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FEASIBILITY STUDY ON APPLYING CLOSE-KIN MARK-RECAPTURE ABUNDANCE ESTIMATES TO INDIAN OCEAN TUNA COMMISSION SHARK SPECIES

FUNDING: The food and Agriculture Organization of the United Nations (“FAO”) under [GCP/GLO/983/EC] - IOTC-Support of the IOTC Scientific Committee Program of Work – strengthening the precautionary approach

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April 29th, 2020

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Cite as	Rodriguez-Ezpeleta N, Patterson TA, Pereda I, Grande M, Davies CR, Lezama-Ochoa N and Zudaire I (2020). Feasibility study on applying Close-Kin Mark Recapture abundance estimates to Indian Ocean Tuna Commission shark species. Final Report to IOTC.
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TABLE OF CONTENTS

1. EXECUTIVE SUMMARY

The Close-Kin mark recapture (CKMR) method is an innovative approach that allows estimating abundance and other important parameters by finding pairs of related individuals in a population based on their genetic make-up. The method that has been demonstrated suitable for application to fish and elasmobranch species and has been applied or is under consideration for application to about a dozen species. A revision of the studies performed or ongoing has revealed that the CKMR method can be applied to species spanning a large variety of life-histories, for which diverse levels of background biological knowledge is available, or with limited or extensive sample accessibility, as long as the model is adapted for each particularity. The compilation of the technical considerations associated with a CKMR study design, the evaluation of alternatives to overcome potential complications, and the review of available biological knowledge, catch data and tissue sampling programs has allowed to perform a preliminary assessment of the potential feasibility of CKMR for IOTC sharks. We have identified the blue shark, the shortfin mako and the silky shark as the most suitable species for application of CKMR. The blue shark has the advantage of having a well-known biology and potential for appropriate sampling logistics, but its large abundance suggests that a potentially impracticable number of samples will be needed to obtain the required number of pairs of related individuals. The shortfin mako has sufficient biological knowledge, potential for appropriate sampling logistics and the advantage of having a previous evaluation done for the South Atlantic suggesting that about 5,000-10,000 samples would be sufficient for finding the required number of pairs of related individuals. The silky shark has sufficient biological background but has the disadvantage of having less catch at size data, which might result in a more uncertain estimation of the samples needed. The next steps for assessing if CKMR is considered feasible for these species is establishing more formal sampling designs which incorporate the relevant biology and available information on likely population size to estimate samples sizes required for varying levels of precision in the abundance estimates.

2. BACKGROUND

Sustainable fisheries management requires, among other things, assessment of population connectivity and estimation of abundance. These, in turn, provide information on spatial distribution, stock size, recruitment and mortality. Direct fishery-independent methods to estimate abundance exist including acoustic or line transect surveys, mark recapture tagging experiments, and daily egg production method surveys. Yet, the required logistics for each of these techniques may make them problematic when used on endangered, deep-water, or highly migratory species. On the other hand, indirect methods such as those based on commercial catch per unit effort (CPUE) suffer from biases due to catchability and/or misreporting. Most Indian Ocean Tuna Commission (IOTC) stocks, particularly sharks, rely on CPUE data, with its associated biases that may compromise stock assessment (Maunder & Piner 2015; Polacheck 2012). Pelagic sharks are landed in IOTC catches and reported to varying degrees, but current catch data is insufficient for conventional stock assessment, and there is concern that this group of sharks may be being depleted at an unsustainable rate. Bycatch data from these fisheries can be reasonably expected to be far less accurate and, therefore unlikely to be useful for constructing reliable abundance indices. In the IOTC, the relative utility of CPUE as an index of abundance is reflected in the fact that, at present, there is only an assessment for one shark species (blue shark) from a targeted fishery. Hence, the need to consider other approaches to establish fisheries independent measures of abundance for assessment and management of pelagic shark populations is a priority for these fisheries.

Either as an alternative or complementary approach, innovative genetic techniques can provide solutions to the estimation of population connectivity and abundance (Casey *et al.* 2016). Genetic data derived abundance estimates are still incipient; yet, they are likely to become a standard approach with the recent development of the Close-kin Mark Recapture (CKMR) method (Bravington 2014). The CKMR is inspired by the principles of classical mark-recapture methods, which are used for estimating abundance and demographic data by marking individuals which are later recaptured. Based on the probability of a fish being recaptured depending on the size of the population and the time of liberty (i.e. from the marking until the recapture), both abundance and mortality are estimated (Cormack 1964; Jolly 2006; Seber 2006). In CKMR, instead of recapturing the marked animals, the aim of the sampling process is to identify closely related individuals. Briefly, the method consists of taking a sample of fish and finding pairs of related individuals based on their genetic make-up. These are called pair-offspring relationships. The number of related pairs detected together with biological parameters of the species, can be used to estimate the population size. In principle, the larger the population size, the lower the probability of finding related pairs, and vice versa (Bravington *et al.* 2016b). The principle of the method is shown in Figure 1, where the number of pair-offspring relationships (P) found among sampled juveniles (mJ) and adults (mA) are used to calculated the total number of adult population size (Na) as:

$$Na = 2mJmA/P$$

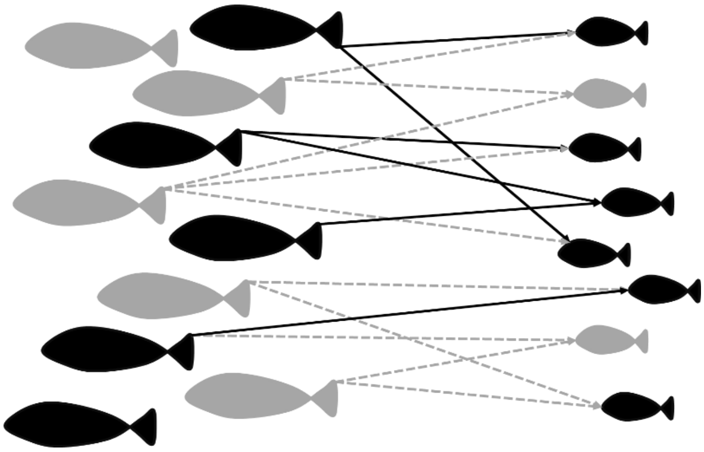

Figure 1. Adults (big fish) and juveniles (small fish) are sampled (black fish) from the total population (black and grey fish) to find POPs (black solid lines) among all the possible ones (grey dashed lines). Here, the total adult population (10) is estimated as two times the number of sampled adults (5) multiplied by the number of sampled juveniles (6) divided by the number of POPs found (6) as indicated in the equation.

The number of kin-pairs in a sample of individuals, is *ceteris parabis*, inversely related to the size of the population from which the sample is drawn. If a study finds kin-pairs to be relatively numerous, it indicates a smaller population than if fewer kin-pairs were obtained from the sampled individuals. This is the central idea of CKMR and in the simplest (and most unrealistic) setting of a closed population with no mortality, recruitment, emigration or immigration, it is a form of Lincoln Petersen estimate (Bravington *et al.* 2016b). Different kinds of kinship pairs can be considered for the CKMR method: (i) Parent-Offspring Pairs (POPs), which provide information on abundance (by number of POPs found) and on fecundity-at-age/size (by differences in numbers of POPs found with parents of different ages), or (ii) Half-Sibling Pairs (HSPs) and Full-Sibling Pairs (FSPs), which provide information on abundance (given the amount of pairs identified) and on survival/mortality rate (given the difference in age between siblings) (Bravington *et al.* 2016b). Finally, (iii) the possibility of GGPs (Grandparent-Grandoffspring Pairs) needs to be considered as they are not genetically distinguishable from HSP (they both share 25% of the genome). Thus, a kinship is considered HSP or GGP based on the age of the individuals in the pair, which also allows for information to be obtained on survival as for an individual to be a grandparent, it must experiment a survival of at least twice the minimum maturity age in theory (in real cases, even more). Apart from these cases, other special cases of kin can also be used. For instance, although finally not used, FOPs (Father-Offspring Pairs) have been considered in some cases (which are just a specific type of POP), as they would provide useful information on abundance, if sex proportions are well known and enough pairs are found. Note also that sex ratios can also be inferred using Maternal/Paternal POPs inferred through mitochondrial DNA (Davies *et al.* 2018a).

Determining the type of relationship that connects two individuals is done by statistical analyses of allele frequencies of polymorphic markers such as microsatellites or single nucleotide polymorphisms (SNPs). First CKMR studies relied on the use of microsatellites, which were (just) suitable to find POPs, and thus recent endeavours have relied on SNPs which have proven to be future-proofed, cheaper and able to find HSP as well as POPs (Bravington *et al.* 2017b). SNPs have become widely used in the study of connectivity in marine fish, which has been facilitated by advent of the Restriction site Associated DNA sequencing (RAD-seq) (Baird *et al.* 2008) and related methods (Davey *et al.* 2011), which subsample putative homologous regions of the genome of several individuals at the same time, with the aim of identifying and genotyping SNPs. Interestingly, the approach can be applied to organisms for which no prior genomic resources are available, which is particularly advantageous for marine fish for which very few complete genomes are available.

Although the principle of the method appears simple and its potential is clear (Martinsohn *et al.* 2015), it is also evident that there is a need to evaluate its viability for each species under study considering the information required to optimize the model, the existing biological knowledge, population size, and the number, location and nature of samples available and needed. All applications of CKMR to date have considered that the probability of detecting a given number of kin-pairs is related to the expected total reproductive output (TRO) of the adult population. Therefore, in relating kinship data to abundance, the modelling usually must account for factors such as maturity schedules, reproductive output, selectivity in the sampling, among other factors. Additionally, the influence of these covariates may need to be sex specific.

These complexities notwithstanding, CKMR is a type of natural tagging experiment which is fisheries independent. Crucially, it is not subject to vagaries of tag reporting rates, fleet/gear/targeting changes, errors in catch reporting and other potential sources of bias associated with more traditional fisheries data. The attractiveness of CKMR is that it can provide an estimate of absolute abundance of the breeding population from a relatively short study (over a few years) and, with enough data, can simultaneously provide estimates of adult mortality rates and population trend. It should be recognised from the outset that for large-scale pelagic fisheries, CKMR is also cost effective relative to other methods such as large-scale conventional tagging programs (Kolody & Bravington 2019). Moreover, when CKMR studies are conducted at appropriate scale, it is also one of the quickest methods for obtaining abundance estimates, as estimates of population size and trend can be produced from a research program of several years. This feature of CKMR derives from the fact that the population has already “tagged itself” through its DNA. A conventional tagging program, even putting aside the aspects of logistics, expense, tag reporting/loss etc., produces data in “real time”. For long lived species such as sharks, this can mean that informative data for abundance estimation are slow to accumulate.

3. OVERVIEW OF PREVIOUS CKMR STUDIES

The CKMR method was originally proposed for the Northeast Atlantic minke whale (Skaug 2001) and has now been fully applied to a handful species and is under consideration for application in several others. The first full application of the method was on the Southern Bluefin Tuna (SBT) (Bravington *et al.* 2016a), a species that fulfilled a series of conditions that were ideal such as absence of population structure, single and known spawning site, extensive species specific biological information and sample availability. Application to other species requires selecting the appropriate form of CKMR method to a apply to the particular context, considering the life-history of the study species and/or the nature of the fishery/practical sampling regime. For example, from the SBT CKMR study, it was discovered that POPs and HSP are required for most teleost species to disentangle confounding between mortality, selectivity and residency assumptions used in the original application (Davies *et al.* 2018b).

In this section, we review all the studies that have applied or that are considering the application of the CKMR method. The information presented in this review has been compiled by examining articles published in indexed journals, as well as reports and working papers of the Commission for the Conservation of Southern Bluefin Tuna (CCSBT) and the international Commission for the Conservation of Atlantic Tunas (ICCAT). Additionally, a summary of the section can be found as a Table 1.

3.1 Tunas

3.1.1 Southern Bluefin Tuna

The first full application of the CKMR method was on the Southern Bluefin Tuna (SBT), *Thunnus maccoyii*. This species supports a high value fishery, and the motivation for applying the CKMR method was that no reliable abundance indices could be derived from Catch Per Unit Effort (CPUE) data (Bravington *et al.* 2016a). Additionally, the SBT seem ideal as case study for the first application of the CKMR because of several characteristics of the species (presence of a single population spawning at a single known spawning ground), previous knowledge of the species (mortality, fecundity), historical investments in research and monitoring, and sample accessibility through an adult fishery on the spawning ground and a juvenile fishery on then nursery ground.

The original application of the CKMR to the SBT relied on POPs identified using 25 microsatellites and involved sampling and genotyping over 13,000 (about half adults and half juveniles) individuals from 2006 to 2010. This resulted in a total of 45 POPs (involving 20 female and 25 male adults) which, combined with individual biological data and species-specific knowledge, allowed to estimate the spawning adult biomass of two or three times larger than the pre-CKMR point estimates (Bravington *et al.* 2016a). The CKMR data has been incorporated into the new CCSBT Operating Models (Hillary *et al.* 2013), the current assessment framework of SBT, with the consequence of reducing the uncertainty related to spawning biomass trend as well as the severity of the estimated depletion level of the stock, i.e, from 5 to 8% of pre-exploitation levels in 2011 (Anon 2013) .

Subsequent applications of the CKMR to the SBT have relied on HSP as well as POPs. This has allowed estimation on total mortality of adults by allowing separation of selectivity and mortality in the estimation model; additionally, including HSP also increases the probability of finding kin-pairs (Davies *et al.* 2018a). Inclusion of HSP for CKMR has been possible through the replacement of microsatellites by SNPs, which, besides providing the information required to estimate the uncertainty in the genotype calls, they reduce genotyping cost and ensure a future-proof tool (Bravington *et al.* 2017b). This study has provided a 10-year time series of abundance (CV<0.2) and estimates of total mortality for the reproductive component of the stock (Bravington *et al.* 2017a; Bravington *et al.* 2015; Davies *et al.* 2019; Davies *et al.* 2018c). The method has now been adopted for monitoring the breeding stock and CKMR data is used in management procedures to set Total Allowable Catches (TACs) (Hillary *et al.* 2017, 2018a).

3.1.2 Atlantic Bluefin Tuna

The Atlantic bluefin tuna (ABFT), *Thunnus thynnus,* is a commercially valuable fish whose status remains vulnerable due to high demand in the growing globalized fish market (Sissenwine & Pearce 2017). Unlike the SBT, the ABFT is composed of at least two partially reproductively isolated populations (Rodríguez-Ezpeleta *et al.* 2019), one spawning in the Mediterranean and another in or close to the Gulf of Mexico, that mix in feeding aggregates throughout the Atlantic. Under this scenario, the CKMR method needs to be applied to each population (West and East) assuming that individuals could be assigned to the population of origin (West or East) prior to the application of the method, which is now possible using diagnostic SNP markers (Rodríguez-Ezpeleta *et al.* 2019). Given this, the CKMR feasibility is currently being evaluated for the Western and Eastern ABFT but has not yet been applied to either of them.

3.1.2.1 Western Atlantic Bluefin Tuna

Applications of CKMR to the Western ABFT should consider that there is a potential genetic differentiation of the two spawning grounds within the West (Rodríguez-Ezpeleta *et al.* 2019): the Gulf of Mexico and the more recently discovered Slope Sea (Richardson *et al.* 2016). Additionally, because there is no fishery operating in the main spawning ground in the Gulf of Mexico, a potential application of the methods should rely on scientific surveys, which are able to provide samples from and larvae, but no juveniles. So far, preparatory work has been done for a POP + HSP based design on larvae from the Gulf of Mexico and adults form the Gulf of Mexico and Gulf of Saint Lawrence (Peter Grewe, CSIRO and John Walter, NOAA; pers. comm.). Davies *et al.* (2018b) conducted an informal design study for Western Atlantic Bluefin tuna and identified a range of pilot studies that needed to be completed as necessary precursors for a full field study (e.g. development of stock structure markers, feasibility of using larvae from larval tows etc). This preliminary work is approaching completion.

3.1.2.2 Eastern Atlantic Bluefin Tuna

Within the Eastern ABFT, although no genetic differentiation has been observed within the Mediterranean sea, the presence of potential behavioural contingents (Arrizabalaga *et al.* 2018) could translate into nonheritable structure that should be considered when applying the CKMR method. This could occur if for example, adults selected a given spawning ground within the Mediterranean Sea (which could be different to where they were born) that they would then use for life. Such scenario, provided a good sampling design, should be possible to detect from CKMR data but require a specific model (Davies *et al.* 2018b). Given the unknowns concerning population structure of Eastern ABFT, the recommendations for a first attempt to apply the CKMR method to this species where that i) adult samples from all potential spawning grounds should be collected, ii) aged juvenile samples from all potential distinct juvenile sites should be collected, iii) HSPs and POPs need to be identified, iv) individual metadata and species-specific biological information should be available. Provided that this data/information is available and assuming two spawning contingents with two juvenile grounds, the scooping study performed for Eastern ABFT estimated that samples in the order of 30,000 – 40,000 juvenile/adult individuals would be required (Davies *et al.* 2018b).

3.1.3 Pacific Bluefin Tuna

The Pacific bluefin tuna (PBFT), *Thunnus orientalis*, is a highly valuable species that perform trans-Pacific migrations. Relative abundance indices used for the assessment of this species are calculated based on CPUE data, which has limitations due to depletion and changes in catchability. Other alternatives for estimating spawning stock biomass have been explored such as aerial or acoustic surveys or mark-recapture approaches, but they are inaccurate and/or expensive. Thus, the CKMR was considered a method to be explored for improving the assessment of the PBFT (Anon 2015). Briefly, the key pieces of knowledge/information needed for application of the CKMR method were examined: age and growth, reproductive output, spawning sites and stock structure, and distribution and movements. Based on this information, they estimated approximative samples per area needed, totalling about 8,000 samples needed (including juveniles and adults) spanning 5 sites to find about 50 same-year POPs. More research on the possible application of the CKMR to the PBFT is ongoing (Anon 2019a).

3.1.4 Yellowfin Tuna

Kolodyand Bravington (2019) presented a discussion paper on the merits of CKMR for Yellowfin tuna (YFT), *Thunnus albacares* in the Indian Ocean, including simple estimates of likely samples sizes required given different spawning stock biomass estimates from the current stock assessments. This simple exercise was aimed at raising awareness of the approach and demonstrating the likely sampling effort and resources required for an abundant species, such as yellowfin. The results suggested CKMR could be a cost-effective alternative, albeit with considerable logistic challenges, given the samples size and geography involved. A more detailed design study is required to provide specific samples sizes and distribution and required covariates and sequencing requirements.

3.2 Sharks

Sharks are particularly challenging for conservation and management due to their low productivity and because they are part of targeted fisheries but also often discarded and unreported. Thus, the CKMR method is very attractive for these species. Yet, the special features of sharks need to be considered since, as opposed to teleost fish, they have much lower litter sizes and lower population sizes. So far, the CKMR method has been applied to or evaluated for five case-studies of sharks.

3.2.1 River sharks

Several euryhaline elasmobranchs in Northern Australia spend their juvenile years within a river system before moving to (and between) estuaries and the open sea as adults, returning to rivers to breed: the largetooth sawsh (*Pristis pristis*), the speartooth shark (*Glyphis glyphis*) and the Northern river sharks (*Glyphis garricki*). Currently no credible quantitative estimates are available for any of them, and thus CKMR studies are ongoing (Toby Patterson, CSIRO; pers. comm.). It is also important to note that there are other applications of kinship data that are particularly interesting for sharks. For example (Feutry *et al.* 2017) use kinship inference to estimate connectivity between speartooth shark populations in Northern Australia, showing that connectivity in populations at a demographically meaningful timescale (i.e. not on evolutionary time scales) can be established.

3.2.2 School Shark

School shark (*Galeorhinus galeus*) is a long-lived slow-breeding demersal shark species fished around southern Australia that has been over-exploited and listed under conservation legislation. The reductions in TACs associated with the implemented recovery plan has invalidated the conventional catch-rate monitoring based abundance index used in the assessment. Thus, determining if the species is recovering requires alternative methods such as the CKMR. Because gear selectivity allows catching juveniles, but not adults, the large scale CKMR project started is based on juvenile samples only. The report of the project on school shark abundance estimation using CKMR is in the final stages of revision and will be available soon (Toby Patterson, CSIRO; pers. comm).

