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CONSIDERATIONS FOR CONDUCTING CLOSE KIN MARK RECAPTURE OF STOCKS MANAGED BY THE IATTC

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"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." (Rodriguez-Ezpeleta et al. 2020)

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SUMMARY

Close Kin Mark Recapture (CKMR) is a new technique to estimate absolute abundance of spawners, adult survival, and relative reproductive output by age. This information is essential for stock assessments and the approach avoids issues of traditional tagging studies such as tagging related mortality, tag loss, and tag non-reporting. It does not require releasing of live individuals and therefore can greatly increase sampling opportunities and improve spatial distribution of tags. It also allows larval and juvenile dispersal to greatly reduce issues with tag mixing and spatial distribution of sampling. The genetic data collected in CKMR also provides information on stock structure, sex, and possibly age. However, stock assessments

also require estimates of juvenile abundance, juvenile survival, and the stock-recruitment relationship. These quantities are not estimated from CKMR and need to be estimated from other data or assumptions made. We evaluate the feasibility of applying CKMR to silky shark and bigeye tuna in the eastern Pacific Ocean (EPO). Both stocks have uncertainty about their absolute biomass levels and therefore CKMR would greatly improve management advice. Tissue sampling opportunities appropriate to CKMR are currently available for both species, but some constraints remain such as non-retention policies for silky sharks and lack of catch date and location information for high-seas longline landings. Any CKMR for these studies will require sampling in the western and central Pacific Ocean (WCPO) and therefore coordination with the Western and Central Pacific Fisheries Commission (WCPFC). Costs of CKMR studies for silky shark are likely to be in the mid to high hundreds of thousands and for bigeye tuna in the low millions of dollars. The next step is to conduct a comprehensive design study for each species and field work to evaluate the practicality of the alternative tissue sampling opportunities and the resultant tissue quality for genetic analysis. CKMR and the associated genetic and statistical analyses are outside the expertise of the current IATTC staff. Therefore, collaboration or contracts with outside experts will be require to conduct the project. The design study and field work could be conducted in 2022 and the sampling for CKMR could start in 2023. Results would not be expected until the 2026 SAC at the earliest. Several recommendations and considerations were identified for each species and are listed below.

Recommendations and considerations for silky sharks:

1. Conduct new silky shark age and growth study in the eastern Pacific Ocean and investigate spatial differences in growth relative to the WCPO.
2. Develop sampling platform for vertebral tissue using the IATTC observer program on the purse seine fishery and shark fishery sampling program in Latin American costal states in the EPO.
3. Expand sampling efforts to WCPO in collaboration with WCPFC/SPC.
4. Use IATTC capacity building funds to support universities/research institutes to conduct biological studies for silky shark.
5. Continue to use the sampling program for shark fisheries in Central America to collect information on maturity and fecundity for the silky shark.
6. Fecundity increases with age indicating that both POPs and HSPs will be needed.
7. Improve catch estimates for the high-seas longline fishery.
8. Improved catch estimates for the purse seine fishery.
9. Consider approaches to estimate juvenile abundance and survival.
10. Approaches to estimate the stock-recruitment relationship are needed.

Recommendations and considerations for bigeye tuna:

1. For high-seas longliners, determine the feasibility and tissue quality of
    a. observers taking tissue samples onboard
    b. sampling at port
    c. sampling at the markets
2. Evaluate the need for date and location information from high-seas longline-caught bigeye sampled at port or in the market.
3. Expand sampling efforts to WCPO in collaboration with WCPFC/SPC.
4. Consider approaches to estimate juvenile abundance and survival
5. The stock—recruitment relationship is uncertain.

6. Sex-specific composition data is needed.

1. INTRODUCTION

Close Kin Mark Recapture (CKMR) is a promising method to estimate absolute abundance and biological parameters based on developments in the analysis of genetic data (Skaug, 2001; Bravington et al., 2016). In addition to estimating the absolute abundance of spawners, it can estimate adult survival and relative reproductive output by age. The approach is like traditional mark-recapture studies, except it uses kin-ship relationships to "tag" individuals based on genetic relatedness. These genetically tagged individuals do not have to be released alive and can be sampled from the retained catch. CKMR avoids several major issues related to traditional mark-recapture studies including: 1) tagging related mortality, 2) tag loss, and 3) tag non-reporting. The tagging effect (e.g. trap-happy, trap-shy) is also reduced because the same individual is not captured twice (Bravington et al, 2016). Tag mixing may be improved using CKMR, particularly for highly fecund pelagic spawners; because individuals are essentially tagged at birth (spawning), and due to factors such as natural larval and juvenile dispersal, the tags are widely dispersed before the individuals are large enough to be caught. Since estimates of spawning abundance are for the birth year of sampled individuals, a single year's sample of multiple aged fish can provide a time series of biomass estimates, which may be indicative of trends (Kolody and Bravington, 2019).

Estimation of absolute abundance is the main task of a fishery stock assessment model, which is the main tool in fisheries stock assessment and management of most commercially valuable species and species of conservation concern. Contemporary stock assessment models integrate multiple data sources to estimate the model parameters and related derived quantities (e.g. absolute abundance, Maximum Sustainable Yield [MSY], biomass corresponding to MSY [B_{MSY}], Maunder and Punt, 2013; Punt et al., 2013). Information about absolute abundance can come from several sources, but generally comes from two sources: 1) indices of relative abundance and 2) catch composition data (Maunder and Piner, 2015).

Information on absolute abundance from indices of relative abundance comes from the effect of catch on the index adjusted for natural mortality (M), growth, and recruitment. This typically assumes that the index is proportional to abundance, which may not be true, particularly if the index is based on catch-per-unit of effort (CPUE; Harley et al., 2001; Maunder et al., 2006), and that the values for natural mortality (M), growth, and recruitment are known, or can be estimated within the stock assessment model, relatively well. Growth may be known well, but natural mortality is seldom known with any reliability and, in general, temporal variability in recruitment cannot be estimated without catch composition data. Knowledge of annual (or quarterly) recruitments is important to differentiate the effect of catch on the index from recruitment variability, particularly for stocks with high recruitment variation (e.g. tunas; Minte-Vera et al. submitted).

Indices of abundance are often uninformative about absolute abundance in stock assessment models and estimates are driven by the catch composition data. The catch curve, as represented by the catch composition data, measures the decline in abundance as a cohort of fish ages and is a measure of total mortality, which can be separated into fishing mortality if the value of natural mortality is known. The fishing mortality (F) in conjunction with catch (C) provides information on absolute abundance (N) as can be illustrated by rearranging the approximate relationship $F = C/N$ to give $N = C/F$. Information on absolute abundance from composition data is therefore dependent on natural mortality being known, or can be estimated within the stock assessment model, relatively well. It is also dependent on the catch composition representing the abundance at age. However, young and/or old fish may not be fully

represented in the fishery due to the characteristics of the gear (contact selectivity) or spatial stratification of ages/sizes of fish (availability). Asymptotic selectivity is often used or needed in stock assessments to ensure reliable estimation of the model parameters, but reduced selectivity for old ages is likely common (Waterhouse et al. 2014) and can cause substantial underestimation of absolute abundance. Additionally, in many cases, reliable growth information is required because age data is not available and length compositions are converted into age using the growth curve. Therefore, estimates of absolute abundance from length composition data are sensitive to uncertainty in growth, particularly asymptotic length (Zhu et al. 2016), which is usually poorly estimated.

