

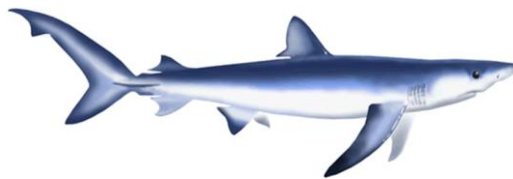
**Checkpoint for the application of close-kin mark recapture for the
North Pacific shortfin mako (*Isurus oxyrinchus*)**

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Abstract

The latest stock assessment of North Pacific shortfin mako was conducted in 2024. Although the stock status was estimated using ensemble approach, some amount of uncertainty, e.g., estimation on the population scale, was recognized and hence the application of close-kin mark recapture (CKMR) method for the future stock assessment was proposed, based on the past successful application to tuna species. However, careful considerations on its application in accordance with the biological aspect of target population is quite important to obtain less uncertain and less biased outputs from this approach. In this document, we listed the check point or issue to be discussed in advance of the development of sampling plan. In addition, challenges for the implementation of international collaborative work, specific to this species, and current work by Fisheries Resources Institute of Japan were also briefly introduced. Understanding these situations would be useful to discuss the future work plan.

From the biological viewpoint,

- Identification of subgroup for each sample is a necessary step in addition to the usual CKMR analysis. Feedback approach of genome-wide nuclear-genome-wide single-nucleotide polymorphism (SNP) data analysis is important phase by phase both for the initial development of sampling design in efficient and robust manner and for understanding the population structure.
- Age information is necessary irrespective of the kinship used for the CKMR estimation. The divergence of individual growth in juvenile stage reported for shortfin mako in the North Pacific still needs to be reviewed and discussed.
- Reproductive variability such as increase of fecundity and maturity rate with maternal body size (or age) affects the probability of finding “kinship”. Development and modification of model to estimate the probability of kinship identification and abundance could be necessary

From the practical viewpoint,

- It is necessary to decide the objective in CKMR and how to integrate the results of CKMR with or apply in the stock assessment. The choice of the periods, the used kinship and the usage of them should be basis to initiate the CKMR study.
- The kinship used for the CKMR estimation (i.e., POP, HSP), estimation method of age (i.e., direct observation, conversion from length, etc.), sample size, and migration pattern and so on are necessarily considered to develop the sampling design.
- The DNA sequences and subsequent genome analysis to obtain the SNPs for the kinship identification is a fundamental technique for CKMR analysis. The sequence data across different methods to get SNP are generally incompatible and the sequence method must be consistent.
- If CKMR study is conducted under the ISC framework, the budget for this study should be considered. Depending on the sampling design, the cost would substantially differ by country. It may be necessary for somebody to pay the costs on behalf.

- Regarding international exchange of samples of this species, we need to understand and consider the effects of international agreements. While the validity of ABS do not relate to the genetic resources collected in high seas, that of CITES (Appendix II) relates to the derivatives collected from any area. Information on this problem from other RFMOs would be useful.

Introduction

In the latest stock assessment of shortfin mako (*Isurus oxyrinchus*) in the North Pacific, model ensemble approach was applied in order to take into account several uncertainties from the catch, standardized CPUE, biology of this population, but still some uncertainty remained in the estimated stock status (ISC 2024). Among several issues specific to this population, lack of information of adult component is prominent and universal in the stock assessment of several RFMOs. As possible reason for their limited information and rare encounter with human activities, mismatch of fishing gear (selectivity: hook size and type of bait etc.), little overlap between their distribution area (both horizontally and vertically) with fishing, practical difficulty in handling and effect of several regulation are suggested. Therefore, it is challenging to obtain meaningful information of reproductive component of this population (e.g., abundance of spawning biomass) using traditional approach (ISC 2024), moreover, critical knowledge, such as spatio-temporal migration of adults including mating/pupping ground, following genetic interaction within the Pacific, and reproductive cycle, is lacking.