3.2.3 White Shark

The white shark (*Carcharodon carcharias*) is an emblematic species listed as vulnerable by the International Union for the Conservation of Nature (IUCN). Several attempts have been performed to estimate abundance of this species, based on photographic records, conventional tag-recapture or historical catches. Yet, obtaining reliable estimations from these methods is challenging due to several factors such as biases introduced by shark´s site fidelity or catchability among others. Thus, the CKMR was adapted for application to this species, taking its biology into account. The white shark CKMR endeavour aimed at finding HSP by sampling juveniles. A total of 183 individuals were collected and genotyped for almost ten thousand SNP markers, which after quality control was reduced to 115 individuals and about two thousand SNPs. From the HSP and UP (unrelated pairs) found, the CKMR method was used to estimate adult abundance, trend and survival rate (Bruce *et al.* 2018; Hillary *et al.* 2018b).

3.2.4 Grey Nurse Shark

The Grey Nurse Shark (*Carcharias taurus*) is considered vulnerable by the International Union for the Conservation of Nature (IUCN) and whose previous abundance estimates relied mostly on photo identification, which suffer from biases associated with non-homogeneous and non-random sampling. Thus, a model similar to the white shark model used for white shark (Bruce *et al.* 2018; Hillary *et al.* 2018b) modification of the model used for the white shark was used to apply the CKMR to this species (Bradford & Thomson 2018). In particular, the model was adapted to the absence of age estimates and reliable length estimates of the sampled individuals. Using 514 samples 108 HSPs, 26 POPs and 11 FSPs were identified, which led to a population estimate of around 2000 adults, more or less depending on the ages for female and male maturities considered (Bradford & Thomson 2018). The Nurse shark example highlight the importance of background reproductive biology (age at maturity, litter size etc) and accurate length and age estimates in CKMR for sharks. Yet, despite the uncertainties, the study resulted in indications of a positive population growth rate, which is important for management decision/policy purposes.

3.2.5 Shortfin mako Shark

The shortfin mako shark (*Isurus oxyrinchus*) is a highly migratory species classified as endangered species under the IUCN classification. Recent research considering the potential for CKMR to estimate the abundance of shortfin mako in the north and south Atlantic is ongoing (Mark Bravington, CSIRO, pers. comm).

3.3 Antarctic Blue Whale

The Antarctic blue whale (*Balaenoptera musculus*) is a heavily depleted species due to Antarctic whaling operations for which no reliable current estimates are available. The CKMR method was evaluated for this species and considered feasible using POPs inferred from large collection of biopsy samples and using methylation to infer age. Briefly, it was found that CKMR offered a significant improvement over traditional Mark-

Recapture methods with the same number of samples, and that it required a shorter time to get realistic results (Bravington *et al.* 2014).

3.4 Brook trout

The brook trout (*Salvelinus fontinalis*) was used as a case study to validate CKMR estimates with standard mark-recapture methods. Seven populations where used and overall, 2400 trout were non-lethally sampled and genotyped for 31 microsatellites. In order to validate the CKMR method, first a mark-recapture alternate technique was used to estimate abundance, and then the CKMR results were validated with the previous one. The estimations derived from the standard mark-recapture and from the CKMR were very similar in the 7 populations, directly validating the CKMR for the first time (Ruzzante *et al.* 2019). Yet, it should be noted that the population structure and life history of this species are unusually simple, and thus not likely to occur in many other species.

3.5 Flatback turtle

Patterson *et al.* (2018) used simple spatial models to determine the likely pattern of POPs expected from sampling various colonies of flatback turtle (*Natator depressus*). Since adult females are readily counted when they nest, the aim was not to determine abundance of the breeding population but rather the connectivity between sites. Therefore, the design exercise was simply aiming to distinguish between various connectivity scenarios through the pattern of cross site POPs.

Table 1. Summary of species for which CKMR has been applied or evaluated.

Species	**Status**	**Genetic markers**	**Kinship pair type**	**Available relevant species-specific data**	**Available relevant individual specific data**	**Additional notes**	**Remarkable specifications of the model**
Southern Bluefin tuna *Thunnus maccoyii*	Applied	Microsatellites; SNPs	POP; POP+HSP	- fecundity-at-age - age at maturity - survival (assumption)	- age (from otoliths) - fecundity (inferred) - sex	Spawning grounds, juvenile and adult locations known; no population structure	-
Atlantic Bluefin Tuna *Thunnus tynnus*	Scoping study performed	SNPs	POP+HSP	- fecundity-at-size - maturity (ogive)	- age (from otoliths) - fecundity (inferred) - sex	At least two genetically isolated populations	Account for alternative stock-structure scenarios
Pacific Bluefin Tuna *Thunnus orientalis*	Initial evaluation	-	-	-	- age (from otoliths) - fecundity (inferred) - sex	-	-
Yellowfin Tuna *Thunnus albacares*	Initial evaluation	-	POP+HSP	- length at age - selectivity at age	- fecundity at age	Expected sample size known	-
River sharks *Pristis pristis* *Glyphis glyphis* *Glyphis garricki*	Application in progress	SNPs	HSP+FSP	- size at maturity	-	Juveniles very rarely move between locations, whereas adult do	Account for kinship finding probability depending on age

School sharks *Galeorhinus galeus*	Application in progress	SNPs	HSP+FSP	-	-	-	-
White shark *Carcharodon carcharias*	Applied	-	HSP	- fecundity (assumption) - age at maturity	- age (from band pairs or ALK) - sex?	Hard to sample adults	Work only with sibling relationships
Grey nurse shark *Carcharias taurus*	Applied	SNPs; mtDNA (for sex determination)	POP+ HSP+FSP +GGP	- fecundity (assumption) - age at maturity (assumption) - survival (assumption)	- age (from ALK) - sex	No real knowledge on maturity-at-age	Need to make assumptions and try different approaches on maturity
Shortfin mako shark *Isurus oxyrinchus*	Under consideration	-	-	-	-	-	-
Antarctic Blue whale *Balaenoptera musculus*	Initial study performed	SNPs	POP	- age at maturity	- age (from methylation) - sex (from mt genome)	Age estimated by methylation (not so accurate)	Account for possible errors or deviations on age estimation
Brook trout *Salvelinus fontinalis*	Applied	Microsatellites	POP	- Fecundity at age - Age at maturity	- age (from ALK) - fecundity (inferred) - catch location	-	-
Flatback turtle *Natator depressus*	Initial evaluation	-	-	-	-	-	-

4. POTENTIAL TARGET SHARK SPECIES

The IOTC listed 55 shark species that are known to occur in the fisheries directed to at IOTC species or sharks (Anon 2019c). The Working Party on Ecosystem and Bycatch (WPEB) is currently focused on species described in Table 2 and recommended the Scientific Committee consider the consolidated set of recommendations, for example, improvements on species identification, data sampling and collection, fill historical data gaps, as well as the management advice for each of these shark species. Historically, low reporting rates of shark nominal catch data occurred in the IOTC. This situation has improved in recent years, by increasing the number of fleets reporting over time since 1950s. Despite the improvement according to WPEB (Anon 2019b), in addition to the low level of reporting, catches that have been reported are thought to represent only those species that are retained onboard without considering discards.

Table 2. List of six shark species of IOTC WPEB interest.

Common Name	Scientific Name	FAO Code
Blue shark	*Prionace glauca*	BSH
Oceanic whitetip shark	*Carcharhinus longimanus*	OCS
Scalloped hammerhead shark	*Spyrna lewni*	SPL
Shortfin mako shark	*Isurus oxyrinchus*	SMA
Silky shark	*Carchiarhinus falciformis*	FAL
Bigeye thresher shark	*Alopias superciliosus*	BTH

Like many other elasmobranchs, pelagic sharks share many of the following life-history strategies: low fecundity/high maternal investment in young, late maturity and high longevity. These aspects of the elasmobranch life history have been widely accepted as putting species within this group at risk of over-exploitation and even extinction (Dulvy *et al.* 2014; Pardo *et al.* 2016). Catch records for sharks are noted to be highly uncertain. Indeed, the patterns in the catch data from the IOTC datasets, are not obviously indicative of any trend and probably reflect factors such as changes to targeting effort, reporting, etc rather than underlying abundance. Hence, the situation for pelagic sharks is that the CPUE data are likely to be of less utility as a consistent, reliable abundance index than the target tuna stocks (Kolody & Bravington 2019). Currently within the IOTC only blue shark has a stock status based on a formal stock assessment. Thus, a significant effort by scientific community and other stakeholders to reverse the situation and work on indicators for providing estimates and scientific advice for these highly vulnerable stocks.

In order to determine if CKMR is applicable to IOTC shark species, the WPEB requested that a feasibility study be conducted for a Close Kin Mark Recapture (CKMR) method applied to a shark species in the Indian Ocean. The aim of this feasibility study is to determine if these methods can be used as an alternative to assessment models for these data poor species. Addressing that aim requires an appraisal of the technical considerations of the CKMR method, the existing knowledge of biological data of the potential shark species and the availability of samples for a potential application of the CKMR method.

5. CONSIDERATIONS FOR CKMR STUDIES

Close kin mark recapture is not applicable to all species (Bravington *et al.* 2016b) and there are aspects that determine whether a species has a breeding biology and population dynamics that makes it amenable to CKMR (Figure 2). For example, species which are semelparous, have weird “clonal” reproductive systems (e.g. parthenogenesis) are ruled out as kinship relationships cannot be reliably identified. Similarly, super abundant species, such as many invertebrates, are infeasible due to the vast amounts of sampling and genetic sequencing that would be required. Another difficult set of species would be long-lived species that display lifetime pair bonds, such as is the case for many seabird species. While CKMR is not impossible for these species, they are more challenging than for species where mating can be regarded as more-or-less random. Examples from marine systems where CKMR is suitable are teleost species (e.g. cod / tuna) where mating is random, but fecundity is related to size or age, and “whale like” species (long lived, random mating and low fecundity) such as cetaceans and sharks. In the teleost case, it is advisable to use samples for both POPs and HSPs. Clearly, for a long-lived species this generally requires sampling of both juveniles and adults. In the case of sharks, this is often infeasible. Adult sharks of several species are rarely encountered (e.g. this was the case for all species investigated in CSIRO studies to date). Luckily, for these species HSPs are sufficient for CKMR.

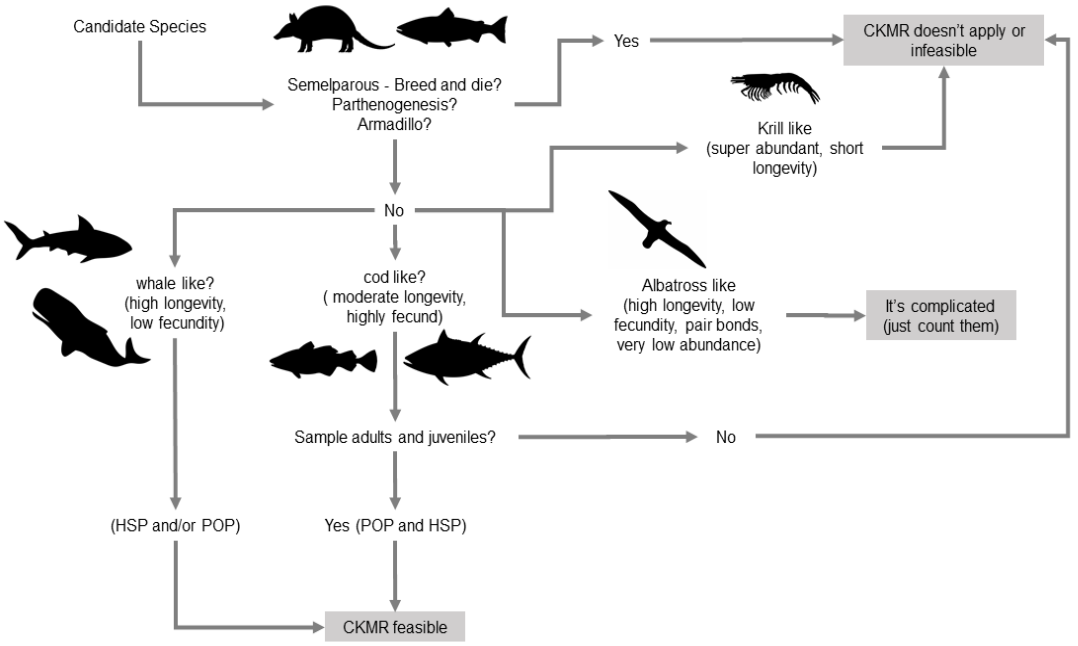

Figure 2. Basic guidelines for determining whether a species may be suitable for CKMR. Additionally, we show whether a study may be successful using Parent Offspring pair matches (POPs), Half-sibling pair matches (HSPs) or both in tandem. As noted in the text, HSPs alone have been successfully employed for sharks where sampling adults is not possible, and POPs are very rarely found and thus uninformative. POP-only studies of teleosts have been conducted, but incorporation of HSP data provides more information on the population and associated parameters.

Even in the case of species that are suitable for CKMR, proceeding without a clear understanding of likely sample size, DNA sequencing requirements and demographic and statistical modelling might well lead to either a clearly unsuccessful study, or even worse, a study which while superficially successful, is actually erroneous in its conclusions.

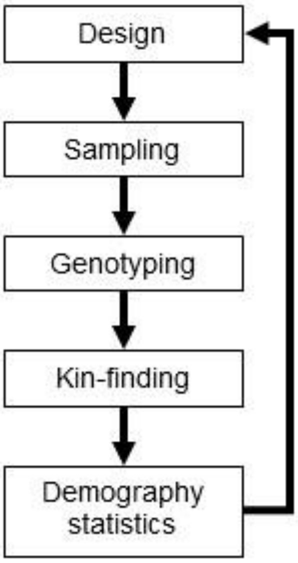

Figure 3. The five components of a CKMR study. The arrows denote the sequence of stages with the loop between the final stage of demography and statistical analysis being connected back to the design stage to indicate that the process is iterative.

There are 5 components to completing a successful CKMR study (Figure 3). The ideal process starts with study design and is followed by sampling, genotyping, demographic/statistical modelling of the population to obtain the abundance estimate and other parameters. Note that there is an arrow leading from the final stage, demography and statistical modelling back to design. This loop back to the start should be emphasized; often the first set of parameter estimates, while highly informative, are typically not the end of the process. Further refinement of the sampling design, collection of more samples etc. will lead to refinement of estimates of abundance and associated demographic parameters. This is particularly likely to be the case in situations like those for pelagic sharks, where the current level of quantitative understanding is low.

5.1 Design

Design of a close kin study aims to use the best available knowledge of the species likely abundance and biology and determine whether a candidate sampling scheme is likely to yield sufficient data for useable (i.e. with useful level of precision) estimates of abundance and other parameters. The process also allows for design of efficient studies that do not over- or under- sample. Undersampling is likely to yield results with high levels of uncertainty (or worse insufficient kin pairs to make a useful estimate) making the results uninformative for management and potentially wasting valuable sampling resources. Over sampling could be inefficient by expending budget and resources on collecting and processing more samples than required for a precise abundance estimate.

CKMR designs use the following logic:

A prior expectation of the likely range of abundance is used as a starting point. While this seems counter-intuitive, given that the point of CKMR is to estimate abundance, some idea of the population size is needed to avoid completely ad hoc sampling and provide a basis for “learning” between iterations of the design exercises in Figure 3 above. In the case of some of the conservation-dependent shark species where CKMR was applied, no formal design was conducted as there was simply no pre-existing data or population estimates to guide the design process. In a commercially targeted, or even monitored bycatch species, the output of a stock assessment based on CPUE data provides a starting point. For design purposes we assume that this number is approximately correct. Clearly, an assessment based on unreliable data and assumptions may be inaccurate. However, by examining sampling designs required to provide a reasonably precise estimate of abundance if the population was of that size, we end up with the following possible outcomes, that are useful in a management context, either: (a) the study finds more kin pairs than expected, in which case we have precise estimates of a smaller stock and clear evidence for management action; or (b) we find fewer kin pairs than expected indicating a larger population size, albeit estimated with a lower level of precision, as the number of kin pairs is related to the precision of the estimate.

In the most detailed examples of design studies (Davies *et al.* 2018b), we combine the abundance estimate with information on growth (potentially by sex), length-weight relationships, maturity schedules, reproductive output (for sharks, litter size and breeding interval) to build a population model. Various scenarios can be built into this - for example that a population is sustainable (at B_{MSY}) or close to unsustainable (i.e. catch rates are at levels equivalent to F_{crash}). Given sufficient ancillary data, spatial models can be used here to test connectivity scenarios as has been applied on Atlantic bluefin tuna (Davies *et al.* 2018b) and also for marine turtles (Patterson *et al.* 2018). Similarly, if there is information on stock structure and individuals can be assigned to a particular stock, then within-stock CKMR is, in principle, feasible.

Using this model and its assumptions, we predict outputs such as the expected number of kin-pairs (either POPs or HSPs) for a range of potential sampling schemes. The number of kin-pairs might be sufficient to proceed with further investigation. This was the case with the study of flatback turtle connectivity (Patterson *et al.* 2018). Typically, the next part of the design is to determine the number of samples required to obtain an abundance with a required level of precision, usually expressed via the coefficient of variation (CV) on the abundance estimate. Design studies also evaluate the efficacy of determining other parameters such as adult mortality and population trend (Davies *et al.* 2018b). In a complicated assessment context, part 3 involves examination of the properties of the likelihood function for the model, conditional on knowledge of the species biology, stock status and of course, the proposed sampling scheme – i.e. number of sampled individuals to be genotyped and if the context involves spatial concerns, their location/stock etc. The goal of the design phase is to estimate the likely uncertainty given the number of samples. This can be done either through simulation or, more efficiently, via the analytical formulae presented in (Bravington *et al.* 2016b).

As shown in Figure 3, a design phase may follow an initial estimate of abundance (Bruce *et al.* 2018) applied an approach following the general analytical design method in Bravington *et al.* (2016b) to the southern-western Australian white shark population, after an initial estimate had been obtained from a CKMR study. The initial population estimate had a relatively large degree of uncertainty and the secondary design study determined the rate of sampling required to halve the CV on the population estimate. This found that if samples of juveniles could be increased then the time required to halve the CV would commensurately reduce from 10 y to 5 y. More recently a design study for north and south Atlantic mako shark stocks by (Bravington 2019) used a fully age and sex structured population model that incorporated catch at age time series to calculate informative sampling designs for these stocks.

It should be clear from the preceding description, that the degree of complexity in a design study can vary considerably depending on the complexity of the underlying stock and the state of knowledge about it and the data series available. While sampling design requirements for sharks can in principle be very straightforward, for the understanding of uncertainty in a "stock assessment" grade CKMR - i.e. one supporting management decisions about for instance, an allowable harvest – design should mostly be based on a fully age structured model which is essentially the same as the final model that uses the real CKMR data. This extra level of model complexity is required to include the effect of various factors such maturity schedules, age or sex specific fecundity, age specific harvest rates, as well as uncertainty in length to age conversion. As noted above, the degree to which this is possible will be determined by the availability of the requisite data.

The key requirements for pre-existing biological knowledge for both design of a study and the modelling required to produce a CKMR estimates of abundance are: (1) a growth model based on adequate age/length samples – e.g. from vertebrae etc. (2) an estimate of age-at-maturity and (3) knowledge of whether the reproductive output is dependent on size. The latter is an influential factor in studies of teleosts where a large female produces disproportionately more gametes than a newly matured female. In sharks, this tends to be less of an influence. Shortfin mako sharks are an example where an older female may produce roughly twice as many pups as a young female. School shark on the other hand have a narrower range of reproductive output of between 20-30 pups. In the former case, this means that reproductive output at age is required to be tracked in the model as older females, by producing more pups, have a higher probability of producing HSPs (i.e. the number of HSPs is systematically inflated).

5.2 Sampling

Obtaining tissue samples in a consistent, structured fashion is obviously a crucial component of CKMR studies. For clarity it is worth emphasizing some of the fundamental aspects of sampling in CKMR.