Estimates of absolute abundance are available, but less frequently, from other methods such as tagging (mark-recapture) studies, surveys with known catchability, depletion estimators, etc. However, many of these are not reliable. Tagging studies are expensive, practicalities of implementation make them unreliable, and they require estimates of tag loss, tag related mortality, and reporting rates. Surveys (e.g. trawl or acoustic surveys) rarely have reliable estimates of the coefficient (catchability) that scales them to absolute abundance. Depletion estimates are only valid in specific cases (e.g. Aires-da-Silva et al. 2016).

There are many stocks for which there is limited information and they lack the time series of catch, relative abundance, and composition data that are used in standard stock assessments. These stocks have to be assessed using data-poor methods (e.g. Pons 2020) or prioritized for management, data collection, and research using ecological risk assessment approaches (Griffiths et al. 2018) and stock status definitions are often either unavailable or unreliable.

CKMR can provide two vital quantities that are uncertain for many stocks: absolute abundance of spawners and survival of adults, which could greatly improve the assessments for many stocks managed by the IATTC. The quantities are useful in themselves and a time series of spawning biomass could be used to monitor a stock, however, a full stock assessment may be preferable and would require additional quantities such as juvenile survival and abundance, and the stock-recruitment relationship. These additional quantities are already estimated or assumed in stock assessment models, and therefore obtaining reliable estimates of absolute spawner abundance and adult survival can only greatly improve current assessments. Combining information from CKMR and data currently used in stock assessments (indices of relative abundance and catch composition data) may allow estimation of juvenile abundance and juvenile natural mortality. Any remaining uncertainties (e.g., the stock-recruitment relationship) can be addressed through choosing robust harvest control rules identified using management strategy evaluation (Butterworth et al., 1997).

The estimates of absolute abundance from CKMR should also help better determine the status of low information species. A current estimate of total catch in association with the estimate of absolute abundance will provide an indication of the fishing mortality rate. The fishing mortality can then be compared with reference points calculated from demographic analysis. Like stock assessment, estimates of juvenile survival, juvenile abundance, and the stock-recruitment relationship are required for the demographic model, and these may need to be based on assumptions, perhaps from similar well studied stocks. A single CKMR study is adequate to estimate absolute abundance, but the sampling could be continued over time increasing precision and estimating a time series of abundance. The time series of abundance could be used in a harvest control rule when a full stock assessment is not possible.

CKMR also provides information on stock structure and exchange rates. Stock assessments typically assume a fully mixed single closed population and some of the unresolved issues in stock assessments

may be due to poor stock structure definitions such that local depletion is occurring within the assumed stock or there is exchange with an unmodelled component of the stock. The genetic information collected in the CKMR study can be used to identify the stock structure and exchange rates among sub-stocks. Therefore, ensuring enough spatial coverage to detect spatial heterogeneity in population structuring is important when designing the study. Spatially stratified CKMR analysis is an area of research (Mace et al., 2020).

Implementation of CKMR requires several considerations. The analysis requires information on the age of sampled individuals (e.g. a growth curve and lengths of sampled individuals) to establish the time at marking (birth). These data are often available, but in some cases may need to be collected as part of the CKMR study. Genetic based methods to age individuals shows some promise (Anastasiadi and Piferrer 2019; Mayne et al. 2020) and may have some practical advantages for some applications. There are also sampling considerations such as the practicality and costs of sampling, and the optimal design. The ability to sample both adults and juveniles is beneficial, but this might not always be practical and sampling juveniles may be adequate for some species (i.e. species (e.g. some sharks) for which reproductive output does not change with age; Bravington 2019). Samples from multiple cohorts can be obtained by sampling multiple ages in a single year or by sampling one age in several years. It is also important that the samples cover the whole spatial range of the stock so that any spatial structure can be detected. However, Conn et. al. 2020 suggest that low to moderate bias in spatial sampling does not greatly affect CKMR estimates.

1.1. Objectives of study

CKMR can be used for a variety of species and life histories, but each may require different refinements to the analysis, data requirements, and sampling design. We discuss CKMR with respect to two different IATTC managed stocks to highlight the potential benefits of CKMR and the challenges that may need to be overcome. The first is the silky shark stock, which is data-poor, although an additional catch sampling program has recently been initiated (Oliveros-Ramos et al. 2020). The second is bigeye tuna, which is relatively data rich and is an important focus for management of the EPO tuna fisheries, but there are still major sources of uncertainty in the assessment (Xu et al. 2020).

2. BASIC CONCEPTS OF CLOSE KIN MARK RECAPTURE

CKMR is based on the same concept as traditional mark recapture (MR) except that genotypes are the “marks” and “recaptures” are inferred by kinship relationships. In MR studies, individuals are marked and released into the population and then the proportion of marked individuals in a subsequent sample from the population is an indication of the population size. The bigger the population, the less marked individuals will be in a sample of a given size. In CKMR, the bigger the population, the less kin (mother-son, brother-sister, etc.) will be in a sample of a given size (assuming the population is sufficiently mixed with respect to kinship relationships).

To use the kinship relationships for estimating absolute abundance, the relationships can be conceptualized as marking individuals (i.e. marking the parent by observing the offspring). This is most clearly illustrated with parent offspring partnerships (POPs). If an offspring is sampled, then both a mother and father must have been alive at birth (spawning, fertilization, etc.) of the offspring and therefore the parents can be considered marked at the date of birth and genetic samples of the parents after that date can be considered recaptures. Standard MR calculations can then be carried out to estimate the absolute abundance (Figure 1).

The use of Full Sibling Pairs (FSPs, share both parents) and Half Sibling Pairs (HSPs, share only one parent) is more complicated (Figure 2). FSPs are often "eliminated" from the analysis to remove the impact of batch spawning and the lucky litter effect (where many individuals from the same spawning/birthing event survive) that can invalidate independence of samples and population processes (e.g. lack of mixing of the "marked" individuals). Analyses using HSPs are based on an offspring-centric view of relatedness that calculates, from a randomly drawn sample, the probability that two randomly chosen juveniles in the sample have the same parent (Rodriguez-Ezpeleta et al. 2020). Further illustration of these concepts can be found in Bravington et al. (2016), Bravington (2019), Rodriguez-Ezpeleta et al. (2020), and other documents.

Having information on age is important to identify the year of birth. Therefore, age data are required. It is preferable to age each sample taken, but taking a size measurement and using a growth curve to estimate age is typical. Uncertainty in aging affects estimates of the rate-of-change in abundance or mortality and makes same-cohort comparisons unavoidable (Bravington 2019). The uncertainty in age can be taken into consideration by doing the calculations across all possible true ages weighted by the probability of the age being correct (Bravington 2019). Uncertainty in aging will reduce precision of all quantities estimated from CKMR data. Genetic approaches to aging are promising (e.g. Epigenetic aging based on DNA methylation; Anastasiadi and Piferrer 2019, Mayne et al. 2020) and may provide a practical approach to age samples used in CKMR (Bravington pers. com.). However, these techniques require a lot of work upfront to develop and calibrate markers. Most of the time, these markers are species-specific, though there are efforts underway to develop more general markers that work for a variety of taxa. Note that length specific selectivity does not impact the CKMR analysis (except in some applications of POP only versions), but age-specific selectivity might (Bravington pers. com.).