In the stock assessment report of North Pacific shortfin mako in 2024, application of Close-Kin Mark Recapture method (hereafter, indicated as “CKMR”) was discussed as a candidate tool to complement the limitation of traditional stock assessment approach (ISC 2024). In advance of application of this approach, it is necessary to establish the clear target (e.g., estimation of adult abundance and how to integrate into the stock assessment) when applying this new approach, with agreement, and to check the biological knowledge necessary for the application (e.g., reproductive schedule with its uncertainty, genetic population structure etc.) one by one. Then, clarification of the important assumption necessary for the estimation, and the development of approach adjusted to this population should be followed, based on this knowledge. In addition, it would be important to identify the influential parameter and setting for the designated estimates and to discuss the appropriate value and or approach, including sensitivity analysis.

Shortfin mako was listed in Appendix II of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 2019 and international trade has been restricted on global scale. Import and export of biological tissue sample, including muscle and vertebra among countries and from high seas (“Introduction from the Sea”) has been also regulated under this rule and thus understanding of current situation and limitation and necessary procedure is important in case that integrated analysis for this highly migratory species is planned.

In this document, we listed 1) the important biological information necessary to be confirmed, 2) discussion points in advance of application of CKMR, and 3) make a brief summary on the current regulation in the exchange of tissue sample and necessary procedure by international agreements. Additionally, feasibility study planned by FRI for the application of CKMR for this species, scheduled in 2025, will be briefly introduced.

1. Biological information necessary for the CKMR analysis

The points below are a portion of fundamental issues and topics necessary to be discussed to launch the CKMR project. Continuous discussion is important, and it is noted that consultation with expertise in statistics and mathematics to develop statistical mark-recapture and population-dynamic models is essential for the successful implementation of this analysis (Casas and Saborido-Rey 2023).

1-1. Population structure

When applying CKMR, definition of target population or sub-population is essential, because the sampling strategy should be discussed and designed according to the population structure and the CKMR estimates of adults depend on it. It is suggested that shortfin mako has multiple pupping areas in the North Pacific and this may invoke the population structure with multiple breeding populations. If multiple breeding populations, which never mate across populations, exist in the shortfin mako in the North Pacific, juveniles from a certain breeding population cannot be a POP with the other breeding populations. In case any adults use every mating ground without any preferences, then the population would be genetically mixtures and the strategy would be different. Thus, spatial bias for sampling for offspring potentially affect to estimate probability of kinship identification depending on the true picture of structure. In such case, phase by phase feedback of genome-wide nuclear-genome-wide single-nucleotide polymorphism (SNP) data analysis (e.g., identification of subgroup for each sample) to the usual CKMR analysis is important both for the initial development of sampling design in efficient and robust manner and for understanding the population structure, otherwise, it may lead to inappropriate sampling and consequently, biased estimation of the probability of kinship identification and abundance.

1-2. Age (growth curve)

Age information is essential for inference of population size with acceptable precision (Bravington *et al.* 2016, Trenkel *et al.* 2022, Petersma *et al.* 2024). For the POP model, age information is necessary to take the reproductive variability by age into account (see next subsection). This can be calculated by years and ages both for adults and juveniles when samples are collected. For HSPs, the cohort of each sample is essential information. The ages estimated by conversion from length is helpful, because direct age observation for every individual is impossible in most cases. The simulation study

with grey reef shark (*Carcharhinus amblyrhynchos*) suggests that incorrect standard deviation of length measurement error has little effect on estimation but having an accurate growth curve is crucial for CKMR when ageing is based on length measurements (Petersma *et al.* 2024). In the case of shortfin mako in the North Pacific, the divergence of individual growth in juvenile stage by study has been reported and still needs to be discussed for the solution.

1-3. Reproductive variability

In case not all adults reproduce annually, and that fecundity and maturity rate increase with maternal body size (or age), it would be suggested that these variability affects the probability of finding “kinship” (Casas and Saborido-Rey 2023, Swenson *et al.* 2024). In the case of shortfin mako, there are currently two different hypotheses both for the fecundity at maternal size (age) (i.e., no relationship or proportional increase) and for the reproductive cycle (i.e., two or three years) (ISC 2024). Depending on the feedback from the observation of kinship, development and modification of model to estimate the probability of kinship identification and abundance could be necessary.