- Samples may be collected over a number of years. For instance, the recent design for shortfin mako in the Atlantic by (Bravington 2019) estimated that 5000 samples were required for the north Atlantic and 10000 for the south Atlantic. These samples could be collected at 1000 per year for 5 and 10 years respectively. Or alternatively they could be collected at 2500 for 2 and 4 years respectively. The result of the analysis will be the same but obviously in the latter case the estimates of abundance are provided in half the time.

- Samples can be stored for long periods under appropriate conditions (e.g. -80°C freezer). Therefore, suitably archived pre-existing DNA samples may be useful, depending on whether other information such as the age or length of the sampled individuals was also available. However, very old samples may not be relevant to the current stock status as the cohorts that they are from will have died out. A CKMR analysis on such samples would only estimate the size of the stock at the time that the samples were collected (actually, CKMR estimates the adult stock size in the birth year of the juveniles). Clearly the stock size could have changed substantially over the intervening period.

Previous studies have encompassed both relatively large-scale samples (1,000's of samples) from commercial fisheries (SBT, school sharks) and also sampling of endangered, low abundance shark species at known aggregation sites (e.g. juvenile white and *Glyphis spp*, 100's of samples). The latter can feasibly be undertaken by experienced researchers actively targeting juvenile sharks for capture specifically for the purpose of CKMR sampling. In these studies, knowledge of where to target sharks and effective capture methods is crucial. In both cases, samples are collected as a biopsy of tissue, commonly as a fin clip. Obviously, care must be taken to avoid cross contamination between different individuals. Samples should be stored in a labelled vial containing appropriate preserving solution (e.g. 90% ethanol or RNA-later) with unique identifiers to associated data on date of capture, location, species, size and sex or any other biological samples retained (e.g. vertebrae). High quality tissue for sequencing is a pre-requisite for CKMR and HSP detection.

Previous experience shows that those new to CKMR often underappreciate the necessity of obtaining reliable age or length estimates. The importance of obtaining information from which the age of an individual may be estimated cannot be over-emphasised. This data is central to reliably estimating the cohort year of each individual and thus, the age difference of two individuals being compared for kinship. Reliable, direct age estimates (from otoliths in the case of teleosts or vertebrae in elasmobranchs) are the preferable source of this information. While perhaps not always practical, this is at least feasible if samples are obtained from (lethal) harvesting. This was the case for school shark.

However, for conservation dependent species where samples from dead individuals are rare, age is inferred from length. This requires that lengths from sampled individuals are accurately measured and that the growth schedules and associated uncertainty are sufficiently well understood to be incorporated into the analysis. Fortunately, juvenile growth rates are relatively fast and so an individual cohort can be assigned sufficiently accurately based on length modes. However, if there is considerable variability in length-at-age, then the uncertainty in the age estimate needs to be accounted for in the CKMR model. This has been done for grey nurse sharks, where the growth models were relatively poor. Additionally, lengths were estimated by divers collecting tissue biopsies. It was clear from the resulting length data that the data were subject to considerable errors which complicated the study further by introducing other sources of bias and imprecision. While these factors led to a relatively uncertain estimate of the eastern Australian GNS population, the data were still able to produce a useful range of abundance estimates and provide evidence of a positive population trend (Bradford & Thomson 2018).

In the case of sampling pelagic sharks from high seas fisheries, where the species are probably more numerous and samples could be obtained either during fishing operations or during portside handling, a key logistical challenge will be the establishment of robust, but relatively simple protocols for sample collection, labelling, gathering of covariate data and storage. Additionally, the CITES listing status and non-retention policies pertaining to some species will introduce further challenges to sampling logistics and potentially shipping of samples to laboratories for genetic processing. Note that detailed consideration of the practical aspects of sampling pelagic sharks (sources of samples, procedures, permits etc) will be considered in the next report from this project which will assess the feasibility of CKMR for IOTC-monitored pelagic shark species.

5.3 Genotyping

- To determine whether two individuals form a kin-pair first requires suitable DNA sequence data in the form of SNP data. By “genotyping” we mean the process that takes raw genetic data from the sequencer and provides an inferred allele (i.e. AA, BB, AB) at each SNP locus. The issues involved in genotyping for CKMR are introduced in (Bravington *et al.* 2017c) and examples of some of the considerations involved are presented in (Bravington et al. 2017b).
- The number of loci genotyped, the quality of genotyping and the information for kin-finding must be of a standard suitable for detecting HSPs.

- Detecting POPs requires less advanced genotyping, but for the reasons outlined above, a “POPs-only” approach is probably not feasible for elasmobranchs. Moreover, even for teleost studies, POPs and HSPs are needed in order to estimate adult mortality.
- For comparison purposes, genotyping must be conducted at the same loci.

Modern genotyping methods are highly complex and substantial investment is required to properly understand the results from any one method. Therefore, genotyping for a given study should use one method. All CSIRO methods to date have relied on either DaRTseq^{TM} or DaRTCap^{TM} which are proprietary techniques developed by Diversity Arrays Technology Pty Ltd., based in Canberra, Australia. Results from this approach have been high quality and cost-effective for large sample sizes. However, other techniques could be employed, and some authors (Bravington *et al.* 2017c; Davies *et al.* 2018b) recommended that an international workshop should be held to specifically discuss genotyping requirements for CKMR applications using available sequencing platforms.

Sequencing mitochondrial DNA (mtDNA) provides valuable additional information for CKMR studies (Feutry *et al.* 2017; Feutry *et al.* 2015). As mtDNA is inherited only from the mother, this allows information on whether two HSPs are likely related via a shared mother or father. Combining this information has proved very powerful and allows estimation of adult population sex ratio, and potentially sex specific allocation of reproductive output over spatially separate elements of connected populations (Patterson et al, in prep).

5.4 Kinship inference

Statistical methods are required that reliably detect kin pairs. While POPs can be detected from widely available software (Jones & Wang 2010), reliably detecting kin-pairs requires development of specifically designed algorithms. There are several stages to the kinship inference process. Prior to starting to look for HSPs and POPs, quality control steps to remove non-biological information (genotyping errors, contaminations, etc.) are necessary. The aim of the kin-finding stage is to compare each the individual sample to all others and determine if the comparison pair in question are kin (HSP or

POPs) or an unrelated pair (UP). The CSIRO CKMR studies have all relied on a calculating a statistic known as a Pseudo log-odds (PLOD) score that is calculated as the log of the ratio of the probability that a given pair is related. So, the PLOD score is a single number (which may be positive or negative) such that smaller values denote UPs and larger values of the PLOD indicate some kin-type of interest (e.g. HSP) (Bravington *et al.* 2017c). The PLOD scores tend to group into distributions and the kin finding relies on identifying threshold PLOD values above which we are extremely confident that all comparison pairs are HSP or POPs (Figure 4). Highly related pairs such as POPs and full siblings (sharing two parents) are generally easily detected as they have very large PLOD scores. HSPs and UPs are more difficult to distinguish. But with a very large number of informative genotyped loci, HSPs can be identified (Farley *et al.* 2019). Crucial to this process is the elimination of false negatives (kin pairs which appear as un-related but are related). When the number of kin-pairs detected is small (as is generally the case), inclusion of spurious kin can have large effects on the resulting estimates of abundance etc. More details on kin-finding can be found in Bravington *et al.* (2017c) and Hillary *et al.* (2018b).

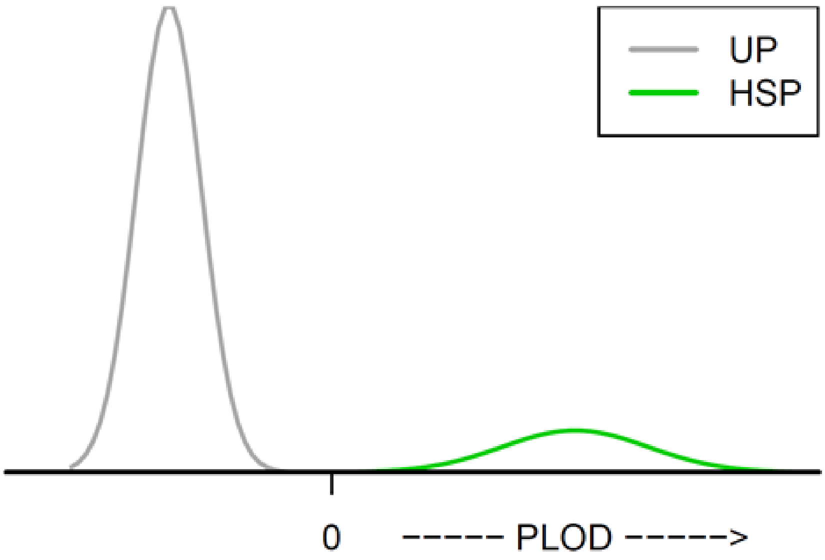

Figure 4. Idealized and schematic representation of the distribution PLOD scores of unrelated pairs (UP) and half sibling pairs (HSP). Note that UP fall to the left and HSP to the right (higher PLOD) scores. POPs (not shown) would have even larger PLOD scores. This figure is reproduced from Bravington, Thomson and Davies (2017).

5.5 Demographic and statistical modelling

The CKMR analysis involves integration of the population model with data on kin-pairs and covariate data. Our description is, for the sake of simplicity and accessibility, somewhat over simplified. It should therefore be recognized that the following is not a guide in any sense, but rather an insight into the linkage between the kinship data and the population models which they inform.

Analysis of HSP matches uses an offspring-centric view of relatedness by seeking the probability that two randomly chosen juveniles will have “chosen” the same parent in a random trial. While this is the reverse of the biological reality of the situation where parent pass on DNA to children, the child-centric view makes for a natural mathematical description without any loss of accuracy. Note that Skaug (2017) has provided details of CKMR from a parent-marking-offspring perspective- but we follow the Bravington *et al.* (2016b) idea of children “marking” their relatives (i.e. parents or siblings). As we have noted POPs tend not to be the most useful type of kin-pair for sharks, but consideration of how CKMR works for POPs is the most easily understood example of CKMR.

If we have a closed population, with no mortality then, analogous to the Lincoln-Petersen estimator of population size, for CKMR we have an estimate of the adult abundance as:

$$N_a = \frac{2m_j\, m_a}{k}$$

where:

- N^a is the estimated number of breeding adults
- m_j and m_a are the number of sampled juveniles and adults, respectively,
- k is the number of observed POPs.

For statistical likelihood calculations we need a probability that a given pair of individuals is a POP given a particular breeding stock abundance (i.e. N^a).

$$\Pr(POP) = \frac{2}{N^a} \qquad (1)$$

However, in sharks it is often very unlikely that we can sample both parents and juveniles. This is not always the case, but for many of the species studied by the CSIRO to date, adults were generally unavailable to sample; either at all, or in useful numbers. In most cases, such as river sharks (*Glyphis spp.*) and white sharks, adults were unavailable, and only juveniles could be sampled. This clearly means that we are highly unlikely to detect sufficient POPs in a sample population to provide a useful estimate of abundance from POPs alone. However, the number of half-sibling pairs (HSPs) is also related to the adult abundance. For a given pair of juveniles, in a simple case the probability that they share a parent is given by:

$$\Pr(HSP) = \frac{4^{-\delta Z}}{N^{a}} \qquad (2)$$

Here we need to introduce two further parameters:

- δ the average cohort difference $mean(|c_i - c_j|)$ where c_i is the cohort of the i and j -th individuals, respectively.
- Z the adult annual survival rate.

Hence, we can link equations (1) and (2) to a population dynamics model of the breeding population through $N_{a,t}$ and implicitly Z. Consider a simple exponential growth model.

$$N_t^a = N_0^a \exp(\lambda t) \qquad (3)$$

where N_0^a is the number of breeding adults in some suitably chosen initial year and λ is the population growth rate. While this model is simple, it has been applied in studies of shark abundance of 4 species; white shark, speartooth shark, northern river shark and grey nurse shark. Although, for studies where there are large numbers of samples a full age-structured population model has generally been employed (Bravington *et al.* 2016a; Davies *et al.* 2018b)

As noted previously, the simple description above, leaves out the complicating factor alluded to the introduction. Namely, that in relating the kinship probabilities to abundance, we must consider the relationship between total reproductive output of the breeding population (aka the spawning stock) and the true abundance. This means incorporating the effects of age, sex, size, sampling/fisheries selectivity into both the population model and therefore the kinship probabilities. Full details of the how this is done in complex models can be found in Bravington *et al.* (2016a) and Hillary *et al.* (2017). Following Bravington *et al.* (2016a), for both the cases of POPs and HSPs figuring in these extra factors (or covariates as they are often referred to) requires an adjustment of the $2/N$ POP probability (equation 1), for each pairwise comparison between two sampled individuals to take account of each's individuals specific sex, year, age etc. Hence, the probability that a pair is an HSP or POP is given by the population dynamics model, which accounts for pair-specific data, and includes demographic parameters such as adult abundance, mortality and perhaps age-specific fecundities. Similar models are the basis of any structured fish stock assessment; however, here the model is used to compute probabilities of kin pairs rather than, say, expected catch rates. For example, when checking whether individual i is the mother of individual j the formula might informally be expressed as:

$$\Pr(\ POP(i,j)|\text{age, length } etc.) = \mathbb{E}\frac{(i\text{ 's reproductive output in } j\text{'s year of birth})}{(\text{ Total female reproductive output})}$$

The general details of the statistical methods for CKMR are provided in Bravington *et al.* (2016b). However, the log-likelihood of these models essentially resolves to a series of binomial probabilities for each sample pair with the probability from equations of the type of (1) or (2).

$$\log\mathcal{L}(N_0^a, \lambda, Z) = \sum_{i\neq j}\sum_{j\neq i}\log\Pr\left(HSP_{(i,j)}\right) \qquad (4)$$

If data from POPs is available, the likelihood function takes a similar form to (4). Additionally, the two likelihoods (POPs and HSPs) can be combined as the they are conditionally independent.

Having a working understanding of CKMR requires having a qualitative understanding of the how the information in the kin pairs, their age, sex etc. flows into estimates of population parameters. Much of the information derives from the rate of HSPs detected through time (known as the "hit rate"). This informs the population growth parameter as a declining population will display an increasing hit rate and the contrary for an increasing population. Mortality rate information comes through the fact that a parent must have been alive at the time of the birth of the youngest individual in an HSP. Hence the adult lived over the period to the birth of the second in the pair and must also have been of breeding age. Put another way; as animals age and die, then the chances of finding a HSP decline. The following, taken from (Bravington 2019) notes the three different pieces of information that inform on abundance, population growth and mortality:

- The average rate of HSP identification (i.e. proportion of comparisons that yield an HSP) is inverse to the average adult abundance;

- How that rate changes for newer cohorts (as they get compared with previous cohorts) shows how fast the adult population is growing or shrinking;
- The HSP-finding-rate will drop with increasing interval between cohorts. The speed at which this happens gives an estimate of the overall adult mortality rate.

Clearly, estimates these parameters requires a spread of samples collected over a number of years. For example, estimates of population growth rate, λ, in white shark in Hillary et al (where essentially λ was zero) were generated from samples which accumulated over several decades. The key advance inherent in the HSP equation (2) above is that we can establish the breeding population size, without ever having to sample an adult breeder. Variants on this model have included splitting the adult population N_t^a by sex and spatially. Additionally, when the time span of samples is over a long period, a time varying trend can be estimated by casting (3) in a state space form with random process error (Bruce et al 2018 applied this for white sharks).

For elasmobranch applications of CKMR conducted at CSIRO it was necessary incorporate more shark-specific biological detail in the population model and kin-pair probabilities to account for particular aspects of the breeding biology of sharks. Sharks, in many ways are more like mammals than teleosts; they produce relatively few young and are late maturing. Additionally, for many species, young are produced in litters. This basic fact of their reproductive biology introduces the potential for considerable heterogeneity in litter survival. Random variation in (surviving) litter sizes will systematically inflate the number of within-cohort HSPs. For example, in a particular year, a random predation event may remove an entire litter of a given mother. This allows for potential for over-representation of within-cohort half siblings. We term this the “litter effect” and a parameter estimate of this is generally included for considering the probability of within-cohort HSPs; additional parameters are included to estimate multiple paternity rates and number of females a male is likely to mate with in a breeding year (see technical appendices in Bruce *et al.* (2018) for full details).

6. REVIEW OF RELEVANT BIOLOGICAL DATA FOR INDIAN OCEAN SHARK SPECIES

Application of CKMR requires a prior knowledge of the species under study. The main parameters of concern for CKMR are age (which is often estimated from length), maturity and reproductive output. Knowing individuals age allows determining the kind of relatedness two individuals can have. For example, if two individuals are born two years apart and the age of maturity of the species is 10, they cannot be parent-offspring. Additionally, knowing the individuals age relative to the age at maturity can be important in determining the effective reproductive output of that individual. Yet, given that most sharks have, relative to teleost fish, generally weak relationship between age/size and fecundity, this is less of an issue for this study. Knowing reproductive output parameters, obtained from information on litter size, gestation period etc. is required to assess the likelihood of a reproductive adult being the parent of a randomly chosen juvenile.

In this sense, a preliminary analysis of the information available is being conducted. Information on the population structure available is summarized by species. In addition, the availability of information on biological traits for each shark species was examined in a literature review, with most of the information gathered already compiled in Murua et al. 2013 and Coelho et al. 2019. Detailed information for each species is provided below and is summarized in Table 3. In general, information on population structure is scarce and only in the case of blue shark there is a stock assessment conducted in the IOTC area. Although there is some information available on life history traits in the Indian Ocean, there are not numerous studies on the issue and the ones performed include a limited number of samples from a small area. If there are marked differences in growth between ocean basins, this may introduce some bias in the CKMR estimates. However, our expectation is that these would need to be extreme to have a substantial influence on a CKMR estimate of abundance.

Table 3 Preliminary review of the available information on shark populations in the Indian Ocean. Population structure (light grey indicates that there is high uncertainty on the population structure in the Indian Ocean), biological traits (dark grey indicates that there are estimates for the Indian Ocean and light grey indicates that there are estimates for other oceans) and stock indicator (dark grey indicates that there is a stock assessment conducted in the Indian Ocean endorsed by the SC and light grey indicates that there are alternative estimations for the vulnerability of the population for the Indian Ocean).

Species	Population structure	Size at age	Weight length relationship	Longevity	Reproductive traits	Sex Ratio	Stock indicator
Blue shark	░	░	▓	░	░	▓	▓
Oceanic whitetip shark	░	░	▓	░	▓	▓	░
Scalloped hammerhead	░	░	▓	░	▓	▓	░
Shortfin mako	░	▓	▓	▓	▓	▓	░
Silky shark	░	▓	▓	▓	▓	▓	░
Bigeye thresher shark	░	▓	▓	▓	▓	▓	░

6.1 Blue shark

The blue shark (*Prionace glauca*) is a widely distributed pelagic species which conducts large vertical and horizontal migrations. The blue shark is classified as near threatened species under the by the IUCN[1], and despite being one of the most productive shark species, the previously conducted Ecological Risk Assessments (ERAs) considered it highly susceptible to longline gear (Murua *et al.* 2018), which is the principal gear contributing to the blue shark mortality in the Indian Ocean (Garcia & Herrera 2018). In the Indian Ocean a single stock is assumed and currently within the IOTC, the blue shark is the only shark species subjected to a stock assessment and shows consistent trends towards the overfished status (Anon 2019c). Although, blue shark is among the best documented species, the uncertainty in catch and bycatch rates is still significant (Coelho *et al.* 2018; Garcia & Herrera 2018). A detailed review on the population structure and

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[1] https://www.iucnredlist.org/species/39381/2915850#conservation-actions

biological traits of this species can be found in Coelho *et al.* (2019); Coelho *et al.* (2018). Significant differences are found in the length-frequency distributions, sex ratios and proportions of immature and mature specimens in the Indian Ocean. Smaller and immature specimens tend to be captured in more temperate waters (i.e. temperate southern waters in the Indian Ocean), while larger and mature specimens are captured more frequently in tropical waters. Therefore, nursery areas seem to occur in temperate waters. Indeed, immature and juvenile sharks concentrate mainly in temperate waters of the south-west Indian Ocean off South Africa, and in the south-east Indian Ocean off south-western Australia, implying that these may be the two main nursery grounds. Overall, sex ratio is close to 1:1, although there are significant spatial differences in the sex ratio, with predominance of females in southern latitudes. As suggested in previous studies, there might be some connectivity between the south-east Atlantic and south-west Indian Oceans. Genetic studies indicate genetic homogeneity and unrestricted female mediated gene flow between ocean basins and suggest that blue shark populations may be connected by gene flow at the global scale (Veríssimo *et al.* 2017). Regarding life history traits, an extensive review can be found in Nakanoand Seki (2003) and Coelho *et al.* (2019). There is information for the Indian Ocean on reproductive biology, age and growth. However, studies focused on the Indian Ocean are not numerous and local in scope. Details can be found in Table 4.