The ages of individuals accessible to sampling gear will determine the segments of the population for which abundance estimates can be obtained. POPs provide information on adult abundance (Kolody and Bravington, 2019). However, POPs require sampling of both juveniles and adults. The use of HSPs means that spawner abundance can be estimated even if the adults are not sampled. But this only works for species for which the reproductive output does not change with age, otherwise assumptions about reproductive output need to be made (Bravington pers. comm.). In addition, the genetic and statistical analyses of HSPs are much more demanding than for POPs, they require higher quality tissue (Rodriguez-Ezpeleta et al. 2020) and may be more expensive to process (Mace et al., 2020).

Sampling of HSPs over multiple cohorts can be used to estimate adult survival, and when combined with catch data, can be used to estimate natural mortality. HSPs from different cohorts share a parent and the parent had to be alive at the time of birth of each sibling. The larger the difference in age between the cohorts relating to the siblings, the longer the parent had to survive. Therefore, sampling multiple cohorts so that there is a range in age differences between HSPs can be used to estimate adult survival. As the difference in age increases, fewer HSPs are expected and the rate of decline is related to adult survival. Multiple cohorts could be obtained either by sampling several ages in the same year or one age over multiple years. For example, if aging is uncertain, sampling the young of the year, which are of a known age, over several years might be appropriate. Having both POPs and HSPs can separate reproductive success from adult mortality, when reproductive success changes with age (Mace et al., 2020). In general, it is better to collect both POPs and HSPs when possible.

Mitochondrial DNA (mtDNA) provides information about the sex of the parents, which might be used for calculating the absolute abundance of adults by sex and survival by sex (Rodriguez-Ezpeleta et al. 2020). This will be important for species that have population and fishing processes that differ by sex and those that show spatial segregation by sex. The relative fecundity effect for males may be different from females and there is often no direct data for males (Bravington 2019). The combination of POP and HSP data allows for direct estimation of the relative fecundity for both sexes (Bravington 2019).

CKMR can estimate the age-structure of the spawning biomass (Richard Hillary pers. com.). The age distribution of the POPs is proportional to N[a] * phi[a] where phi[a] is the relationship for how reproductive success changes with age. Since phi[a] can be estimated if both POPs and HSPs are sampled (also with the additional information about survival from composition data when integrated into the stock assessment model), then the age-structured of the spawning population can be estimated.

3. SAMPLING AND GENETIC ANALYSIS

There are several other aspects of sampling that need to be considered. For example, the whole spatial domain of the stock should be sampled. Also, any possibility of the sampling of kinship relationships being non-random needs to be avoided. Essentially, the event of one individual of a kinship pair being sampled should be independent of the other individual of the pair being sampled, conditional on covariates (Bravington et al. 2016). For example, care should be taken when sampling individuals in a single fishing set as they may be more related than by random chance (e.g. if mother-calf pairs are caught together; Bravington et al. 2016). Thus, taking a single sample per set, possibly of each age, but sampling more sets, might be preferable, particularly when there is no prior information available on kinship relationships relative to sampling units. For species with tight parent-offspring bonds or in areas of birth (e.g. pupping for some sharks and rays), POPs may be biased (HSP are not considered from the same cohort so this issue is minimized unless family groups stay together). Other examples that require specific modelling consideration are when large fish are more likely to breed as well as to be caught and when there is persistent heterogeneity in individual's reproductive success (Bravington et al. 2016). Therefore, the life history of the species should be taken into consideration when designing the sampling program.

Developing a sampling design that explicitly specifies how many individuals, of what age, and in how many years they are to be sampled, is important to ensure the goals of the study are achieved. The resulting precision of the estimates of spawner abundance and adult survival is highly dependent on the population size. Therefore, a good approximation of the spawning abundance is needed to develop a sampling design that will achieve a predetermined level of precision. This could be taken from existing stock assessments. However, in the absence of abundance estimates, a maximum desirable fishing mortality (e.g. the limit reference point taken from spawner per recruit studies of similar populations), in combination with known catch, could be used to define a minimum estimate of abundance and, from that, the sample size calculated that would obtain estimates with the desired precision. If the abundance is larger than assumed, then despite the estimate being imprecise (and the confidence intervals do not include the minimum estimate of abundance), the population is not in imminent danger and additional sampling can be conducted in the future if more precise estimates are desirable. The most comprehensive sampling design studies involve using age structured models that are the same as used to do the CKMR analysis (Rodriguez-Ezpeleta et al. 2020).

There are several practical considerations of sampling and high-quality tissue samples for sequencing is a pre-requisite for CKMR, particularly for HSP detection as explained by Rodriguez-Ezpeleta et al. (2020).

Samples should be stored in a labelled vial containing appropriate preserving solution (e.g. 90-95% ethanol or RNAlater) with unique identifiers so that samples can be reliably cross-referenced to data on date of capture, location, species, age/size and sex, and to any other biological samples collected (e.g. vertebrae). Care must be taken to avoid cross contamination between the samples of different individuals. Preferably, new gloves and clean tools should be used each time a sample is collected, but simply wiping the scalpel clean between samples may be enough. The tissue removed from the sample for analysis should not be taken from the area that was cut to remove the sample from the fish. If contamination is suspected, there are methods that can be used to filter out the samples that are contaminated. Samples can be stored for long periods under appropriate conditions (e.g. -80◦C freezer; in practice, samples preserved in ethanol and stored in a freezer tend to work out better than samples stored in ethanol at room temperature), which means that sample collection can occur prior to development of a program for sample analysis. However, repeated freezing and thawing of samples can degrade the tissue and make it unusable for high-throughput genomics methods like restriction-site associated DNA sequencing (RAD-Seq). DNA capture panels can be developed to mitigate issues associated with DNA quality, but these require an initial investment to identify appropriate markers. A single flash freeze, as is done with fish caught on longline vessels and available for sampling at port, with tissue sample taken when frozen and put in ethanol might provide tissue of high enough quality. Therefore, the sampling of tissue from different fleets needs to be carefully considered and preferably tested in advance of the initiation of any large scale sampling program.

When it is not possible to consistently obtain tissue of high enough quality for RAD-Seq, there are a couple workarounds (John Swenson pers. com.):

First, a subset of high quality tissue can be used to do an initial smaller RAD-Seq run and those data used to develop RAD-Capture or GT-Seq panels. These methods allow the use of RAD-Seq with lower quality tissue samples, but would require work upfront to develop the panels (especially GT-Seq). Once the panels are developed, however, they can be used repeatedly and are likely to work with samples of lower quality (especially GT-Seq).

A good workflow would be:

1) use RAD-Sequencing on a small number of high-quality samples,
2) identify useful genetic regions for kinship assignment (i.e. regions with SNPs),
3) develop capture panels to target these regions,
4) use the capture panels in conjunction with RAD-Seq on the full set of samples.

This is likely–though not guaranteed–to mitigate many issues arising from low quality tissue. Also, capture panel development can occur as soon as a few dozen high quality samples are collected, so it can be parallelized with sample collection. This would be a good workflow regardless of tissue quality, because both RAD-Capture and GT-Seq substantially reduce subsequent sequencing costs (>50%). See Table 1 of Meek and Larson (2019).