1-4. Other issue

Understanding of migration of the target species is also important, because sampling without the consideration of movement pattern may lead to the bias of sampling, and consequent biased estimation of abundance (Irie 2016). Conceptual life-history model considering sex-specific movement would be useful to develop a sampling design. As other limitations, species with very long-life span and those displaying parthenogenesis are inadequate species to apply CKMR (Bravington *et al.* 2016). Although no information is available for shortfin mako, Casas and Saborido-Rey (2023) noted many cases of parthenogenesis in elasmobranch species and caution when applying CKMR to this group in order to avoid wrong estimation of abundance with CKMR. Demographic modelling should consider the sex-specific life history characteristics, if strong sexual dimorphism in growth rates, fecundity-at-age and different catchability between sexes (gear selectivity pattern) exist among other particularities (Trenkel *et al.* 2022).

2. Discussion points for the application of CKMR

The following issue was listed based on the experience in Japanese scientists, including Pacific bluefin tuna. These issues are related to each other and hence some of them may need to be considered simultaneously.

2-1. Setting of the objective

It is necessary to decide the objective in CKMR and how to integrate the results of CKMR with or apply in the stock assessment. POPs with age information can infer the age structure of adult

population, while HSPs can estimate mortality level of adults by the decreasing the finding probability of HSPs by death of adult over time. Long term CKMR analysis can provide the population scale and its trends by itself, while CKMR can be an information on the population scale for that period in the integrated model. Also, using CKMR data solely could assess the stock status without any fishery data, although this would require high sample size to be less uncertain CKMR estimation. The choice of the periods, the used kinship and the usage of them should be basis to initiate the CKMR study.

2-2. Sampling design

The kinship used for the CKMR estimation (i.e., POP, HSP), estimation method of age (i.e., direct observation, conversion from length, etc.), sample size, and migration pattern and so on are necessarily considered to develop the sampling design. Depending on the sampling design and sampling area, we can discuss the sampling allocation by ISC members.

2-3. Decision of approach to obtain genetic marker necessary for the kinship analysis

The DNA sequences and subsequent genome analysis to obtain the SNPs for the kinship identification is a fundamental technique for CKMR analysis. There are several methods for getting SNPs from wide genome information. However, the sequence data across different methods are generally incompatible. Ideally the sequence should be conducted by one method in one place. Even if not, the sequence method must be consistent. In the case of Pacific bluefin tuna, which is the species dealt with ISC, they use GRAS-Di method. They have already combined the sequence data by this method from two different labs and succeeded SNP call (Tsukahara *et al.* 2025).

2-4. Budget

If CKMR study is conducted under the ISC framework, the budget for this study should be considered. Depending on the sampling design, the cost would substantially differ by country. And some DNA sequence methods can be conducted in limited lab so far and then it may be necessary for somebody to pay the costs on behalf.

The necessary cost for the implementation would be budgets for the collecting sample (tissue) by purchase from fisherman, consumables for the experiment including reagent, and sequencing are necessary at least.

Although not listed above, the process of kin-identification from genome-wide SNPs dataset and the development of age-structured population dynamics model are necessary steps to obtain the estimates of abundance.

3. Restriction of sample exchange by the regulation of international agreement

Aside from technical issues, there are several issues considered in the case of shortfin mako. As general procedures, we need to confirm the necessary process according to the ABS (Access and Benefit-Sharing) protocol when genetic resources is moved from a certain country to another country. In case of marine species, it relates to the interchange of samples collected in EEZ and Japan has continued the communication with Mexican colleague, which was not permitted yet. In the case of ISC Shark WG, some countries are Party to the Nagoya Protocol (e.g., Japan, Mexico) and others are not. Thus, case-by-case clarification of the procedure would be necessary to exchange the samples.