Table 4. Life history indicators for blue shark. F: females; M: males; C: both sexes combined; IO: Indian; AO: Atlantic Ocean; PO: Pacific Ocean; RND: round weight; DWT: dressed of carcass weight; TL: Total Length; FL: Fork Length; PCL: precaudal length; L50: size at which the 50% of the population is mature.

Reference	**Ocean**	**Linf (cm)**	**K (yr^{-1})**	**t0 (yr) or Lo (cm)**	**W-L Conversion**	**Longevity (yr)**	**Maturity (cm and yr)**	**Fecundity (n)**	**Sex ratio (F:M)**
Andrade *et al.* (2019)	IO	283.8 (M) 290.6 (F)	0.13 (M) 0.12 (F)			25			
Ariz *et al.* (2007)	IO				RND = 1,33x10^{-6} T^{L3,20} RND= 2,80x10^{-6} FL^{3,17} DWT = 1,69x10^{-7} TL$^{3.42}$ DWT =4,02x10^{-7}FL^{3,36}				0.2:1
Compagno (1984)	ALL				LogW=-5.396+3.134logTL	20	182-281 (M) 221-323 (F)	4 - 135	1:1
Francisand Duffy (2005)	PO				FL = –1.615 + 0.838 TLnat FL = 0.745 + 1.092 PCL		190-195 (M) 170-190 (F)		
Fujinami *et al.* (2019)	PO	284.9 PCL (M) 257.2 PCL (F)	0.117 (M) 0.146 (F)	-1.35 (M) -0.97 (F)					
Hazinand Lessa (2005)	AO	352.1	0.1571	-1,01	EW=0.010TL$^{2.8592}$		225 TL (M) 228 TL (F)	30	1.8:1.0
Jolly *et al.* (2013)	IO/AO	294.6 (M) 334.7 (F) 311.6 (C)	0.14 (M) 0.11 (F) 0.12 (C)	−1.66 C		16	201.4 (M; L50) 194.4 (F; L50)	43	
Joung *et al.* (2011)							193 TL (F)		
Joung *et al.* (2017)	AO	352.1	0.13	–1.31		21.4 - 26.6	6.5 y. (M) 6.7 y. (F)		
Joung *et al.* (2018)	PO	376.6 (M) 330.4(F)	0.128(M) 0.164(F)	-1.48(M) -1.29(F)					0.3:1
Nakano (1985)	PO				WT = 2.838x10^{-6} TL$^{3.174}$ (M) WT =2.328x10^{-6}PL^{3,294}(F)		199 TL		

Nakano (1994)	PO	289.7 (M) 243.3 (F)	0.129 (M) 0.144 (F)	-0.756M) -0.849(F)			203 (M; LT50) 186-202 (F; LT50)	1-62	
Rabehagasoa *et al.* (2014)	IO	258	0.16			15			
Romanovand Romanova (2009)	IO				$TW=0.159x10-^{4}FL^{2.84554}$(C) $W=0.160x10^{-4}FL^{2.84153}$ (M) $TW=0.835x10^{-5}FL^{2.97234}$ (F)				
Varghese *et al.* (2017)	IO						207.11 (LT50) (M)		1:5.5

6.2 Oceanic whitetip shark

The oceanic whitetip shark (*Carcharhinus longimanus*) is a highly migratory species with a circumglobal distribution in tropical and subtropical seas, occupying the epipelagic column and with complex vertical movements (Tolotti *et al.* 2017). The oceanic whitetip shark is classified as critically endangered under the by the IUCN[2] and received a medium vulnerability ranking in the ERA rank for longline gear because, despite being characterized as medium susceptibility to longline gear, it was estimated as one of the least productive sharks species (Murua *et al.* 2018). There is no quantitative stock assessment and limited basic fishery indicators currently available for oceanic whitetip sharks in the Indian Ocean. The genetic analysis evidenced low levels of genetic diversity for the oceanic whitetip shark and moderate levels of population structure with restricted gene flow between the western and eastern Atlantic Ocean, and a strong relationship between the latter region and the Indian Ocean. This can indicate that some specimens of tropical and sub-tropical fish found in the Indian Ocean may cross the barrier of the Benguela current (Camargo *et al.* 2016). Indeed, the absence of significant genetic structure can indicate the existence of only one genetic stock of oceanic whitetip sharks around the African continent (one for the eastern Atlantic and western Indian Ocean) (Camargo *et al.* 2016). The low genetic diversity observed mainly in the eastern may represent a dramatic risk to the adaptive potential of the species leading to a weaker ability to respond to environmental and anthropogenic pressures (Camargo *et al.* 2016). In the Indian Ocean differences in length, sex and reproductive phase distribution were observed among areas suggesting a segregation due to migratory patterns (Garcia-Cortes et al., 2012). Despite some studies that have been conducted exploring the biology of this species, its life history information available in the Indian Ocean is still limited. Details can be found in Table 5.

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[2] https://www.iucnredlist.org/species/39374/2911619

Table 5. Life history indicators of whitetip shark. F: females; M: males; C: both sexes combined; IO: Indian; AO: Atlantic Ocean; PO: Pacific Ocean; RND: round weight; DWT: dressed of carcass weight; TL: Total Length; FL: Fork Length; PCL: precaudal length; L50: size at which the 50% of the population is mature.

Reference	**Ocea**	**Linf (cm)**	**K (yr-1)**	**t0 (yr) or Lo (cm)**	**W-L Conversion**	**Longevity (yr)**	**Maturity (cm and yr)**	**Fecundity (n)**	**Sex ratio (F:M)**
Ariz *et al.* (2007)	IO				RND = 4.91x10-6 TL3,07 RND= 1.84x10-5 FL2.92 DWT = 2.40x10-5 TL2.59 DWT =8.04x10-5FL2.45				0.2:1
Bass (1973)	IO						185-198 TL (M) 180 – 190 TL (F)		
D'Alberto *et al.* (2016)	PO	315.6 (M) 316.7 (F)	0.059 (M) 0.057 (F)	74.7 (M) 74.7 (F)		18 (M) 17 (F)	193 / 10 yr. (M) 224 / 15 .8 yr. (F)		
Coelho *et al.* (2009)	AO						160-196 (M) 181-203 (F)	1-14	1:1.2
(Tambourgi *et al.* 2013)	AO						170-190 (M) 170 (F)	1-10	1:1
(Joung *et al.* 2016)	PO	309.4	0.085	64	W = 1.66 × 10−5TL2.891		194.4 (L50) /8.9 yr (M) 193.4 (L50) /8.8 yr (F)	10–11 (n=2)	1:1
(Lessa *et al.* 1999)	AO	325.4	0.075	-3.342		14 (M) 17 (F)	180-190 /6-7 yr.		
(Romanov & Romanova 2009)	IO				TW=0.386x10-4FL2.75586 TW=0.120x10-4FL2.98524 (M) TW=0.508x10-4FL2.70428 (F)				
(Saika 1985)	PO						170-180 (M) 171 (F)		
(Seiki *et al.* 1998)	PO	244.58	0.103	2.698			168–196 (M) 175–189 (F)	1-14	
(Varghese *et al.* 2017)	IO						207.19 (L50) (M) 187.74 (L50) (F)	3-9	1:0.93

6.3 Scalloped hammerhead

The scalloped hammerhead (*Sphyrna lewini)* is a species of shark that has a circumglobal distribution classified as critically endangered by the IUCN[3]. Upon the ERA assessment in the Indian Ocean, the scalloped hammerhead received a low vulnerability for longline due to lower susceptibility to this type of gear but higher vulnerability is estimated for gillnets (Murua *et al.* 2018). There is no quantitative stock assessment or basic fishery indicators currently available for scalloped hammerhead shark in the Indian Ocean therefore the stock status is unknown; however, studies reported that their populations have declined around the world and in the Indian Ocean in particular (Anon 2019b). This species is observed in open ocean, but it is abundant along continental margins linked ontogenetically to coastal areas, bays and estuaries for parturition and juvenile development. It shows a fidelity to nursery grounds for reproductive females (Daly-Engel *et al.* 2012; Duncan & Holland 2006). Scalloped hammerheads utilize different habitats depending on the moment of the day. During the night, they inhabit offshore pelagic areas to actively search for food whereas during the day, they migrate to seamounts, bays, estuaries or reefs where females from schools for social interaction (Schluessel *et al.* 2008). Population subdivisions with a genetic discontinuity within oceans barriers, with an exchange between Indian and Atlantic Ocean throw Southern Africa was suggested (Duncan & Holland 2006). In contrast, Daly-Engel *et al.* (2012) observed connectivity and in some cases nonsignificant population structure across ocean basins. Male-mediated dispersal and gene flow has likely facilitated the connectivity observed among global populations, while the maternal lineages indicates strong restrictions to dispersal between discontinuous coastlines (Daly-Engel *et al.* 2012). Across the Indian Ocean, no population structure was observed, mainly due to male-mediated gene flow across large expanses of open ocean (Daly-Engel *et al.* 2012), while highly significant mtDNA structure was observed between Seychelles and West Australia. Regarding life history traits, an extensive review can be found in Miller *et al.* (2014). There are few studies exploring life history traits of scalloped hammerhead in the Indian Ocean (Table 6)

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[3] https://www.iucnredlist.org/species/39385/2918526

Table 6. Life history information of salloped Hammerhead shark. F: females; M: males; C: both sexes combined; IO: Indian; AO: Atlantic Ocean; PO: Pacific Ocean; RND: round weight; DWT: dressed of carcass weight; TL: Total Length; FL: Fork Length; PCL: precaudal length; L50: size at which the 50% of the population is mature.

Reference	Ocean	Linf (cm)	K (yr^{-1})	t0 (yr) or Lo (cm)	W-L Conversion	Longevity (yr)	Maturity (cm and yr)	Fecundity (n)	Sex ratio (F:M)
(Anislado-Tolentino & Robinson-Mendoza 2001)	PO	336.4 (M) 353.3 (F)	0.13 (M) 0.16 (F)	–1.1 (M) –0.63 (F)		8.8 (M) 18.6 (F)			
(Tolentino *et al.* 2008)	PO	364 (M) 376 (F)	0.12 (M) 0.1 (F)	1.18 (M) -1.16 (F)					
(Ariz *et al.* 2007)	IO				$RND = 3{,}2510x10^{-6} TL^{3{,}0957}$ $RND = 9{,}1646x10^{-6} FL^{3{,}0300}$				
(Chen *et al.* 1990)	PO	321 (M) 320 (F)	0.22 (M) 0.25 (F)	-0.7(M) -0.4 (F)		10.6 (M) 14 (F)	198 (M) 210 (F)		
(Chodrijah & Setyadji 2015)	IO				$CL = 0.0971*TL^{-2.8435}$			16-38	1.64:1 (2010) 2.40:1 (2013)
(Drew *et al.* 2015)	IO	289.6 (C) 259.8 (M) 289.6 (F)	0.159 (C) 0.15 (M) 0.16 (F)	54.2(C) 56.8 (M) 53.2(F)		19 (M) 35 (F)	8.9 yr.(M) 13.2 yr.(F)		
(Harry *et al.* 2011)	PO	331.2 (C)	0.076 (C)	0.58 (C)		21 (M)			
(Kohler *et al.* 1995)	AO				$RND=7.77x10^{-6}(FL)^{3.0669}$				
(Kotas *et al.* 2011)	AO	266.0 (M) 300.0 (F)	0.05 (M) 0.05 (F)	-3.9 (M) -3.7 (F)					
(Piercy *et al.* 2007)	AO	214.8 (M) 233.1 (F)	0.13 (M) 0.09 (F)	−1.62 (M) −2.22 (F)		30.5			
(White *et al.* 2008)	IO						175.6 (M, LT50) 228.5 (F, LT50)	14-41	

6.4 Shortfin mako

The shortfin mako (*Isurus oxyrinchus*) is a highly migratory species found in tropical and temperate waters worldwide (Compagno 2001). It is classified as endangered species under the IUCN classification[4]. In the Indian Ocean it is the second most important species and it is mainly caught by gillnets followed by longlines (Garcia & Herrera 2018). These species is usually retained for their valuable meat and fins (Coelho *et al.* 2019; Compagno 2001). However, the catch levels are highly uncertain and underreported longlines (Coelho *et al.* 2019; Garcia & Herrera 2018). Indeed, main obstacles preventing quantitative advice is the uncertainty in catches and the limit availability of abundance trends (Coelho *et al.* 2019). Trends in the CPUE of the Japanese and EU Portugal longline fishery suggest that biomass has decline from the 90s to 2003/2004 and has been increasing since then (Anon 2019b). Shortfin mako sharks received the highest vulnerability ranking in the ERA rank for longline gear because it was characterized as one of the least productive shark species and has a high susceptibility to longline gear (Anon 2019b). There is no quantitative stock assessment currently available for shortfin mako shark in the Indian Ocean therefore the stock status is unknown (Anon 2019b). A quantitative stock assessment has been planned by the Working Party on Ecosystems and Bycatch (WPEB) for 2020. Ecological risk assessment conducted in the Indian Ocean considered the shortfin mako as one of the most vulnerable species due to its high susceptibility and low productivity (Murua *et al.* 2018). Fishing reduction to the levels observed during the early years in the 1990's would likely be sustainable (Coelho *et al.* 2019). As in other species, there is a weak evidence of population structure at global scale (Schrey & Heist 2002). Differences observed between north Atlantic and Pacific Ocean indicate that shortfin mako move across ocean basins at a rate sufficient to reduce genetic differentiation (Schrey & Heist 2002). Nuclear DNA data indicate shortfin mako may constitute a globally panmictic population (Corrigan *et al.* 2018). Across Indian Ocean connectivity has been also detected (Corrigan *et al.* 2018). However, (Taguchi *et al.* 2011) reported that the eastern Indian Ocean sample was highly differentiated from

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[4] https://www.iucnredlist.org/species/39341/2903170

most other sampling sites. Mitochondrial DNA data suggest matrilineal substructure across hemispheres in the Indian Ocean (Corrigan *et al.* 2018). Male-mediated gene flow across the ocean basins could be occurring while females showing philopatry for parturition sites (Corrigan *et al.* 2018; Schrey & Heist 2002). Indeed, significant matrilineal sub-structure has been reported (Corrigan *et al.* 2018; Michaud *et al.* 2011; Taguchi *et al.* 2015). According to Corrigan et al., 2018, populations in southern Australia and the western Indian Ocean appear to be distinct; however further research is needed to explore the population structure in the Indian Ocean. Ontogenic, seasonal and gender differentiated migrations has been detected for this species (Groeneveld *et al.* 2014; Mucientes *et al.* 2009; Semba *et al.* 2011). These characteristics imply differences in both the life history traits and the behaviour of the sexes in this species which sexual segregation becoming increasingly prominent according to growth (Semba *et al.* 2011). Females can delay maturity and grow larger compared with males which mature earlier and slowdown of growth at their size at maturity (Semba *et al.* 2011). Mature makos move closer to the coast in eastern South Africa, where some females give birth (Groeneveld *et al.* 2014). Regarding life history traits, and extensive review can be found in (Coelho *et al.* 2019). More details can be found in Table 7.

Table 7. Life history information of shortfin mako. F: females; M: males; C: both sexes combined; IO: Indian; AO: Atlantic Ocean; PO: Pacific Ocean; RND: round weight; DWT: dressed of carcass weight; TL: Total Length; FL: Fork Length; PCL: precaudal length; L50: size at which the 50% of the population is mature.

Reference	Ocean	Linf (cm)	K (yr^{-1})	t0 (yr) or Lo (cm)	W-L Conversion	Longevity (yr)	Maturity (cm and yr)	Fecundity (n)	Sex ratio (F:M)
(Ariz *et al.* 2007)	IO				RND = $1.05x10^{-5}$ $TL^{2.96}$ RND = $1.116x10^{-5}$ $FL^{3.0}$ DWT =$5,20x10^{-6}$ $TL^{3.02}$ DWT $6.7x10^{-6}FL^{3.02}$				
(Barreto *et al.* 2016)	AO	328.7 (M) 407.7 (F)	0.08 (M) 0.04 (F)	-7.08		29 (M) 38 (F)			
(Bishop *et al.* 2006)	SPO	302.2 (M) 820.1 (F)	0.05 (M) 0.01(F)	−9.04 (M) −11.30 (F)		28			
(Bustamante & Bennett 2013)	PO						180 (M)		1:1
(Cerna & Licandeo 2009)	NPO	274.3 (M) 299.7 (F)	0.08 (M) 0.07 (F)	−3.58 (M) −3.18 (F)		25			
(Francis & Duffy 2005)	PO				FL = 0.821 + 0.911 TLnat		80–185 (M, L50) 275 –285 (F, L50)		
(Groeneveld *et al.* 2014)	IO	285.4	0.113	90	WW = $8x10^{-6}FL^{3.0412}$ (M) WW = $1x10^{-5}FL^{2.9596}$ (F)	19.5 (M) 18.5 (F)	190.2 (L50) / 7 y. (M) 294.8 (L50) / 15 y. (F)	9-14	1:1
(Liu *et al.* 2018)	IO	267.6 251.6 (M) 323.8 (F)	0.12 0.15 (M) 0.08 (F)	−2.49 −1.99 (M) −3.86 (F)	GW = $1.0×10^{-4}$ $CFL^{2.517}$	14-18			
(Maia *et al.* 2007)	AO				Wt = $0.0000244*FL^{2.8289}$ (M)		180 (M)		1.18:1
(Mollet *et al.* 2000)	Overall						298 in WNA (F, L50) 273 in SE (F, L50)	4-27.5	
(Natanson *et al.* 2006)	AO	253.3 (M) 365.6 (F)	0.12 (M) 0.08 (F)	72 (M) 88 (F)		29 (M) 32 (F)	185 / 8 y. (M) 275 / 18 y. (F)		

(Romanov & Romanova 2009)	IO				$TW=0.349x10^{-4}FL^{2.76544}$ $TW=0.177x10^{-3}*FL^{2.4601}$ (M) $TW=0.148x10^{-4}FL^{2.9249}$ (F)				
(Semba *et al.* 2009)	PO	256.5 (M) 340.4 (F)	0.16 (M) 0.09 (F)	67					
(Semba *et al.* 2011)	PO						156 / 5.2 y. (M) 256 / 17.2 y. (F)		
(Varghese *et al.* 2017)	IO						189.05 (M) 266.42 (F)		1:0.96

6.5 Silky shark

Silky sharks (*Carcharhinus falciformis*) are listed as vulnerable species under the by the IUCN Red List of Endangered Species. In the Indian Ocean, previous Ecological Risk Assessments (ERAs) identified silky sharks as species with high risk of overexploitation (Murua *et al.* 2018). Silky sharks are targeted by artisanal small-scale fisheries and as bycatch in industrial fisheries (longline and purse seiners). The silky shark is the second shark species with the highest catch estimates in the Indian Ocean (Coelho *et al.* 2019). However, there is not currently quantitative stock assessment conducted by the IOTC WPEB, mainly due to the poor quality and reliability of the recorded catch statistics. Thus, the stock status for this species remains unknown (Coelho *et al.* 2019). Nevertheless, it was suggested that maintaining or increasing fishing likely to lead to declines in biomass, productivity and CPUE in the Indian Ocean (Coelho *et al.* 2019). As in the case of mako, (Coelho *et al.* 2019) assessed the stock in order to provide in order to provide a preliminary stock status in the Indian Ocean. According to the catch MSY results give the perception that the stock biomass is still above BMSY (stock is not overexploited), but the current fishing mortality is high, around 2 times higher than FMSY (stock is currently under over-exploitation) (Coelho *et al.* 2019). The exploitation rate for year 2015 was predicted to be well above the MSY-level (Coelho *et al.* 2019). At present the silky shark stock would be subjected to overfishing but not overfished.