In the absence of the above, it may still be possible to assign kinship using microsatellites, which do not require high quality DNA (John Swenson pers. com.). Microsatellites are not as powerful as genomics-based methods like RAD-Seq, but they have been used with CKMR before (e.g. Bravington et al. 2016). The problems with microsatellites are that 1) it is very difficult or impossible to identify half-siblings, and 2) they require significant upfront investment to develop. While genomics-based methods like RAD-Seq,

RAD-Capture, and GT-Seq are preferable (especially since they allow identification of half-siblings), if DNA degradation is unavoidable, microsatellite markers are still likely to allow assignment of Parent-offspring relationships. A suite of microsatllite markers for silky sharks was developed by O'Bryhim et al. (2015). A suite of microsatllite markers for tuna was developed by Clark et al. (2004).

Avoiding false positives (i.e. excluding less related kin) is important due to the rarity of positives (e.g. 50-100 in a study), but this also produces false negatives. However, a known false-negative rate, which can be estimated, can be accounted for in the calculations to remove bias (Bravington 2019). False positives can be minimized by computing the likelihood ratios of identifying different kinship relationships and then only counting as positives those that are above a certain ratio. The R program CKMRSim is designed to run these calculations and assign kinship based on a user-specific likelihood ratio. This allows the user to decide on an acceptable false positive rate and to evaluate the likely proportion of false negatives that arise as a result, which can then be accounted for.

4. SILKY SHARK

Silky shark is a cosmopolitan species throughout the tropical Pacific but its stock structure remains poorly understood. It is a moderately productive shark species with litter sizes of approximately 2 to 16 (average size of 6; Garcia-Cortes et al., 2011) and grows to lengths of about 250 cm total length (Oshitani et al., 2003). Juveniles are found predominantly north of the equator in the EPO (Roman-Verdesoto and Orozco-Zoller, 2005), but the young of the year are observed in the landings of coastal artisanal fisheries in the EPO (Salvador Siu pers. com.). The majority of the catch in the EPO is estimated to be taken by coastal nation-based longline artisanal fisheries, where the silky shark is one of the target species (SAC-05-INF-F; Siu and Aires-da-Silva, 2016). However, substantial catches are also taken by the tuna purse seine fishery across the EPO (Figure 3) and the distant water nation longline fleets. Both combined are estimated to represent less than 10% of the total catches in the EPO (SAC-05-INF-F), but the estimates may be biased low. A assessment of the EPO stock was attempted in 2014, but lack of a reliable time series of data (catch, index of abundance, and catch composition), particularly catch, resulted in unreliable results (SAC-05-INF-F). A Pacific-wide assessment was conducted in 2018 but suffered from similar issues and thus its results were not used to formulate management advice (WCPFC-SC14-2018/SA-WP-08). Catch sampling is possible for the purse-seine fishery through the AIDCP onboard observer program and collaboration with national observer programs is also possible. An in-port pilot study sampling program for Central American coastal nation-based fleets is ongoing by IATTC to improve the estimates of total catch and to obtain length composition samples for those fisheries (Oliveros-Ramos et al., 2020). Interim management is based on closed seasons for longline shark targeted fisheries, which include bans on wire leaders, as a precautionary measure. Additional bycatch mitigation measures are in place (IATTC Resolution C-19-05).

The following provides a detailed description of the current state of knowledge of the silky shark life-history parameters and fishery that are relevant for the application of CKMR to a stock assessment for the species in the EPO, and outlines the required data for CKMR that could be collected as part of existing data collection programs.

4.1. Age of sampled fish

Aging of silky sharks is conducted by reading annuli in vertebra and it is therefore not feasible to age each sample collected for the CKMR study. In principle, collection of length measurements for each sample (from dead sharks) is feasible, but these lengths would need to be converted to age using a growth curve.

Currently, there are two growth studies relying on the interpretation of vertebral band counts which could be considered to describe the age and growth of silky sharks in the EPO (Oshitani et al., 2003; Sanchez de Ita et al., 2011). Unfortunately, there are some challenges and concerns with both studies. Although Oshitani et al. (2011) relies on direct empirical counts of vertebral bands, samples are predominantly taken in the western and central Pacific Ocean. With respect to latter study, Sanchez de Ita (2011) relied on the use of back calculation to increase sample size of their age-at-length data and the samples are very limited in terms of their spatial coverage in the EPO. In addition, these curves differ greatly from each other including juvenile ages. Therefore, a new age and growth study for silky sharks should be conducted. A sampling design could be developed by IATTC staff to take advantage of sampling opportunities in the purse seine tuna fishery as well as the pilot study for Central American shark fisheries in the EPO (possibly expanding to other coastal countries). In collaboration with WCPFC/SCP, sampling efforts should also expand to the WCPO to investigate stock structure/spatial heterogeneity in growth at the Pacific wide scale. Latin American universities/research institutes are well positioned to conduct this study with support of IATTC (e.g. Capacity Building Funds). Aging based on genetic samples should also be considered.

4.2. Maturity and fecundity at age

Maturity and fecundity at age are useful for developing the sampling design, but are not necessary for the final analysis because when both POPs and HSPs are available, the analysis can estimate the effective reproductive output at age for both males and females. Information on maturity at age and an assumption of reproductive output being proportional to weight might be enough for some (teleost) species (at least for females), but it may not be appropriate for sharks.

Maturity at length estimates are available from the collaborative work between IATTC staff and scientists from member countries (S. Soriano and L. Castillo, Mexico). Maturity at age estimates are available from the existing growth curves but there are challenges with these curves (see above). Considering that the Soriano-Castillo samples were taken in Mexican waters, additional maturity at length data should be collected in other regions of the EPO. The sampling program for shark fisheries in Central America is already collecting maturity information for the silky shark. Opportunities for collecting maturity information in the industrial fisheries (purse seine, longline) are more limited.

Fecundity at length estimates are available for the silky shark in the EPO (Garcia-Cortes et al., 2011), but there are concerns with the age and growth curves available (see above). Fecundity increases with age indicating that both POPs and HSPs will be needed. Knowledge of the gestation period and breeding cycle is also an important component of reproductive output and some information is available for silky sharks in the EPO (Garcia-Cortes et al., 2011). Information on the reproductive biology of the silky shark is currently being taken by the sampling program for shark fisheries in Central America.

4.3. Stock structure

Although there have been some mitochondrial DNA studies for silky shark in the EPO (e.g. unpublished work by J. Hyde, SWFSC-NMFS), stock structure for the silky shark in the EPO and the wider Pacific Ocean remain poorly understood. Mixing between the EPO and WCPO probably occurs (WCPFC-SC14-2018/SA-WP-08).

4.4. Sex determination

Sex of silky sharks can easily be determined externally. Sex information is being collected by all sampling programs. There appears to be some differences in the distribution by sex in the EPO.

4.5. Approximate abundance

The stock assessment for the silky shark in the EPO was not successful in obtaining reliable estimates of abundance given the limitations of some important datasets (SAC-05-INF-F). A Pacific-wide assessment also suffered from similar challenges (WCPFC-SC14-2018/SA-WP-08), and therefore taking any estimate of abundance from these assessments should be regarded with caution. Alternative methods are desirable to define an approximate abundance for developing sampling designs. A maximum fishing mortality (e.g. based on spawner per recruit from a meta-analysis of similar stocks (e.g. Bravington 2019)) in combination with the catch information could be used to define minimum abundance to use for the sampling design. However, these will be sensitive to the assumptions of natural mortality and they do not explicitly take into consideration the stock-recruitment relationship for the specific stock (Bravington 2019). An estimate for the harvest rate may also be possible from recent archival tagging programs (Schaefer et al. 2021). This estimate could be used along with an estimate of catch to derive a broad estimate of abundance.