Another issue is the regulation by CITES. CITES works by subjecting international trade in specimens of selected species to certain controls. As described, shortfin mako has been listed as Appendix II of CITES and thus, export, re-export and introduction from the sea of species (IFS) covered by the Convention has to be authorized through the granting of an export permit or re-export certificate . The international transfer of derivatives (including muscle tissue, vertebrae etc.) becomes “international trade” and each Party of export needs to follow the required formalities of export. The regulation by CITES would be related to most member countries of ISC Shark WG, because it covers derivatives collected in high seas. Each Party must designate one or more Management Authorities competent to grant permits or certificates and one or more Scientific Authorities to advise that such export or IFS will not be detrimental to the survival of that species. I summarized the necessary permission in the trade (export or IFS) of derivatives (Table 1, Appendix Table1). In case of Japan, total of nine types of paper needs (Appendix Table2) to be prepared for obtaining the Legal Acquisition Findings (LAF) at a minimum. For Non-Detrimental Findings (NDF), each country needs to develop its own NDF for each species including information of its stock status. In case of Japan, NDF for shortfin mako in the North Pacific was developed based on the stock assessment in ISC. It is necessary that the Scientific Authority advices that each export of the tissue sample is not detrimental to the survival of this species.

4. Introduction of FRI study and workplan

Fisheries Resource Institute (FRI) of Japan has tackled the clarification of genetic population structure of shortfin mako on global scale using a cutting-edge approach by newly developed reference genome and individual-based analysis of mitochondrial and nuclear genomes of this species (Takeshima *et al.* 2022). The initial analysis focused on the Atlantic and Indian Ocean but recently, the analysis included information of individuals from four localities of the Pacific Ocean (Northwest and central, Northeast, Southwest, Southeast). In a series of analysis, we performed mitogenomics and nuclear-genome-wide SNP genotyping with a genotyping-by-sequencing (GBS) method, namely GRAS-Di (Enoki and Takeuchi 2918, Enoki 2019). The SNPs data which has already been accumulated by our work (i.e., GRAS-Di data) is more than useful to identify related individuals if CKMR is performed for this population.

FRI has collected tissue samples of shortfin mako from wide size range, including neonates and adults. With the GRAS-Di as GBS, we plan to find kin-relationship (full-sibling or half sibling) from neonate sample in order to feedback for the development of sampling plan and as a material for the further discussion of CKMR. Depending on the budget allocated, more than twenty samples will be analyzed by collecting several thousand SNP data. The derived results will be shared in future shark WG meeting.

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Tables

Table1 Outline of necessary permission in the export of derivatives listed in Appendix II.

Appendix II		Contact
Objective	species not necessarily threatened with extinction, but in which trade must be controlled in order to avoid utilization incompatible with their survival.	
Regulation	Trade for commercial purpose and landing from high seas are possible*	
Necessary permission	Permissions (below) from Management Authorities and Scientific Authorities of exporting country are necessary.	
	1. Legal acquisition findings (necessary information that the specimen was not obtained in contravention of the laws of that State for the protection of fauna and flora)	Management Authority
	2. Non detrimental findings (Scientific advice that export is not detrimental to the survival of the species)	Scientific Authority
	3. Introduction from the Sea (the prior grant; advice from Scientific Authority** and satisfaction of Management Authority is necessary)	Management Authority

*At present, exchange of derivatives for the purpose of research has not been exempted.

**In case of Japan, permission of NDF is not issued but Scientific Authority conduct NDF according to the philosophy that it is a necessary process.

In addition, for the IFS of living specimen, the Management Authority of the State of introduction need to be satisfied that the specimen will be so handled as to minimize the risk of injury, damage to health or cruel treatment.

Appendix Table 1. Necessity of certificates or permission for species listed in Appendix II. No and Yes denote “not necessary” and “necessary”, respectively in Japan.

Event	Without reservation	With reservation
Landing the catch from EEZ domestically	No	No
Landing the catch from high seas domestically	Yes	No
Export	Yes	Yes

Appendix Table 2. Necessary preparation for obtaining LAF in case of Japan.

1. Application for export approval
2. Instructions of application for export approval
3. Invoice of export
4. CITES permit/certificate : (each government)
5. Certificate of harvest
6. Commitment form
7. Photograph of derivatives
8. Certificate of Transfer or Certificate of Sale
9. Statement of mutual agreement of collaborative study