The silky shark is a circumglobally distributed, tropical and subtropical species (Rabehagasoa *et al.* 2012). It is essentially pelagic species distribute from slopes to open ocean (Anon 2019b), from the surface (18 m) down to at least 500 m of depth (Compagno 1984). Adults and older juveniles of this species are found in deep waters just off continental and insular shelves but also commonly in open-ocean waters (Clarke *et al.* 2015). Stable isotope analysis performed on muscle tissues revealed that silky sharks have a more inshore foraging habitat (Rabehagasoa *et al.* 2012). Smaller specimens are typically found in coastal waters (Anon 2019b). High fidelity of adults associated with seamounts, and juveniles with floating objects has been described (Ebert *et al.* 2013). In that sense, this species is the most commonly caught in the tropical FAD purse seine fisheries (Gilman 2011). Although there has been recorded long-distance movements in large-body specimens, most of the tagging studies described more limited movements

(Clarke *et al.* 2015) or aggregation behaviours around FADs (Filmalter *et al.* 2015; Filmalter *et al.* 2011). There is almost no information about the stock structure of silky sharks worldwide (Bonfil 2008). In the Indian Ocean, the population structure of silky shark is unknown, but a single stock may be assumed (Coelho et al., 2019). However, recent genetic studies on mitochondrial DNA showed that despite its large population size, silky sharks in the Indian Ocean appear to be isolated on relatively small spatial scales, showing certain genetic differentiation between sampled regions (Clarke *et al.* 2015). Although information on life history traits is available for this species worlwide, in the Indian Ocean the studies directed to explore growth and reproductive traits are not numerous and limited in spatial scope (Table 8).

Table 8. Life history information of silky shark. For females (F); males (M) or both sexes combined (C) in the Indian, Atlantic or Pacific Ocean (IO, Ao or PO, respectively). RND: round weight; DWT: dressed of carcass weight; TL: Total Length; FL: Fork Length; PCL: precaudal length L50: size at which the 50% of the population is mature.

Reference	Ocean	Linf (cm)	K (yr^{-1})	t0 (yr) or Lo (cm)	W-L Conversion	Longevity (yr)	Maturity (cm and yr)	Fecundity (n)	Sex ratio (F:M)
(Ariz *et al.* 2007)	IO				RND = $6{,}51x10^{-6}$ $TL^{2{,}99}$ RND = $4{,}72x10^{-6}$ $FL^{3{,}18}$ DWT =$5{,}66x10^{-6}$ $TL^{2{,}89}$ DWT =$1{,}30x10^{-5}FL^{2{,}83}$				
(Galván-Tirado *et al.* 2015)	PO						180 (M, L50) 190 (F, L50)	2-14	0.81:1
(García-Cortés & Mejuto 2002)	IO				DWT=$1.1x10^{-5}(FL)^{2.915}$				
(Grant *et al.* 2018)	PO	261.3	0.14	82.7 cm		23 (M) 28 (F)	183 (L50) / 11.6 yr (M) 204 (L50) / 14 yr (F)	3-13	
(Hall *et al.* 2012)	IO	299.4	0.066	-5,12		20 (M) 19 (F)	207.6 (L50 / 13 yr. (M) 215.6 (L50) / 15 yr.(F)	2-14	1:01
(Joung *et al.* 2008)	IO	332.0	0.0838	−2.761	W = 2.92×10^{-6} $TL^{3.15}$	28.6 (M) 35.8 (F)	212.5 (L50 / 9.3 yr. (M) 210–220 (L50) / 9.2–10.2 yr.(F)		
(Romanov & Romanova 2009)	IO				TW=$(0.160x10^{-4})$*$FL^{2.92}$				
(Stevens 1984)	IO						239 (M) 216 (F)		
(Varghese *et al.* 2016)	IO	309.80	0.10	-2.398			217.0 (M, LT50) 226.5 LT (F, LT50)	3-13	1:0.83

6.6 Bigeye thresher shark

Bigeye thresher sharks (*Alopias superciliosus*) are listed as vulnerable species in general worldwide under the by the IUCN5. In the Indian Oceans, from the previously conducted Ecological Risk Assessments (ERAs), identified this species as one of the most vulnerable species for longline fishing fleet, characterized by its low productivity and high susceptibility to this gear type (Murua *et al.* 2018). Despite its low productivity, bigeye thresher shark has a low vulnerability ranking to purse seine gear due to its low susceptibility to this particular gear (Murua *et al.* 2018). In IOTC resolution 12/09 it was noted that this species is particularly endangered and vulnerable (Coelho *et al.* 2019). However, there is not currently quantitative stock assessment conducted by the IOTC WPEB, mainly due to the poor quality and reliability of the recorded catch statistics. Thus, the stock status for this species remains unknown (Coelho *et al.* 2019). Nevertheless, it was suggested by WPEB that in the Indian Ocean maintaining or increasing fishing would be likely to lead to declines in biomass, productivity and CPUE. The bigeye thresher shark is a circumglobally distributed, tropical and temperate species (Compagno 2001). This species inhabits coastal areas and especially the open ocean in the pelagic zones, occurring at depths of 50 to 300 m, although mostly occur in depths below 100m (Compagno 2001; Morales *et al.* 2018; Young *et al.* 2016). This shark species is considered a highly migratory species. According to (Young *et al.* 2016), results support shallow population structure between Indo-Pacific and Atlantic populations of Bigeye thresher shark, but not among populations spanning the entire Indo-Pacific Ocean. In the Indian Ocean, the population structure of Bigeye thresher sharks is unknown, but a single stock may be assumed (Coelho *et al.* 2019). Recent genetic studies using partial sequence of mitochondrial DNA in the Atlantic and Indian Ocean, showed absence of population structure, even between both oceans, which would corroborate the high dispersal ability of this species (Morales *et al.* 2018). Regarding life history traits, some details can be found in Table 9. The information available for the Indian Ocean is limited.

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[5] https://www.iucnredlist.org/species/39381/2915850#conservation-actions

Table 9. Life history information of bigeye threster (Alopias superciliosus). Females (F); males (M) or both sexes combined (C) in the Indian, Atlantic or Pacific Ocean (IO, Ao or PO, respectively). RND: round weight; DWT: dressed of carcass weight; TL: Total Length; FL: Fork Length; PCL: precaudal length L50: size at which the 50% of the population is mature

Reference	Ocean	Linf (cm)	K (yr^{-1})	t0 (yr) or Lo (cm)	W-L Conversion	Longevity (yr)	Maturity (cm and yr)	Fecundity (n)	Sex ratio (F:M)
(Chen *et al.* 1997)	PO						270.1-287.6 (M, LT50) 332-341.1(F, LT50)	2	
(Fernandez-Carvalho *et al.* 2011)	AO	206 (M) 293(F)	0.18 (M) 0.06 (F)	93 (M) 111 (F)		17 (M) 22 (F)			
(Fernandez-Carvalho *et al.* 2015)	AO					25 y	159.2 (M, FL50) 208.6 (F, FL50)		1.1:1*
	PO								
(Kohler *et al.* 1995)	AO				W= 9.1069 x $10FL^{3.0802}$				
(Liu *et al.* 1998)	PO	218.8 (M) 224.6 (F)	0.088 (M) 0.09 (F)	-4.24 (M) -4.21 (F)	$W=3.73*10^{-5}*TL^{2.57}$ (M) $W=1.02*10^{-5}*TL^{2.78}$ (F)	19 (M) 20 (F)	270-288 (M) 332-341 (F)	--	--
(Moreno & Morón 1992)	AO						270 (M) 340 (F)		
(Varghese *et al.* 2017)	IO						263.5 (M, LT50) 320.7 (F,LT50)	2	1:1.4
(White 2007)	IO						279-283 (M)	2	

7. REVIEW OF CATCH DATA FOR INDIAN OCEAN SHARK SPECIES

In the following sections we examine nominal catch data from the IOTC catch data sets for the shark species of interest. Note that the goal is not to look at standardized trends as a relative abundance index, since several studies have noted the likelihood for the reported catches of sharks to be low relative to true catch and the most recent includes sensitivity tests for alternative catch series. Here, we are simply concerned with the amount of catch in terms of the potential for large scale sample collection of juvenile/sub-adult and adult sharks to provide a fisheries independent estimate of abundance via CKMR. In fact, the more important consideration here, from a CKMR perspective, would be a situation where reported catches were, for whatever reason, overestimates of the true catches. This could lead to a situation where a project could be initiated based on expectation of achieving sample sizes that could not in fact be obtained. Therefore, as far as sampling for CKMR is concerned, feasibility gauged on conservative estimates of the catch is probably erring on the side of caution.

Times series of annual reported catch by species show marked variation between the species under consideration (Figure 5). Two species, blue shark and silky shark are reported as the highest-caught by a considerable margin. Blue shark reported catch has more than doubled between 2000 and 2017. Silky shark catch was reported to have had a peak catch in 1999 at over 24000 tonnes, but reported catches have since declined to roughly 6% of this (2017 reported catch 1491.3 t). Other shark species generally have much lower reported catch. Thresher sharks are reported in larger numbers than silky sharks in more recent years and the non-identified thresher sharks are nearly doubling over the years 2000-2017.

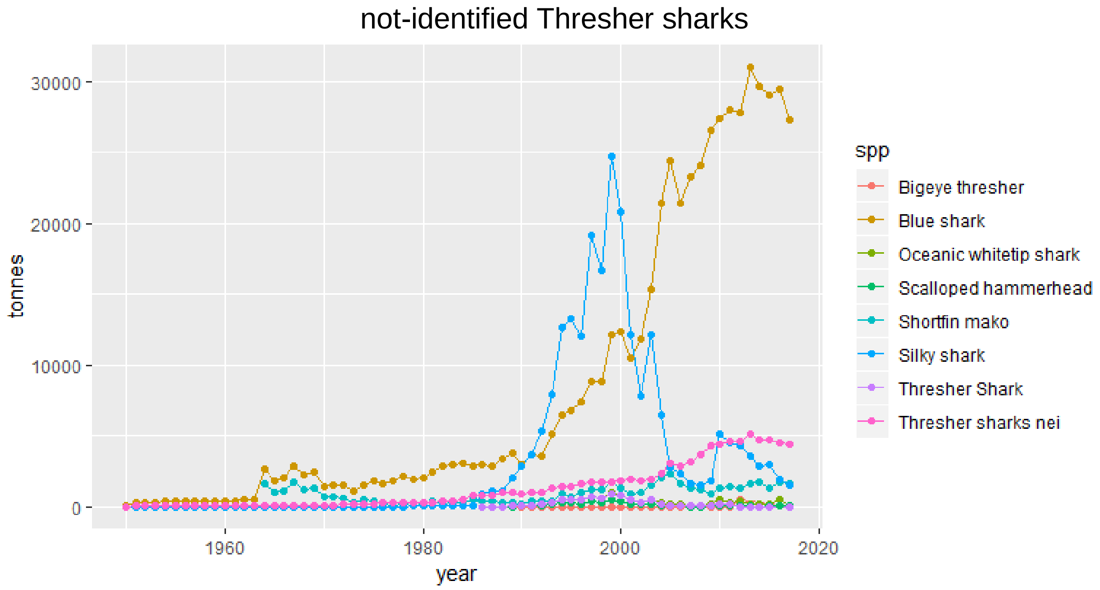


Figure 5. Catch of pelagic sharks reported in the IOTC catch records.

IOTC catch data for sharks was examined by fleet. This has been done in detail in the studies by (Garcia & Herrera 2018). Our focus here is not to use these data as an accurate estimate of the catch, but rather as a guide determining which fleets and gear types are catching sufficiently large numbers of sharks that, in principle they may be a source of samples. Note that it is clear to the authors at the outset, that an actual CKMR study would require detailed examination of sample collection procedures and investigate quantitative sample sizes in a statistical design framework. For this exercise and as an approximate guide to future work, we considered the average and range of catches over the last decade (2010 and onwards) on the basis that a hypothetical sampling program, putting aside issues of logistics etc., could in principle have a degree access to catches at least at the average nominal level.

7.1 Reported catches by country

Indonesia reported the largest mean annual blue shark catch (Figure 6) over the most recent decade. Although many fleets reported blue shark catch (N= 26), four had average reported catches >1000 tons. The next highest reporting fleets were Portuguese and

Taiwanese, followed by Spanish and Japanese fleets. The average catch over the period by Indonesian was over 4500 t.

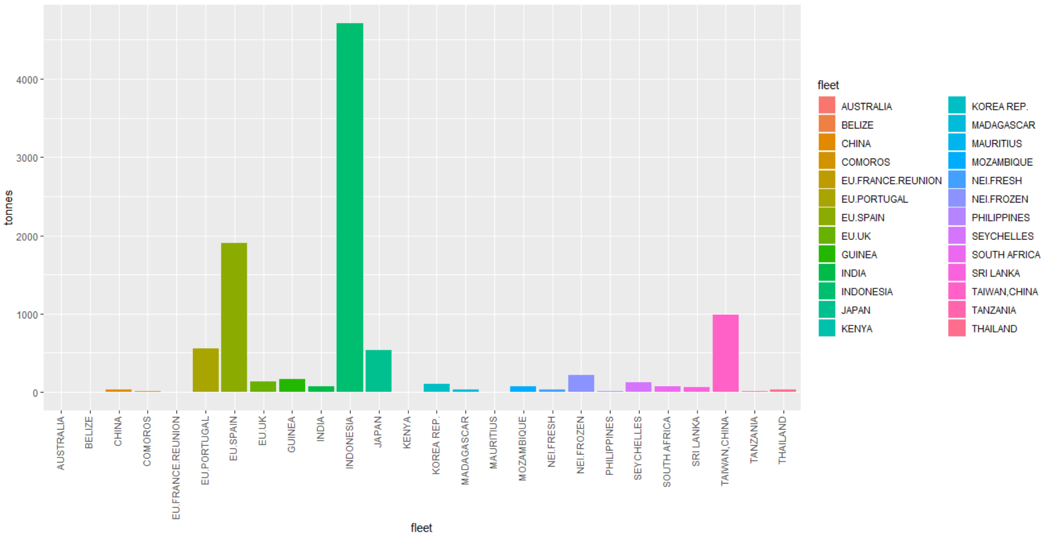


Figure 6. Blue shark annual mean catch 2010-2017.

The catches of the oceanic whitetip shark in the Indian Ocean are highly uncertain (Garcia & Herrera 2018), but most of the mortality of this species in the Indian Ocean comes mainly from longline fishery followed by the gillnets (Garcia & Herrera 2018). Average catches of oceanic whitetip shark were low relative to other species (Figure 9). The highest average catch was from the Comoros and Madagascan fleets. Spain and China were the larger of the distant water fleets, but each reported less than 30 tons.

In the Indian Ocean, scalloped hammerhead are often bycatch of recreational and industrial fisheries (longline, purse seine and gillnet), most of the mortality coming from gillnets (Garcia & Herrera 2018). However, there is a high uncertainty in catch estimates as hammerhead shark catches are under-reported and unregulated. There are not estimates of the population size for the Indian Ocean. Reported catches of scalloped hammerhead were small compared to other species (Figure 8). Sri Lanka reported the largest amount with mean catch of around 17 tons.

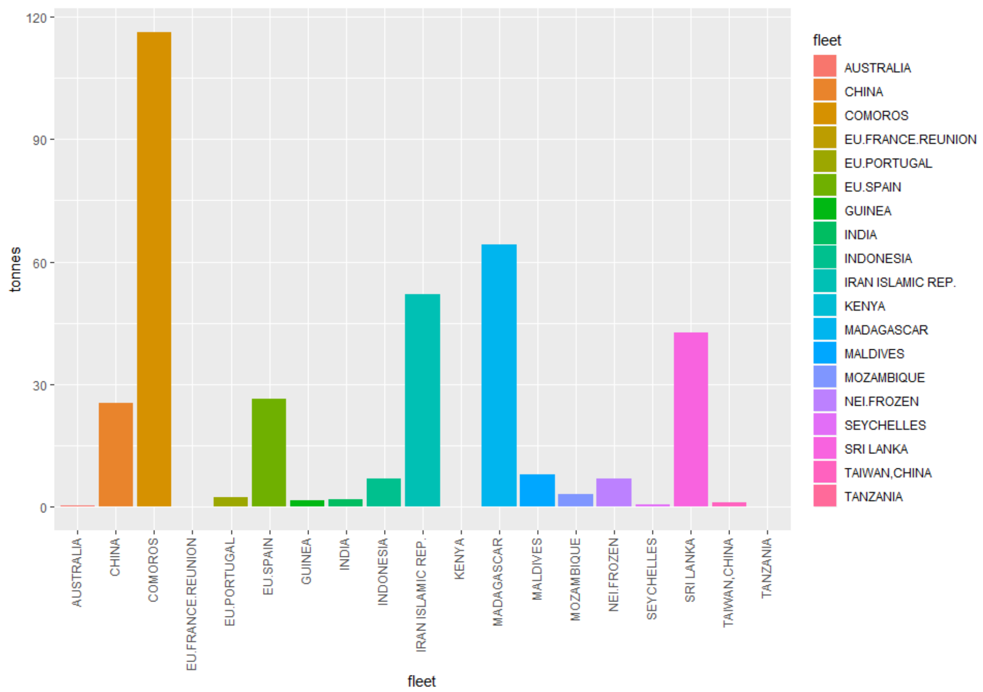


Figure 7. Oceanic whitetip annual mean catches 2010-2017

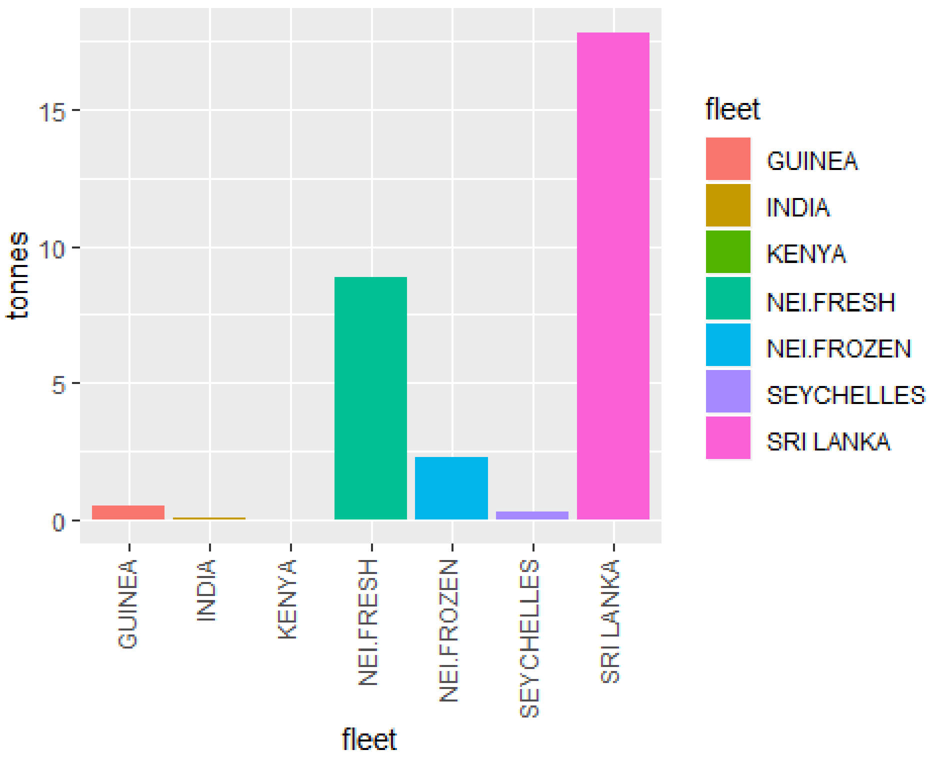


Figure 8. Scalloped hammerhead annual mean catch 2010-2017

Reported catches of shortfin mako were largest from Spain, South Africa and Portugal. Indonesia also reported nearly 100 tons (Figure 9).