4.6. Catch data

Historical catch data are unreliable for several components of the fleet (SAC-05-INF-F). The most complete times series of catch data is available from the purse seine fishery. The newly developed coastal nation sampling program has been designed to provide data for estimation of the catch for artisanal fisheries, including longline and gill net fleets. Only limited catch data for the distant water longline fleets are available to IATTC staff. A preliminary estimate of catch is available from a genetic analysis with shark trade fin data (Clarke et al., 2006). Surprisingly, the estimate derived from the genetic analysis is very consistent with preliminary EPO catch estimate derived by IATTC staff and collaborators (SAC-05-INF-F).

4.7. Assessment

Since 2009, IATTC staff, national observer program staff, scientists of member countries, nongovernmental organizations, and industry collaborators have worked together to accumulate, process, and analyze data for the silky shark (*Carcharhinus falciformis*) in the EPO. This collaborative effort has produced a great deal of fishery data and information on stock structure, biological parameters, and size selectivity of different fisheries catching silky sharks in the EPO, whether as a target or as bycatch. A stock assessment covering the 1993-2010 period was attempted using Stock Synthesis. Unfortunately, the model was unable to fit the main index of abundance adequately, and therefore the results were not reliable since relative trends and absolute scale are compromised in the assessment. The poor performance of the model was probably due to incomplete information on the total catch in the EPO, particularly for the early period of the assessment (1990s and early 2000s). An alternative approach based on indicators has therefore been used to provide management advice for silky sharks (SAC-05-11a).

Even with an estimate of absolute spawner abundance from CKMR or any other method, a typical stock assessment would not be possible due to the lack of reliable time series of catch data for all fisheries. Alternatively, an estimate of fishing mortality based on the absolute estimate of spawning biomass and the recently available estimate of total catch, can be compared with fishing mortality rates from demographic analysis or from spawner per recruit from similar species to establish stock status. However, the close kin analysis estimates absolute abundance of the spawners, while the fishing mortality rate needs to be calculated based on the vulnerable biomass. Therefore, the absolute abundance of the juveniles that are vulnerable to the fishery also needs to be calculated. It may be desirable to conduct a full assessment for the time period that catch is available, even if it is just a single year, so that the

unknown model parameters can be estimated in an integrated approach. This may be possible since the main task of a stock assessment is to estimate absolute abundance, which will be available for the spawners from the CKMR analysis.

Demographic analysis requires estimates of all the population and fishing processes. Essentially, it requires all the parameters used in a standard stock assessment, except for the absolute biomass estimates. These include growth, natural mortality, the stock-recruitment relationship, and selectivity. Growth of individuals representing most of the catch and biomass is the easiest to estimate and should be possible by sampling vertebrae and validating the aging. Adult survival can be estimated from CKMR, but estimates of juvenile survival are needed. Hillary et al. (2018) used telemetry to estimate survival for juvenile white shark when combining CKMR estimates of abundance with demographic analysis. The stock-recruitment relationship might be the most difficult component of the demographic analysis to estimate. Appropriate stock-recruitment models are available for low fecund species like sharks that take the litter size into consideration (e.g. Taylor et al. 2013), but the strength of the density dependence is unknown. Selectivity can be easily calculated from the estimates of absolute abundance at age, which CKMR can provide information on, particularly if integrated with other information (Bravington pers. com.), and the catch composition samples. It may be possible to estimate some of the unknown parameters by fitting the model to a limited series of catch composition data conditioned on catch. Due to the differences in population and fishing processes between males and females, a sex structured model and sex specific parameters would be needed for the CKMR and demographic analysis. This would also require sex composition of the catch, which is currently being collected in all fisheries.

4.8. Sampling considerations

Biological sampling should be conducted throughout the tropical Pacific to ensure that the full spatial range of the silky shark is covered and to minimize any bias that might be caused by non-random spatial distribution of kinship relationships and to evaluate stock structure. The Appendix provides information on the sampling opportunities in the different fisheries. In principle, within the EPO, sampling can be conducted by existing onboard observer programs of the purse-seine and the high-seas longline fisheries, and by the newly-developed port-sampling program for coastal nation shark fisheries (Oliveros-Ramos et al., 2020). This coastal nation sampling program covers the central region of the silky shark distribution in the coastal EPO. However, options for sampling at the northern and southern coastal limits of the tropical EPO are being explored. Collaboration with the WCPFC will be needed to obtain samples through monitoring programs of fisheries operating in the central and western tropical Pacific Ocean. Exemptions for sampling to non-retention policies (e.g. IATTC C-19-05 for purse-seine vessels operating in the EPO) may need to be established so that biological samples can be collected from dead silky sharks before they are discarded or released alive (live releases may require genetic aging if length measurements cannot be safely taken).

There are two main additions to existing sampling programs that will need to be considered to obtain all the biological data required for CKMR. First, the collection of length data by all sampling programs operating in the Pacific Ocean should be reviewed to determine which length measurement are being used by each program, and whether additional data collection will be needed to convert different length metrics to one common metric. At present, total length (TL) is recorded by the IATTC purse-seine observer program in the EPO. The high-seas longline observer data provided to IATTC contain length information as fork length, precaudal length or total length, depending on the Members and Cooperating Non-

Members (CPC). Silky shark catch unloaded by coastal nation fisheries is typically pre-processed, and thus the most widely available length measurements are interdorsal length, although fishers have periodically provided whole animals for special sampling projects (genetic aging and species identification may be beneficial for these samples). Therefore, with cooperation from fishers and CPCs, it is likely that the coastal nation sampling program could be expanded to include collection of length data from unprocessed fish for some fraction of the catch. The other primary addition to existing sampling programs concerns invasive sampling for biological data such as tissue and vertebrae. Currently, the sampling program for the purse-seine fishery does not routinely collect such biological data and protocols would need to be developed that could be integrated into existing catch and effort data collection. Sampling is more difficult on the distant water longline fishery and the costal based fisheries. Silky sharks are not retained and often released before being landed on board, and therefore changes in management practices that allow silky sharks to be landed and sampled may be needed (silky sharks are prohibited from being retained on board in the both the EPO and WCPO). The longline observer coverage is only 5% and it is not clear if enough samples and spatial coverage would be obtained (Figure A.10). Japan also has research and training vessels that might be able to take samples, but their spatial coverage is limited in the EPO and they are also restricted by the silky shark no retention measure in the WCPO. For coastal nation fisheries, some routine biological data collection is underway, but those data do not include vertebrae so additional sampling protocol would need to be developed. Also, consideration should be given to the catch conservation methods in the coastal fisheries; some coastal nation fisheries bring catch to port fresh or iced, as opposed to frozen.