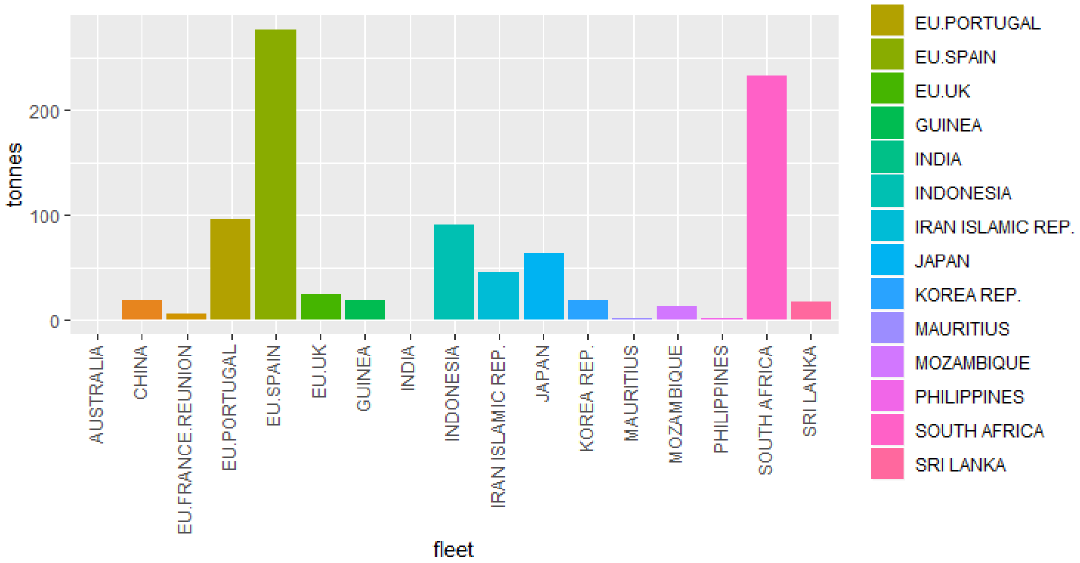


Figure 9. Shortfin mako mean catches by fleet 2010-2017

A large number of fleets reported silky shark catch. Iranian fleets reported mean catches over 600 tonnes, followed by Sri Lanka at just over 200 tonnes mean catch (Figure 10).

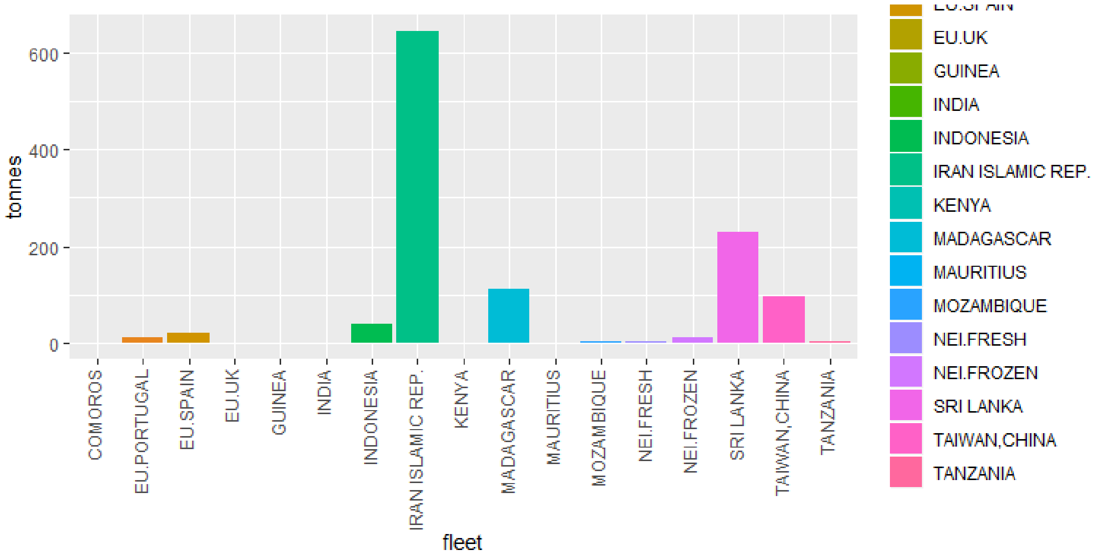


Figure 10. Silky shark annual mean catch 2010-2017

The largest catch of bigeye thresher was from Sri Lanka which reported an average catch of 76.6 tons (Figure 11). Other fleets show minimal catch.

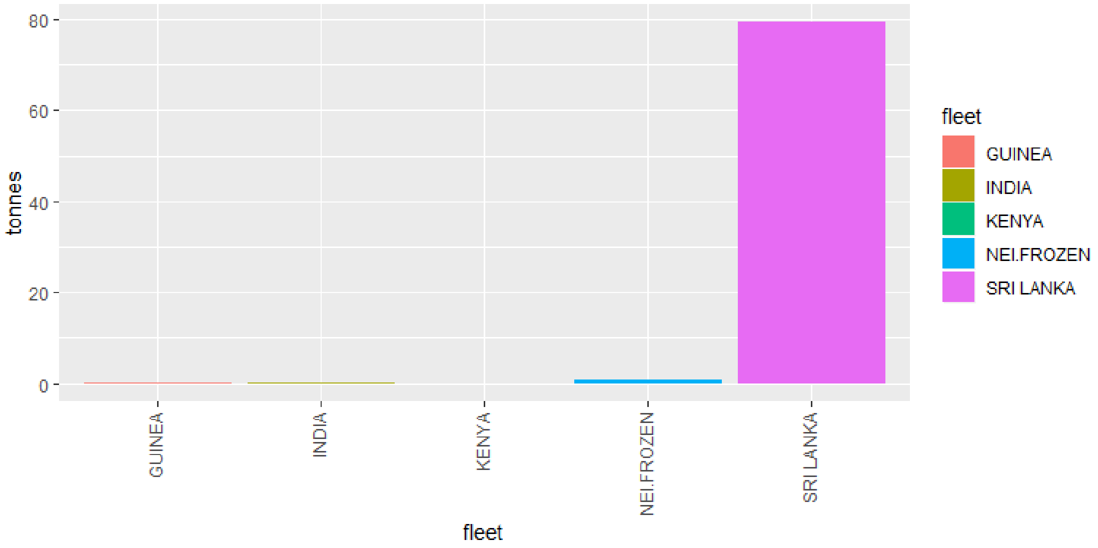


Figure 11. Bigeye thresher annual mean catch 2010-2017

While identified thresher sharks were a small proportion of the catch, the data indicated a much larger amount of thresher sharks listed as NEI (non-identified species), largely reported from Indonesia (Figure 12).

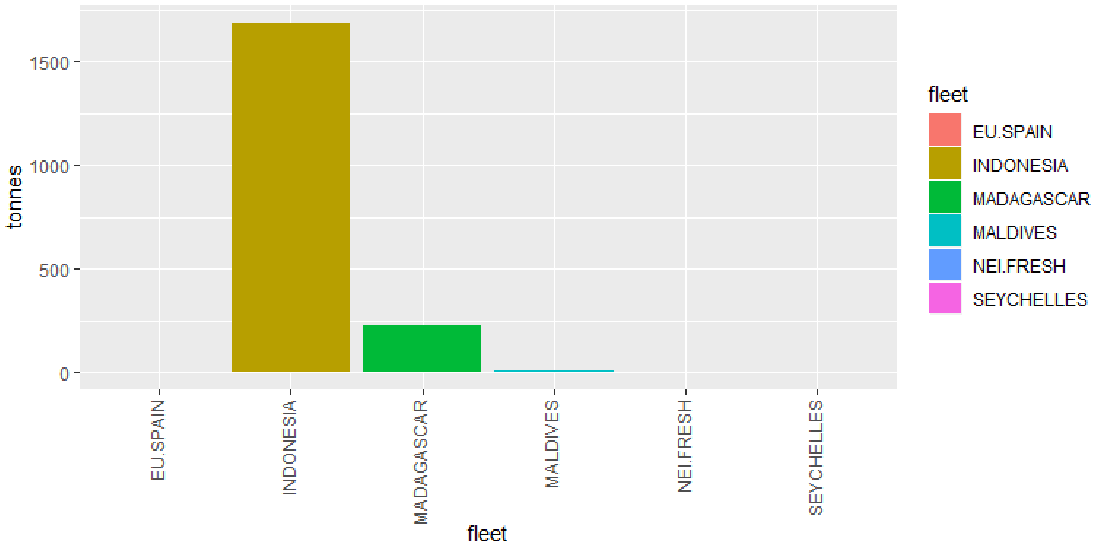


Figure 12. Thresher shark listed as NEI by fleet.

7.2 Reported catches by gear

Sharks catches were reported from a range of gear types for recent (2010 onwards) years (Figure 13). The top 10 gear types, by catch, were generally longline (overwhelmingly coastal longline sets) and to a lesser extent gillnets. Purse seine catches were around 50 tons of sharks, in total, over the past decade.

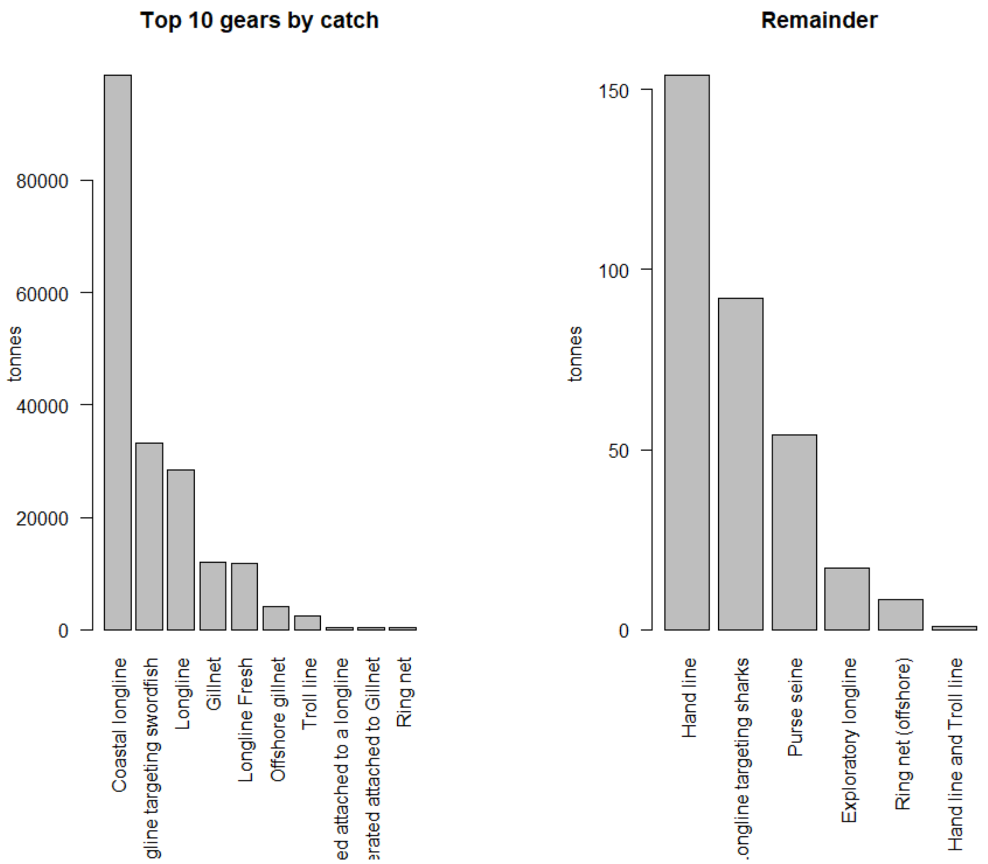


Figure 13. (Left) total catch (2010 onwards) for top 10 gear types. (Right) remainder of gear types.

We can further break these catches down by gear and species and examine catches from relatively recent years in order to understand the potential sources of shark samples. The notion here is that recent activity may be a more up to date guide for targeted sample collection for CKMR studies. This section therefore considered catches from 2013 onwards.

Again, the catch is dominated by blue shark (Figure 14) which is mostly reported as coastal longline catch. Reported catch of blues hark also dominated the catch in other longline metiers. Offshore gillnet seems to be made up largely of silky shark.

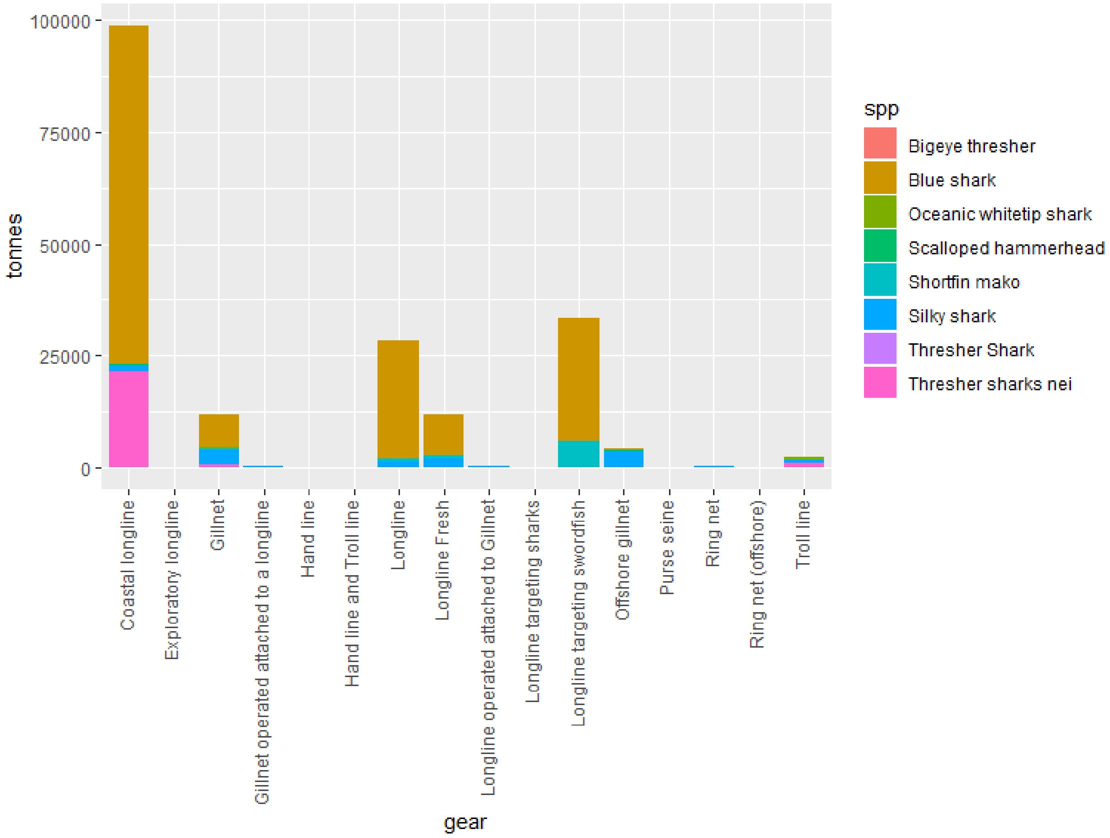


Figure 14. Total catch (2013-2017) by gear type and split by species.

As per the previous plots of the catch times series, it is informative to examine the species/gear reported catch composition with blue shark removed to clarify the contribution of the other species (Figure 15). Here we see that not elsewhere identified thresher sharks are identified as the next largest component of the coastal longline catch. Silky sharks are the second most numerically dominant component. Gillnet gears (both “Gillnet” and “Offshore gillnet’’) account for a large proportion of the silky shark catch. Oceanic whitetip is also reported on these gear types but is a relatively minor component of the total catch. Shortfin mako were mostly reported on longline gears with ``Longline targeting swordfish’’ having the highest catches of this species.

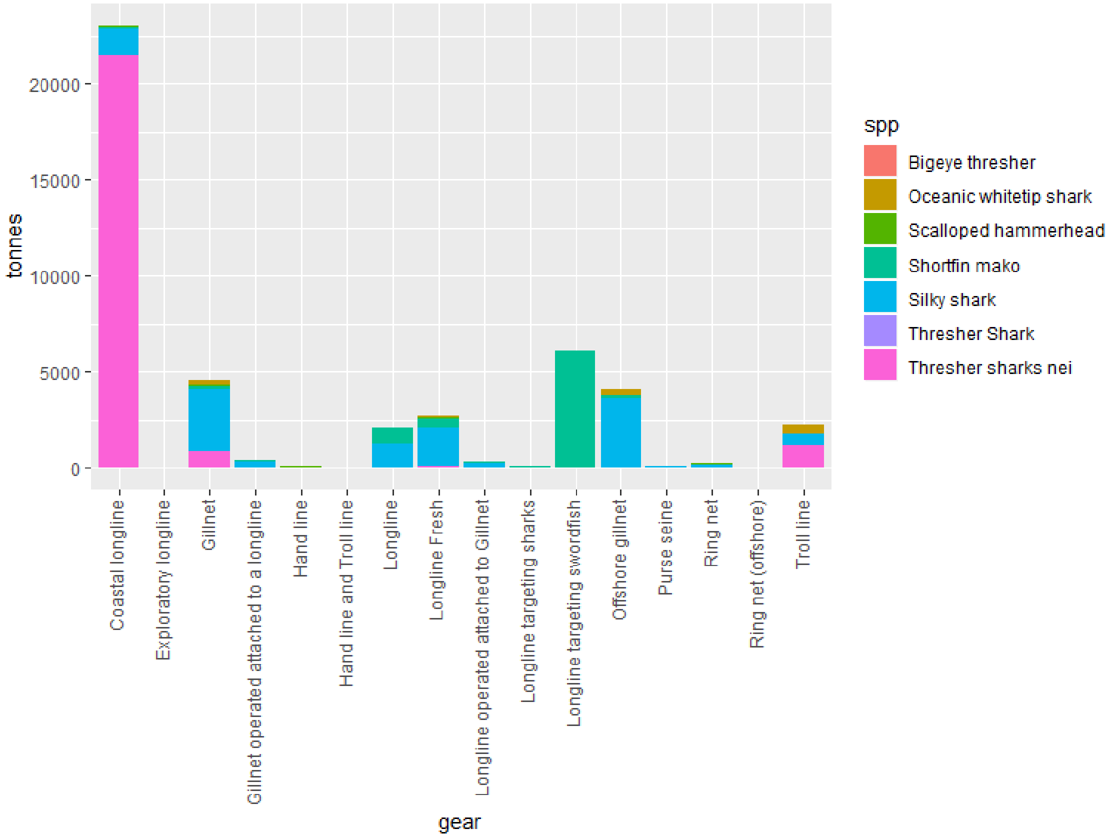


Figure 15. Catch of species (excluding blue shark) by gear type for years 2013 onwards.

Given that longline gears of various types are catching the majority of reported landed shark tonnage, the following few plots show the three species which are the most represented in the reported catch (blue shark, shortfin mako and silky shark) and display how these catches are distributed by fleet/metiers. Again, the figures show total tonnage reported between 2013-2017. Notable in the data pertaining to blue sharks (Figure 16) is that the majority of coastal longline catch is reported by Indonesia.

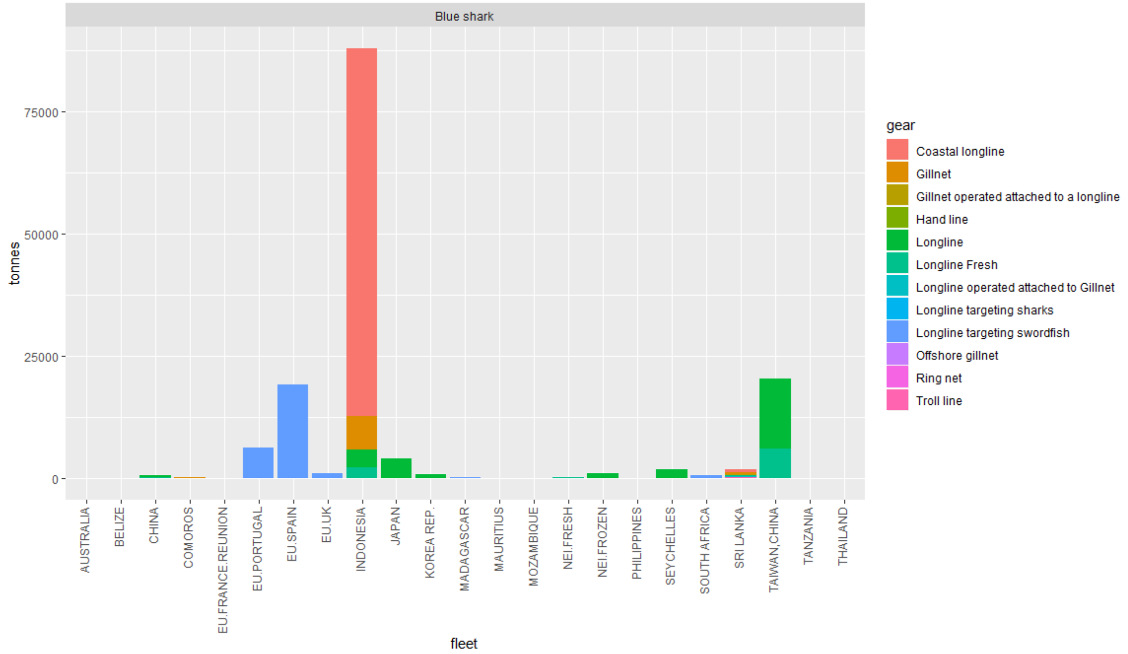


Figure 16. Blue shark total catch (2013-2017) by gear type and fleet.

Next largest catches are the Taiwanese (longline/longline fresh) and Spain and Portugal - both designated as longline targeting swordfish. The growth of the Indonesian coastal longline catch is responsible for this large component of the reported / nominal catch over this period. Figure 17 shows the development of this fleet from levels <5000 t prior to approximately 2000. From then the catches have increased at a rapid rate.

Longline gear targeting swordfish was responsible for most of the shortfin mako catch (Figure 18) which was from Spain, Portugal and South Africa. Silky shark (Figure 18) is split across a different set of gear types (coastal longline, various gillnets) and mostly reported by the Iranian, Taiwanese and Sri Lankan fleets.

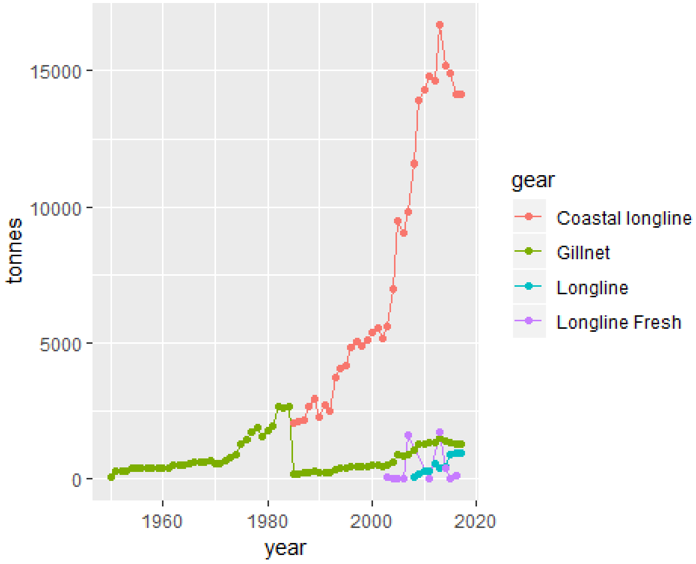


Figure 17. The time series of Indonesian reported nominal catch showing the increase in the Coastal longline component of the catch.

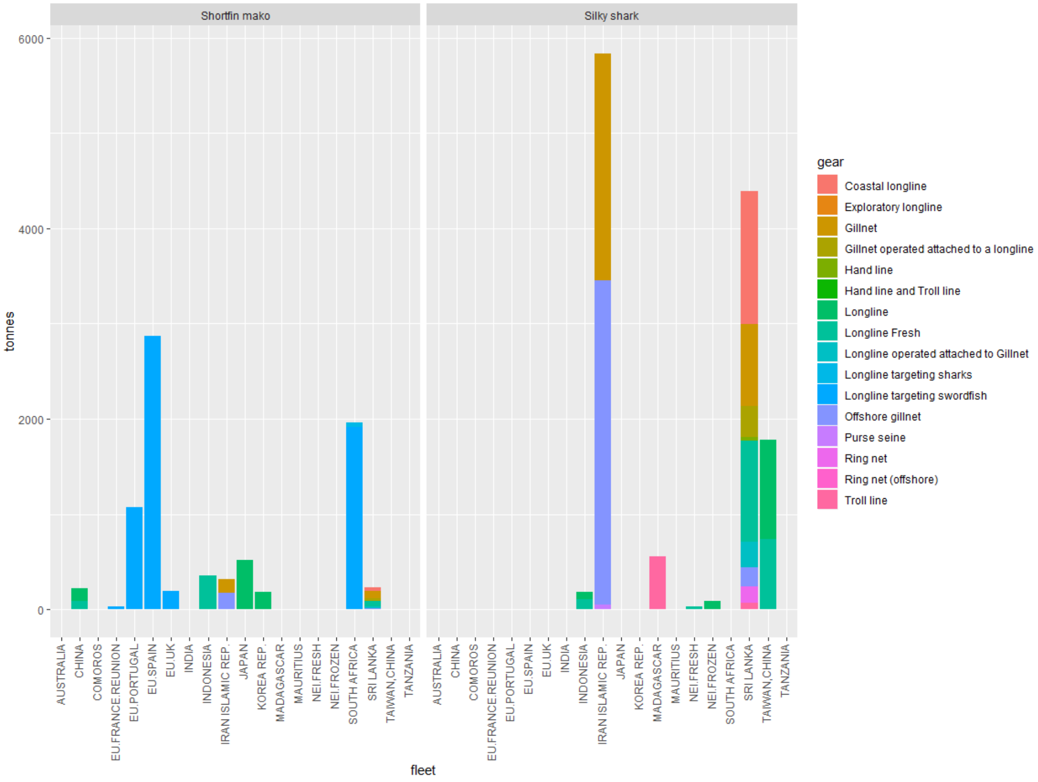


Figure 18. Shortfin mako and silky shark total catches (2013-2017)

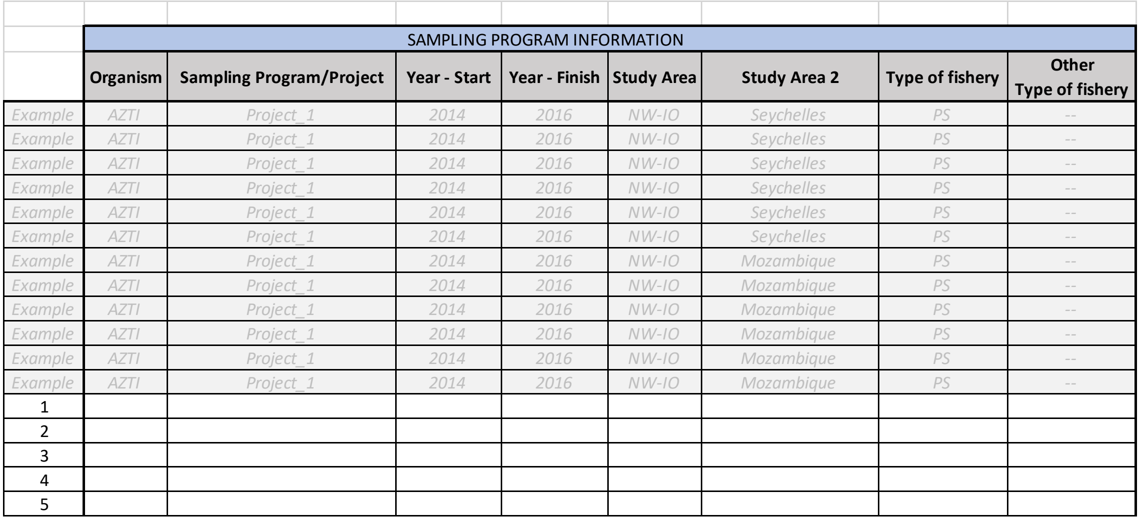
8. INVENTORY OF TISSUE SAMPLING PROGRAMS FOR INDIAN OCEAN SHARK SPECIES

Besides knowledge of several biological characteristics of the species under study, application of CKMR requires availability of tissue samples from hundreds or thousands of specimens. The CKMR method can also analyze samples from dead animals, which opens up its applicability to other species of bycatch like sharks. This specificity of the method makes possible also the analysis previously sampled and stored tissues. However, obtaining tissue samples in a consistent, structured fashion is a crucial component of CKMR studies. Thus, this feasibility study has as goal to develop an inventory of available tissue samples and ongoing sampling programs of different shark species studied in the past and currently in the Indian Ocean. For that a questionnaire including relevant information regarding i) sampling program information, ii) studied species information, and iii) data and samples availability was defined to collect required information. This questionnaire was forwarded to researchers attending IOTC WPEB, Scientific Committee meetings and other researchers involved in shark species sampling programs. The contact list was extracted from the attendance list of the meeting reports documents. IOTC Secretariat was also involved in contacting interested people. The questionnaire was defined in three sections to collect required information:

8.1 Sampling program information

This section aims at collecting information about the samples collection programs, leading research centre, year of sample collection, type of fishery used for sampling and the area covered by the program. This information will allow us to identify covered areas and gap areas for the design of a future CKMR sampling program in the Indian Ocean (Table 10).

Table 10. Section of questionnaire requesting information related to sampling programs.

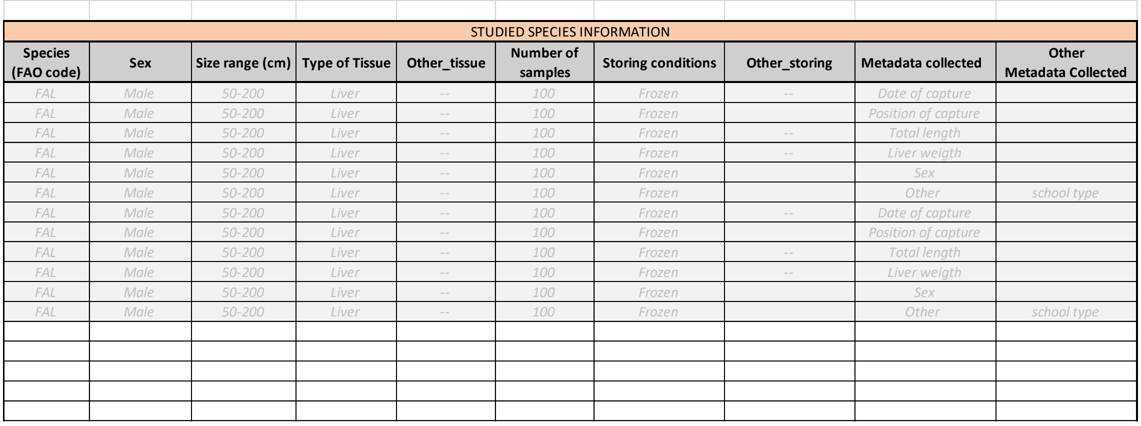


	SAMPLING PROGRAM INFORMATION							
	Organism	Sampling Program/Project	Year - Start	Year - Finish	Study Area	Study Area 2	Type of fishery	Other Type of fishery
Example	AZTI	Project_1	2014	2016	NW-IO	Seychelles	PS	--
Example	AZTI	Project_1	2014	2016	NW-IO	Seychelles	PS	--
Example	AZTI	Project_1	2014	2016	NW-IO	Seychelles	PS	--
Example	AZTI	Project_1	2014	2016	NW-IO	Seychelles	PS	--
Example	AZTI	Project_1	2014	2016	NW-IO	Seychelles	PS	--
Example	AZTI	Project_1	2014	2016	NW-IO	Seychelles	PS	--
Example	AZTI	Project_1	2014	2016	NW-IO	Mozambique	PS	--
Example	AZTI	Project_1	2014	2016	NW-IO	Mozambique	PS	--
Example	AZTI	Project_1	2014	2016	NW-IO	Mozambique	PS	--
Example	AZTI	Project_1	2014	2016	NW-IO	Mozambique	PS	--
Example	AZTI	Project_1	2014	2016	NW-IO	Mozambique	PS	--
Example	AZTI	Project_1	2014	2016	NW-IO	Mozambique	PS	--
1								
2								
3								
4								
5								

8.2 Species information

This section aims to collect information about studied shark species, sex, sampled size range, number of samples analysed, and sampled type of tissue (Table 11). Besides, it is requested used conservation method and the type of metadata collected during the sampling. As described previously obtaining tissue samples in a consistent, structured fashion is obviously a crucial component of CKMR studies. This questionnaire will allow us to identify available samples and relative data that fits with the CKMR program requirements.

Table 11. Section of questionnaire requesting information related to species information.

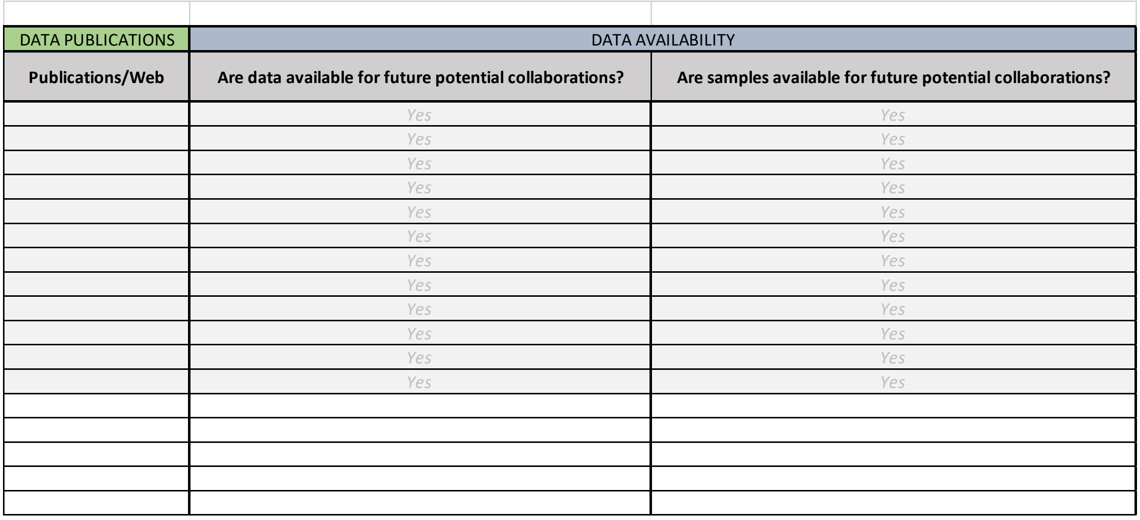


STUDIED SPECIES INFORMATION									
Species (FAO code)	Sex	Size range (cm)	Type of Tissue	Other_tissue	Number of samples	Storing conditions	Other_storing	Metadata collected	Other Metadata Collected
FAL	Male	50-200	Liver	--	100	Frozen	--	Date of capture	
FAL	Male	50-200	Liver	--	100	Frozen		Position of capture	
FAL	Male	50-200	Liver	--	100	Frozen	--	Total length	
FAL	Male	50-200	Liver	--	100	Frozen	--	Liver weigth	
FAL	Male	50-200	Liver	--	100	Frozen		Sex	
FAL	Male	50-200	Liver	--	100	Frozen		Other	school type
FAL	Male	50-200	Liver	--	100	Frozen	--	Date of capture	
FAL	Male	50-200	Liver	--	100	Frozen		Position of capture	
FAL	Male	50-200	Liver	--	100	Frozen	--	Total length	
FAL	Male	50-200	Liver	--	100	Frozen	--	Liver weigth	
FAL	Male	50-200	Liver	--	100	Frozen		Sex	
FAL	Male	50-200	Liver	--	100	Frozen		Other	school type

8.3 Data availability

This section aims to collect information about the data and samples availability for potential collaborations in a future CKMR program in the Indian Ocean, and weather the works derived for the analysis of samples have been already published (Table 12).

Table 12. Section of questionnaire requesting information related to data availability.



DATA PUBLICATIONS	DATA AVAILABILITY	
Publications/Web	**Are data available for future potential collaborations?**	**Are samples available for future potential collaborations?**
	Yes	Yes
	Yes	Yes
	Yes	Yes
	Yes	Yes
	Yes	Yes
	Yes	Yes
	Yes	Yes
	Yes	Yes
	Yes	Yes
	Yes	Yes
	Yes	Yes
	Yes	Yes

8.4 Result of the questionnaire

Two questionnaires have been received. One from Research Institute for Tuna Fisheries (RITF) from Indonesia and the other from the National Fisheries Research Institute from Mozambique. In the case of RITF samples were collected in the frame of a Scientific Observer Project during 2018 and 2019 in the South East Indian Ocean in the Longline fishery. Muscle and fin samples of 13 species (i.e. *Prionace glauca, Alopias superciliosus, Carcharhinus brevipinna, Carcharhinus falciformis, Isurus paucus, Carcharhinus longimanus, Lamna nasus, Pseudocarcharias kamoharai, Alopias pelagicus, Isurus oxyrinchus, Sphyrna lewini, Sphyrna zygaena, Galeocerdo Cuvier*) are stored frozen (Table 13). Information on sex, length, location and capture date is available for specimens sampled.

Table 13. Samples available at Research Institute for Tuna Fisheries in Indonesia

Species	FAO code	Area	Fishery	Fin	Muscle
Prionace glauca	BSH	SE IO	LL	20 (F); 9 (M)	11 (F); 13 (M); 3 (NR)
Alopias superciliosus	BTH	SE IO	LL		1 (F); 1 (M)
Carcharhinus brevipinna	CCB	SE IO	LL		1 (F); 1 (M)
Carcharhinus falciformis	FAL	SE IO	LL	1 (F); 3 (M)	4 (F); 2 (M)
Isurus paucus	LMA	SE IO	LL	1 (F); 1 (M)	
Carcharhinus longimanus	OCS	SE IO	LL	1 (M)	3 (F)
Lamna nasus	POR	SE IO	LL		3 (F); 1 (NR)
Pseudocarcharias kamoharai	PSK	SE IO	LL	1 (F)	1 (F); 3 (M)
Alopias pelagicus	PTH	SE IO	LL		1 (F)
Isurus oxyrinchus	SMA	SE IO	LL	1 (F); 2 (M)	7 (F); 1 (M)
Sphyrna lewini	SPL	SE IO	LL		2 (F)
Sphyrna zygaena	SPZ	SE IO	LL		1 (F)
Galeocerdo cuvier	TIG	SE IO	LL	1 (F); 1 (M)	

On the other hand, in the case of National Fisheries Research Institute at Mozambique 18 fins of 8 shark species (i.e. *Carcharhinus albimarginatus, Carcharhinus amblyrhynchos, Carcharhinus brachyurus, Carcharhinus amboinensis, Rhynchobatus djiddensis, Isurus oxyrinchus, Sphyrna lewini, Galeocerdo cuvier*) are stored dry (Table 14). The species were identified by genetic analysis at Department of Genetics at Stellenbosch University. These samples were collected in Longline or Gillnet fishery in routine monitoring at the airport between 2018-2019.

Table 14. *Samples available at the National Fisheries Research Institute in Mozambique*

Species	FAO code	Area	Fishery	Fin samples
Carcharhinus albimarginatus	ALS	SW IO	GL/LL	1
Carcharhinus amblyrhynchos	AML	SW IO	GL/LL	1
Carcharhinus brachyurus	BRO	SW IO	GL/LL	1
Carcharhinus amboinensis	CCF	SW IO	GL/LL	1
Rhynchobatus djiddensis	RCD	SW IO	GL/LL	3
Isurus oxyrinchus	SMA	SW IO	GL/LL	3
Sphyrna lewini	SPL	SW IO	GL/LL	7
Galeocerdo vuvier	TIG	SW IO	GL/LL	1

Considering the number of responses received shark samples available on research institutions involved in shark research in the Indian Ocean is very low which highlight the need of considering a coordinated tissue sampling program for the application of CKMR. During the enquiry various research institutions in different countries express their interest on future collaborations on the CKMR.