Sampling designs for collection of CKMR data will need to be developed and may differ by fishery due to fishery-specific differences in selectivity. For at least some fisheries, the length range of silky sharks is quite broad. Length measurements from the purse-seine fishery in the EPO range from about 50 cm TL to over 200 cm TL, and length composition varies by set type (SAC-05-INF-F). A similar range in lengths is seen for the purse-seine and high-seas longline fisheries in the WCPO (Clarke et al. 2018). The estimated length composition of the Costa Rican longline fishery ranges from about 60 cm TL to just over 200 cm TL (Pacheco Chaves et al. 2020). Absent information on fishing gear-specific age and sex selectivity, the length composition of the catch could be analyzed to determine whether length (as a proxy for age) varies more within or among fishing “units” (e.g. a set or an unloading), and hence whether sampling should be more intensive within or among fishing units. Contributing to variability in length composition among fishing units may be factors such as gear differences (e.g. purse-seine set type) and fishing location, which may need to be considered in the sampling design. Regardless, it is anticipated that emphasis would be placed on sampling more fishing units and fewer fish per fishing unit to try minimize bias caused by non-independent sampling of kinship relationships. The sampling should be designed to cover all areas and seasons of operation of each fishery; sets that are made in “hotspots” or, in the case of the purse-seine fishery, on fish-aggregating devices (FADs), may be more likely to involve related individuals if the mixing rate of the population is low relative to the persistence of aggregations. Particular consideration should be given to whether sampling in pupping grounds is appropriate, since the mother and offspring may be more likely to be sampled, or the mother caught and not available for sampling at a later date. In addition, the sampling protocol should provide specific guidelines to sampling technicians on sampling frequency in both space and time (e.g. the minimum spatial and temporal separation between samples) because the locations and dates of fishing operations will not be known in advance. These guidelines may need to be updated in near real-time to avoid collection of too many samples for certain areas and time periods, if fleet effort is highly concentrated. A practical field consideration is that access to fish to collect samples

will require cooperation from fishers, as will sample storage for onboard observers, and thus vessel owners/companies should be consulted during the development of sampling protocols.

4.9. Recommendations and considerations

1. Conduct new silky shark age and growth study in the eastern Pacific Ocean and investigate spatial differences in growth relative to the WCPO.
2. Develop sampling platform for vertebral tissue using the IATTC observer program on the purse seine fishery and shark fishery sampling program in Latin American costal states in the EPO.
3. Expand sampling efforts to WCPO in collaboration with WCPFC/SPC.
4. Use IATTC capacity building funds to support universities/research institutes to conduct biological studies for silky shark.
5. Continue to use the sampling program for shark fisheries in Central America to collect information on maturity and fecundity for the silky shark.
6. Fecundity increases with age indicating that both POPs and HSPs will be needed.
7. Improve catch estimates for the high-seas longline fishery.
8. Improved catch estimates for the purse seine fishery.
9. Consider approaches to estimate juvenile abundance and survival.
10. Approaches to estimate the stock-recruitment relationship are needed.

5. BIGEYE TUNA

Bigeye tuna is a valuable species common throughout the tropical Pacific and there is some information about stock structure, but there is still uncertainty (Schaefer, 2009; Schaefer et al., 2015; Moore et al., 2020). Bigeye is caught in the purse seine and longline fisheries of the EPO. It is a moderately productive species and grows to lengths of about 200 cm. Stock assessments of EPO bigeye tuna have been conducted since 2000. The most recent stock assessment cannot differentiate between optimistic results relating to large biomass and pessimistic results relating to low biomass (Xu et al., 2020). Estimates of absolute biomass from CKMR would help to differentiate between these two possibilities. There is also uncertainty in the stock structure, which genetic data used in CKMR could help define, but would require samples from both the EPO and the WCPO. Catch and associated composition data are available from all the fisheries and are used in the stock assessment. Only longline based CPUE indices of relative abundance are used in the assessment. Indices of relative abundance from the purse seine fishery are complicated by vessels making multiple set types and the use of echosounder buoys, and they only index juveniles. Management is based on reference points and a harvest control rule that considers all three tropical tunas.

The following describes the stock characteristics and information available for application of CXKMR to bigeye tuna in the EPO.

5.1. Age of sampled fish

Aging of bigeye tuna is based on daily increment for fish 4 years of age and younger (Schaefer and Fuller, 2006). Aging of older fish is challenging (IATTC 2019a). It is not practical to age all the samples. A growth curve is available based on daily increments and tagging growth increment data, but there is uncertainty for large fish (Aires-da-Silva et al., 2015). The continuous spawning of bigeye will generate additional variation in aging because of the birth timing. However, the uncertainty about aging and birth timing can be integrated into the CKMR analysis. Genetic aging should be considered.

5.2. Maturity and fecundity at age

Maturity at length is available for females, but not for males (Schaefer et al. 2005). Fecundity at length/age is not available and it is assumed proportional to weight in the stock assessment (Xu et al. 2020).

5.3. Approximate abundance

A stock assessment is available (Xu et al., 2020). However, the population size is uncertain with two different orders of magnitude possible. Basing the sampling on the lower biomass may allow the confirmation or rejection of that scenario, but rejection would result in an imprecise estimate of abundance because the abundance is higher.

5.4. Stock structure

Stock structure is uncertain. Collaboration with WCPFC to include the central and WPO is needed. The best information available on stock structure of bigeye in the Pacific Ocean is that provided by Schaefer 2009, Schaefer et al 2015, and Moore et al., 2020. However, there are still uncertainties about stock structure.

5.5. Sex information

Sampling of sex information requires looking at gonads and may not be possible for juveniles. A genetic test may be possible as part of the CKMR study, but this may only be feasible for the samples taken for the CKMR study and not the catch sampling. There is only limited sex composition sampling from the longline fisheries.

5.6. Catch data

Reliable catch data, including size composition, is available from all the fisheries. There is only limited sex composition sampling from the longline fisheries. The purse seine fisheries generally catch juveniles and the longline fisheries catch adults. Few bigeye are caught in the coastal nation fisheries.

5.7. Assessment

An age and sex structured integrated stock assessment is available for bigeye tuna in the EPO (Xu et al., 2020). The CKMR estimates of spawner abundance and adult survival should be integrated into the stock assessment. There is little information on juvenile natural mortality, but it might be estimable from the assessment model, which includes length composition data from several fisheries. Conventional data tagging may be useful in estimating juvenile survival. The stock-recruitment relationship may be a remaining uncertainty in the assessment for which MSE may be needed to define robust harvest control rules. Juvenile abundance would be estimated inside the stock assessment model.

5.8. Sampling considerations

Many of the sampling considerations that were discussed for silky sharks are also relevant for bigeye tuna. The Appendix provides information on the sampling opportunities in the different fisheries. Both parents and offspring need to be sampled for BET because fecundity increases with age. Sampling is possible on the purse seiners by observers. This will cover the juvenile component of the population. Sampling is more difficult on the distant water longline fishery, which catch adult bigeye, and the costal based fisheries. The observer coverage is only 5% on the distant water longline fishery and it is not clear if enough samples and spatial coverage would be obtained. There is potential for sampling frozen fish at port, including age determination using genetic methods, but trials need to be conducted to determine if tissue samples would be of high enough quality to provide the required genetic material. Sampling at port is problematic

because the spatial and temporal information about the catch (e.g. set information) is not known on an individual basis. Some of the catch is landed by carry vessels with catch from several longline vessels further complicating obtaining this information. Therefore, location might be limited to northern EPO, southern EPO, or certain spatial ranges such as 0-20 degree. Japan also has research and training vessels that might be able to take samples, but their spatial coverage is limited in the EPO. Few bigeye are caught in the coastal nation fisheries.