9. FEASIBILITY OF CKMR FOR INDIAN OCEAN SHARK SPECIES

This study assesses the feasibility and suitability of CKMR to estimate abundance of IOTC pelagic shark species, for which three aspects are considered:

- Suitable and known biology: That is, is there sufficient background understanding of the key reproductive biology and age and growth data to support fitting of a close kin model assuming sufficient kin pairs can be identified? Additionally, are there any peculiarities of the biology of the species in question that would compromise/need to be addressed to be able to design and implement a full CKMR study?

- Feasible sampling: That is, are the species caught in large enough numbers to permit a sufficient number of samples to be obtained - at least in principle? Which fleets/gear types catch sharks and from the collected size frequency data? Which sharks tend to be sampled by observers the most?

- Priority: That is, is the population of that species considered to be of concern (i.e. through listing) or does it have life history characteristics that would make it more or less vulnerable to over-exploitation?

A validated age and growth study based on bomb radiocarbon is the gold standard for ageing that would provide the length-at-age relationship required to allow lengths of sampled sharks to be converted to an age and thus estimation of the year of birth/cohort of the individuals. There are existing studies (mostly not validated) for all species of sharks considered here which provide estimates of growth, maturity, litter size and breeding frequency. Many of the studies are not based on IO data and it is possible that there is regional / ocean basin specific growth schedules. We expect that the influence of regional growth would be relatively minor, but this expectation could be examined statistically in full a design study. The main conclusion therefore is that there is enough existing knowledge in the literature to proceed with statistical design studies for priority species - but where IO specific studies are lacking, errors in age/growth should be considered in the designs. Potentially, a later full CKMR study should consider options for (ideally validated) direct ageing as a component of the project. Similarly, maturity studies exist for most species but are often from the Pacific or Atlantic Oceans. As per the situation with growth curves, we expect that the sensitivity of different sampling design options to these parameters would be examined in any future quantitative design work for a full implementation project. One aspect of reproductive biology that can be influential in CKMR is differential reproductive output with age. This is generally more of a factor with teleosts than elasmobranchs. The latter appear less variable in their reproductive output with age than teleosts (Stevens *et al.* 2000). Note that our criterion for considering the existing information adequate for design purposes should not be taken to mean that there is not room for improvement in estimating life history parameters of sharks. Rather that the understanding of sharks abundance is generally so poor that these uncertainties, which might be problematic for management of a targeted teleost species, are likely to be relatively minor compared to the potential improvement to be gained by having an abundance estimate from a moderately well informed CKMR study on a pelagic shark species.

In summary, most species have some estimate of growth and some idea of the maturity and litter size. For several species there appears to be a lack of studies specific to the Indian Ocean populations. Other studies of the growth/maturity and size-at-birth of pelagic sharks have indicated that these species can display a relatively large degree of variation between regions. Therefore, it would be advisable to conduct IO studies of growth and reproduction. However, given the current lack of data on population size for management, we would suggest that such work be conducted simultaneously with a

CKMR study and take advantage of the associated sampling logistics. Our expectation would be that any biases due to regional variation in biology are likely to be minor compared to the information gain which would stem from the availability of a CKMR abundance and total mortality estimate, which can be improved upon over time as more samples and improved understanding of the specifics of biology accumulate. Furthermore, the likely scale of these potential biases can be examined as part of species-specific design studies.

9.1 Sampling intensity from size frequency data.

Size frequency data is informative in the CKMR context for three reasons:

1. Potentially, fleets/metiers reporting large numbers of measurements are useful as a source of samples for an actual CKMR project.
2. The size and thus, age typically selected by these fisheries will determine whether it is likely that juveniles or adults are captured. Essentially this requires determining if large/old sharks make up an appreciable proportion of the catch. If many adults and juveniles are captured it is possible that some POP matches might be found. While these can be highly informative, the typical situation with sharks is that most frequently, pre-breeding adults are captured by fisheries operations. Potentially this is due to selectivity differences, distributional/habitat differences between juveniles and adults, or simply because breeding adults are much scarcer. As we outlined earlier, the expectation with sharks is that juveniles are routinely encountered and HSP based CKMR is likely to be the only viable route.
3. In order for a full statistical design, it is desirable to have a series of catch-at-age data (Bravington 2019). However, age distributions can be estimated from an age-length model, and therefore used to compile a rough estimate of catch-at-age.

Here we examine available size frequency data from the IOTC.

In the following we present two tables which detail the intensity of samples of length through time and by fleets (correlated with country). The aim of this is to examine whether existing rates of sampling, as reported by member countries in the IOTC, is likely to be sufficient for a CKMR study. Clearly this does not automatically imply that a CKMR study could easily access sampled sharks through time for genetic samples and associated covariate data (size, sex etc.). Nonetheless, the data are one of the few IO-wide sources of information on current data collection rates. While they are likely to be imperfect and should be refined by further consideration and advice from scientists involved in the IOTC Ecosystems and Bycatch WG, there is merit in their consideration if for no other reason than as a rough guide to fleets that might be sources of samples for each species.

Table 15 indicates the following:

(a) Blue shark is numerically dominant in the size frequency data reported to the IOTC.

(b) Shortfin makos and silky shark are the next most numerous species.

(c) Oceanic whitetip and scalloped hammerhead length frequency data is particularly sparse. This limits the scope for a detailed statistical design on these species.

Table 15. Number of sharks in the size frequency data set by species and year for the species of interest to this report. BSH-blue shark, FAL-silky shark, OCS-oceanic whitetip, SMA-shortfin mako and SPL-scalloped hammerhead

	2005	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**
BSH	28	150	813	383	2235	3835	7546	23325	19651	20932	19522	24677	28338
FAL	44	38	20		3			948	1630	1963	514	836	2257
OCS			1	1	11	1		76	106	1	18	10	72
SMA	11	10	79	9	104	262	1525	4245	2366	1699	630	357	1672
SPL										3	8		2

The size frequency data for the species which have the most numerous records are shown below. The size frequencies for each year are plotted. The point of the year by year breakdown is not to examine yearly variation in size frequency distributions, but rather to note that the patterns of catch are relatively stable with respect to size. For CKMR purposes, where the biggest issue is whether mature or likely immature individuals are captured, then the data indicate that for blue shark there is a relatively large number of animals larger than the size at 50% maturity for both species reported in the IOTC supporting information (Figure 19 Top). For shortfin mako (Figure 19 middle) and silky sharks (Figure 19 bottom) it is clear that the majority of the catch is of immature individuals.

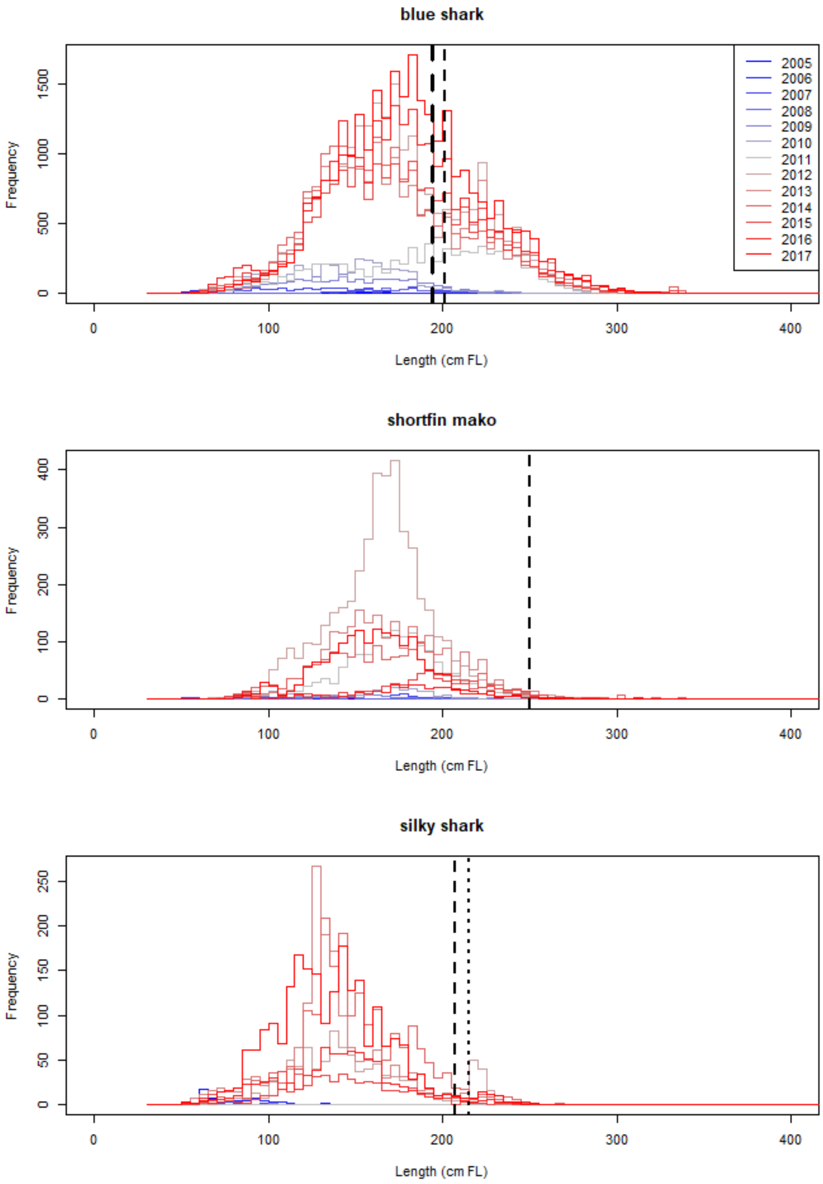


Figure 19. size frequency for (TOP) blue shark (MIDDLE) shortfin mako and (BOTTOM) silky sharks. These species are dominant in the size frequency data. All other species contain significantly fewer records. Vertical lines indicate size at 50% maturity by sex (IOTC supporting information, 2019)

Without a detailed age-specific design study it is not possible to determine whether CKMR for blue shark might expect to detect a useable/informative number of POPs. However, the available catch size distribution data suggests that the potential for POP and HSP CKMR approach (Davies *et al.* 2018b) is something to consider in a design study for blue shark. For the other species it seems clear (assuming that this data is representative), that the samples likely to be available for CKMR studies would be sub-adult and hence design work would. expect to be restricted to CKMR inferences based on HSPs only.

Broken down by fleet it is clear that there are notable fleets which are the source of the size frequency data (Table 16). For blue shark TWN (Taiwanese) fleets report the largest number of observations (over 100K). Other large contributing fleets are Portugal (>19K) and Japanese (~14K). Most silky shark length frequencies are reported to be from the Taiwanese fleets (>6K samples). Shortfin mako observations are reported to be contributed from mostly Portuguese fleets and Taiwan - each contributing between 4.6 to 5 K samples.

Table 16. Number of sharks in the size frequency data set by species and fleet

	CHN	EUESP	EUFRA	EUGBR	EUPRT	EUREU	IND	JPN	KOR	LKA	MOZ	MUS	SYC	TWN	ZAF
BSH	584	2282	13	409	19256	4		14047	7395	181	68		4308	101996	892
FAL	42	3	2	7		20	173		310	1426	2			6268	
OCS	67	1	8			5			10		18		18	170	
SMA	50	469		135	4687	59	2	766	395	5	28	8		5083	1282
SPL			1				1		3		8				

9.2 Expected number of kin-pairs

A full statistical design in the sense of (Farley *et al.* 2019) is beyond the scope of this feasibility study. However, we can give some idea (using equation 2) of the sort of sample size required. Again, we must stress that this is not in any way a rigorous design and should not in any sense be considered as anything other than an illustrative depiction. The key thing to consider is how sample size scales with current/recent population size.

Figure 20 shows an estimate of the number of samples required as a function of the required number of HSPs for given population sizes. Noting that the CV on an abundance estimate scales with $1/\sqrt{(N_{hsp})}$, then a study should aim for at least 50 kin pairs to obtain a CV of less than 20%. The properties of the population (mortality rates, age-structure etc) are important to factor into a full design, so again, we stress that this example is simply illustrative. In the cases depicted, one with total mortality Z=0.25 and the other with $Z = 0.1$ but a common average time between HSP cohorts δ, we find that in the case of higher mortality rates, with sample sizes up to 5K we only expect obtain 50 HSPs for breeding populations less than approximately 0.5M individuals. For a population with lower total mortality, 50 HSPs or above would be expected for population size up to 0.95M breeding individuals.

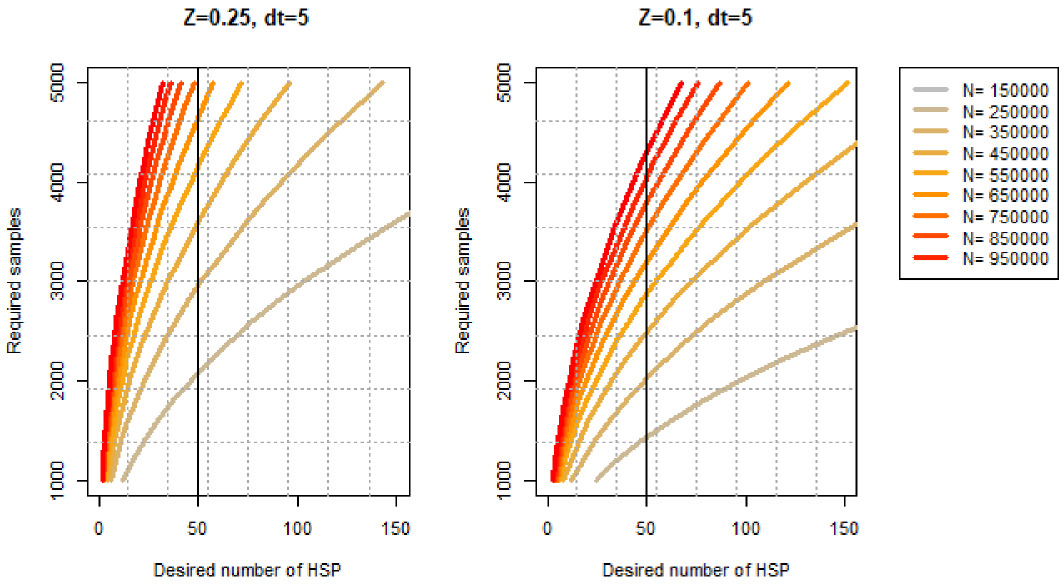


Figure 20. Number of samples as a function of required number of HSPs in two hypothetical situations where there are relatively higher (left) and lower (right) total mortality rates Z.

9.3 Conclusions

This feasibility study indicates that from a biological perspective, and relative to the starting point for studies of other elasmobranchs, all species considered here had no biological characteristics that would rule out the use of CKMR. Further investigation of a small number of tissue samples would allow for scrutiny of individual species genotypes to determine if there are any issues with finding suitable markers for designing cost-effective sequencing approaches for large scale kin-finding.

The background biology was for all species sufficient for further investigation via more specific and detailed statistical design. In some cases, the available age/growth/maturity studies are from other oceans and there were no IO studies in the literature. Assuming that biological studies like this for the IO are not likely in the near future, it would be worth considering the effect of length at age uncertainty in design work. This could inform on the extent of likely benefit from basic biological work is required and provide a justification/priority for such work in the future.

Nominal catches of blue shark, shortfin mako, silky shark and thresher sharks indicate that the fisheries for these species are taking large numbers of individuals. The NEI thresher shark catch was relatively large indicating that species ID for reporting may be an issue. This is likely to be addressed by genetic methods where a species ID test could be designed and used as part of the genetic workflows.

What we term “full” statistical design – in other words a design for a stock-assessment grade CKMR model of the sort developed for SBT - would likely only be possible for the species which are caught in the largest numbers and have requisite data and information available:

Blue shark:

- Biology relatively well-known, including age and growth and well observed catch-at-length time series (which would be used to estimate catch-at-age). Additionally, the assessment should be able to output an estimate of catch-at-age for use in a detailed CKMR design study.

- A blue shark CKMR design study could investigate the sample sizes required to obtain an informative number of POPs in addition to HSPs. If a study could be designed to find POPs in informative numbers for a reasonable cost, POPs would be expected to improve the accuracy and precision of resulting estimate of abundance.

- Nominal catches indicate a large proportion of the catch being taken by relatively few countries – Indonesia, Spain and Portugal with sufficiently broad spatial coverage for CKMR purposes. This could indicate that support from these nations would be sufficient to obtain requisite samples. Taiwan, Portugal and Japan contribute the bulk of the size frequency data. If this data collection effort could be expanded to obtain tissue for genetic analyses, then this species is likely to be a viable option.

- Clearly blue sharks are one of the most abundant pelagic sharks. This means that a large number of samples would be required and that a large project would be needed to be successful. A statistical design study would provide a much clearer indication of whether this species is actually a viable candidate for CKMR and specific estimates of the costs associated with alternative design options.

Shortfin mako:

- Catch at size time series data is available over the last decade – which is short but possibly workable for further design work. The growth and biology are sufficiently known to permit further design work. Given the size distribution of catches, this design would likely focus on finding sufficient HSPs and not POPs.

- The major countries reporting shortfin mako catch in the IO are France (Reunion), Spain, Portugal and South Africa. Therefore, we would expect that sampling from these fleets could yield sufficient samples for CKMR. Note that the target sample size from full statistical designs in the North and South Atlantic (Bravington, 2019) were in the order of 5,000 and 10,000 individuals, respectively. We would expect that roughly comparable sample sizes would be needed for the IO to provide an equivalent level of precision on the abundance estimate (i.e. a CV of ~0.2).

Silky shark:

- Background biology is known to a reasonable degree. As with the other species there would likely be a need to consider regional differences in growth.
- The catch at size data is probably the poorest of these 3 species with the highest reported catches. While we would consider this species as worthy of consideration, design estimates may end up being more uncertain as a result of the nature of the available data and information.
- Silky shark is caught in large numbers, according to the IOTC nominal catch, by Iran, Sri Lanka and Taiwan.

The data from the other species are far more uncertain. We think design targets for these species may be developed but would be far more uncertain.

- NEI Thresher sharks appear to be a sizeable component of the catch, but species-specific breakdown of the catch data indicated small catches resolved to species level. Additionally, the size frequency data is poor.
- Oceanic whitetip and scalloped hammerheads have long been considered as potential species in need of management action to ensure the size of historical catches and their potential vulnerability. It would therefore be advisable to consider data and tissue collection which might accumulate sufficient samples (e.g. in the order of 2-4 K) over time. At such a point, a less formally designed CKMR project could proceed to identify kin among the available samples for fairly moderate cost.

ACKNOWLEDGEMENTS

We would like to thank Hilario Murua (ISSF), Gorka Merino (AZTI), Josu Santiago (AZTI), Jessica Farley (CSIRO), Paul de Bruyn (IOTC), Fahmi Zulkarnaen (RITF, Indonesia), Jorge Mafuca (IIP, Mozambique) and Stela Fernando (IIP, Mozambique) for contributing with background information, facilitating obtaining data used for this study and/or providing comments on earlier versions of this report.

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ANNEX A - ACRONYMS

ABFT: Atlantic Bluefin Tuna

ALK: Age-Length Key

BMSY: biomass that enables a fish stock to deliver the maximum sustainable yield

CCSBT: Commission for the Conservation of Southern Bluefin Tuna

CKMR: Close-Kin Mark Recapture

CPUE: Catch Per Unit Effort

ERA: Ecological Risk Assessment

FOP: Father Offspring Pair

FSP: Full-Sibling Pair

GGP: Grandparent-Grandoffspring Pair

HSP: Half-Sibling Pair

ICCAT: International Commission for the Conservation of Atlantic Tunas

IOTC: Indian Ocean Tuna Commission

IUCN: International Union for the Conservation of Nature (IUCN)

MSY: Maximum Sustainable Yield

mtDNA: mitochondrial DNA

PBFT: Pacific Bluefin tuna

POP: Parent-Offspring Pair

RAD-seq: Restriction Site Associated DNA sequencing

SBT: Southern Bluefin Tuna

SNP: Single Nucleotide Polymorphism

TAC: Total Allowable Catch

TRO: Total Reproductive Output

WPEB: Working Party on Ecosystem and Bycatch