5.9. Recommendations and considerations

1. For high-seas longliners, determine the feasibility and tissue quality of
   a) observers taking tissue samples onboard
   b) sampling at port
   c) sampling at the markets
2. Evaluate the need for date and location information from high-seas longline caught bigeye sampled at port or in the market.
3. Expand sampling efforts to WCPO in collaboration with WCPFC/SPC.
4. Consider approaches to estimate juvenile abundance and survival
5. The stock—recruitment relationship is uncertain.
6. Sex-specific composition data is needed.

6. DISCUSSION

CKMR should be feasible for both silky shark and bigeye tuna in the EPO, as well as other commercially important species or species of conservation concern for IATTC. Estimates of spawner abundance and adult survival from CKMR will greatly improve stock status estimates. However, each application will have its particular uncertainties and data requirements. CKMR requires sampling the whole stock and, for most EPO stocks, this will require sampling outside the EPO to ensure adequate coverage as well as to clarify stock boundaries. CKMR does not estimate juvenile abundance or survival, and these will have to be obtained from other data (e.g. conventional mark recapture or telemetry data), estimated with the stock assessment, or assumptions made (e.g. taken from stocks of the same or similar species in other oceans). The stock-recruitment relationship is likely to remain a major uncertainty and may require defining harvest control rules, through MSE, that are robust to the uncertainty.

A good CKMR design amounts to working out what precision is likely to be achieved for various quantities of potential interest (e.g. absolute adult biomass) with alternative specifications of the design parameters including total sample size, duration of study, range of sizes sampled, and precision of age estimates. The whole "design process" leads to choosing a single design that is likely to answer management questions at lowest cost and satisfying logistic constraints (Bravington 2019). Total sample size has the greatest effect on precision (e.g. Bravington 2019). Bravington (2019) suggests initially focusing sampling of older individuals and then focusing on young individuals in later years. An initial program to evaluate the practicality of sampling and to test the tissue sample quality should be strongly considered before starting any full sampling program.

Adequate sampling of both the adult and juvenile components of the stock across its entire range over several years is desirable for CKMR. In general, this requires sampling from several different fisheries. The IATTC has a comprehensive observer program on large purse seiners that can be used to sample tissue for genetic analysis. However, tissue samples are not part of the current sampling program and previous sampling projects (e.g. those for gonad and stomach contents) should be evaluated to improve sampling.

Sampling is more difficult on the distant water long line fishery and the costal based fisheries. Observers should be able to take samples of bigeye tuna. However, silky sharks are not retained and often released before being landed on board, and therefore changes in management practices that allow silky sharks to be landed and sampled may be needed (silky sharks are prohibited from retaining on board in both the EPO and WCPO). The longline observer coverage is only 5% and it is not clear if enough samples and spatial coverage would be obtained (Figure A10). There is potential for sampling frozen fish at port, but trials need to be conducted to determine if tissue samples would be of high enough quality. Sampling at port is problematic because the spatial and temporal information about the catch is not known for each individual fish (i.e. set locations and times are only known for the trip and not for each individual fish). Japan also has research and training vessels that might be able to take samples, but their spatial coverage is limited in the EPO and they are also restricted by the silky shark no retention measure in the WCPO. A sampling program for the EPO coastal based fisheries has been initiated and could be used to sample tissue. The sampling will need to be coordinated with the WCPFC to obtain samples from the whole Pacific. Sampling could be started in advance of the genetic analysis since sample collection and archiving is relatively inexpensive and there are options if tissue quality is not perfect such as using microsatellites to identify POPs and/or developing RAD-Capture panels or GT-Seq panels if half-sib relationships are required (John Swenson pers. com.). Although, the study design needs to be considered and tissue quality needs to be tested.

It should be noted that it may be more useful to evaluate the precision on quantities used for management advice (e.g. Fcur/Fmsy) rather than the outputs of CKMR (e.g. adult abundance) (Bravington 2019). This requires including the full assessment process in the study design.

Spatial distribution of samples is still a concern with CKMR as it is with traditional tagging studies. It is possible that siblings born a few years apart will be found in the same area and therefore a given sample size in a local area may find more HSPs than a more spatially distributed sample (Bravington 2019). However, with reasonable sample sizes such patterns should be detectable. This is likely a bigger problem with species that have low larval/juvenile dispersal rates or natal homing compared to highly fecund pelagic spawners like tuna.

The type of genotyping kinship finding algorithms used is important to ensure success of the CKMR study (Bravington 2019). They need to distinguish HSPs from other less related kinships. It is also important that the samples are analyzed consistently, which is best achieved if they are all done by the same lab. This is an area of expertise outside the knowledge of the IATTC staff.

Stock assessments require estimates of juvenile abundance, juvenile survival, and the stock-recruitment relationship. These quantities are not estimated from CKMR and need to be estimated from other data or assumptions made. Conventional or genetic tagging might be possible in some cases to estimate juvenile survival and abundance. They may also be estimable inside the stock assessment model with typical data used in assessments (indices of relative abundance and composition data). Robust harvest strategies evaluated using MSE may be needed to deal with the uncertainty about the stock-recruitment relationship.

One of the main considerations in conducting a CKMR study are the costs and how they compare to alternatives (e.g. conventional mark-recapture). Several rough estimates of cost have been estimated for other stocks. Bravington et al. (2016) give a rough sample size calculating for a single sampling event of

$10\sqrt{N}$ with an equal mix of juveniles and adults to give a CV of 15% in POP studies. Rodriguez-Ezpeleta et al. (2020) suggest that to achieve a CV of less than 20%, about 50 HSPs are needed, although 100 should be considered (Bravington 2019). They give an example where a sample of 5,000 individuals could provide this level of precision for populations of 0.5 and 0.95 million for total mortalities of Z = 0.25 and Z = 0.1, respectively. The cost of analyzing the genetic samples is approximately US$30 (Bravington 2019), with a total cost of US$150,000. Kolody and Bravington (2019) give lower costs of US$17.5 for an example using Indian Ocean YFT with a sample size of 64,000 (US$1,120,000). Bravington (2019) puts the genetic analysis at AU$20 or less and budget a total cost of $30-35 to include everything from sampling to genetic analysis. The costs may be double if both 50 POPs and 50 HSPs are required for estimation (e.g. separating adult survival from reproductive success). Bravington et al. (2020) found from initial exploration that 20-25,000 South Pacific albacore spread over 3-4 years might need to be sampled. Other costs include sampling, DNA extraction, transportation, storage, close kin analysis, and population dynamics modelling. There are a variety of options for the genetic analysis ranging from outsourcing the whole project to doing all the prep work in house and outsourcing just the sequencing, and the option chosen will determine the costs. If RAD-Capture or GT-Seq panels are developed and the labwork is completed in-house by a post-doc or PhD student rather than sending to a sequencing center, these costs can be substantially reduced (see Meek and Larson, 2019). Obviously, population specific calculations should be done to design the sampling strategy. The per unit costs of genetic analysis is probably dependent on the number of samples analyzed and costs may be reduced as the lab becomes more experienced with the process. However, based on these rough estimates, CKMR studies are likely to run in the mid to high hundreds of thousands of dollars for silky sharks and the low millions of dollars for bigeye tuna, which is the order of magnitude for traditional mark-recapture studies. In cases where the population size is uncertain and required sample sizes are unknown, it may be possible that collecting and storing samples is relatively cheap compared to the genetic analysis, so more samples can be collected than needed and then only enough samples analyzed to give the required precision.

Several components of the study can be carried out while the sampling is in progress. For example, if a few good samples are collected, the RAD-Seq libraries could be created and sequenced to identify genetic markers for kinship analysis. The lab-work would take about a month for a small sample set (96 or less) for a person who is familiar with the protocol, has all the necessary reagents, and is able to dedicate most of their time to the process. The analysis (e.g. identifying appropriate markers) will take longer. The quality control routines and the algorithm for kinship finding can be created and RAD-Capture or GT-Seq panels can be developed to reduce costs of sequencing the full dataset and likely permit the use of lower quality samples when the full dataset is analyzed (Ali et. al. 2016; Campbel et. al. 2015).

For species that are listed there may be requirements for permits for collecting and transporting samples among countries. Inquiries into obtaining these permits should be initiated at the start of the project because the permitting process may take a long time. Alternatively, it may be possible to find laboratories for analysis in countries where the samples are collected.

The priority would be to commission a comprehensive design study to identify i) the specific assessment questions to be addressed, and ii) the best sampling programme(s) to achieve these outcomes (i.e. in terms of age composition, years sampled, and spatial distribution). We would expect such a design study to cost around USD 50-100K based on other estimates (e.g. Kolody and Bravington, 2019; Bravington et al., 2020). One approach would be to follow that outlined for South Pacific albacore (Bravington et al., 2020).

1. Convene a workshop of relevant experts to examine the feasibility and costs of applying the close-kin mark-recapture estimation of the population size to species caught within the EPO.
2. Identify the scientific issues that conducting such a study would help address.
3. Identify those species in the EPO for which it may be appropriate to conduct a close-kin mark-recapture study.
4. Outline the elements of a small project, identifying possible project investigators and associated costs, aimed at conducting a feasibility study in the EPO.

The detailed feasibility study for each species should include (based on information provided for South Pacific albacore by Bravington et al., 2020):

1. An evaluation of the fisheries and locations where useful quantities of samples can be collected, noting that samples must include approximate capture location information, and some information on fish age. This should include:
    a) Ensuring enough spatial coverage to detect spatial heterogeneity in population structuring.
    b) The "population" representativeness of fish unloaded at ports, and the practicality of sampling at these locations.
    c) Evaluation of tissue sample quality and recommendations for testing tissue sample quality
2. Determine the needs for aging and develop a sampling program if needed.
3. Realistic consideration of achievable precision in a stock assessment context of key management parameters, such as BMSY, besides abundance and natural mortality per se;
4. Develop the necessary collaborative and stakeholder consultation arrangements to move to full-scale implementation.
5. Develop a panel of genetic markers that can be used for determining kinship and sex, incorporating any likely markers of population structure. (also consider age and species identification)
6. A costs and benefits comparison of adopting CKMR as a fishery monitoring tool

CKMR and the associated genetic and statistical analyses are outside the expertise of the current IATTC staff. Therefore, collaboration or contracts with outside experts will be require to conduct the project. The design study and field work could be conducted in 2022 and the sampling for CKMR could start in 2023. Results would not be expected until the 2026 SAC at the earliest. Several recommendations and considerations were identified for each species and are listed below.

7. ACKNOWLEDGMENTS

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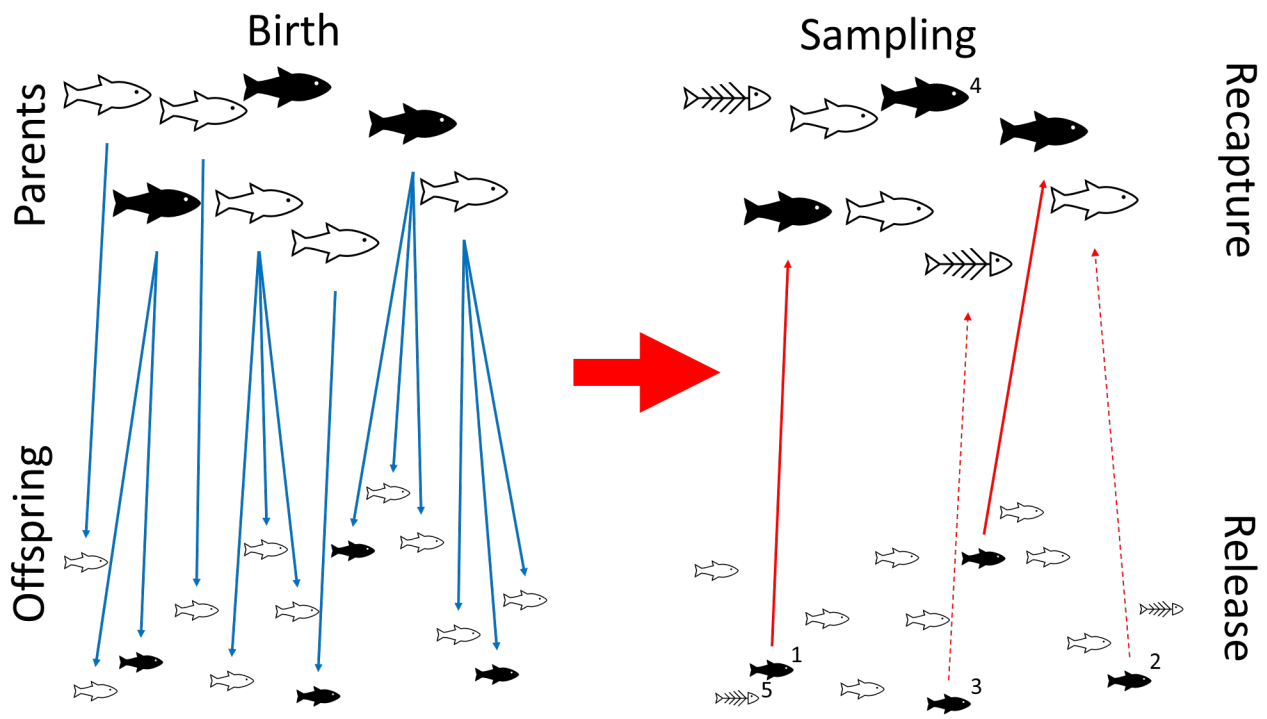

Figure 1. Example of parent offspring pairs to show how sampling of offspring "tags" parents at the time of birth. The diagram is simplified by showing only mothers. The solid fish indicate fish that were sampled. The blue lines indicate parent offspring relationships. The solid redlines indicate tagging and recapture. The dashed red lines indicate tagging without recaptured. The parents and offspring can die between the time of birth and the time of sampling (indicated by skeleton fish). Offspring and parents that die between the time of birth and the time of sampling cannot be sampled. 1) The offspring was sampled (tagged) and the parent was sampled (recaptured); 2) The offspring was sampled (tagged) and the parent was alive at sampling, but not sampled (recaptured); 3) The offspring was sampled (tagged), but the parent was dead at sampling and therefore could not be sampled (recaptured); 4) The parent was sampled, but its offspring was not sampled, and therefore the parent was not a tagged fish; 5) The offspring was dead so it could not be sampled. (note that with large populations the probability of sampling two offspring from the same parent is rare.) In this example, R=4 fish are tagged, n=3 fish are sampled for tags (the sampled parents), and r=2 fish had tags and were recaptured (the sampled parents that had offspring sampled). So, a simple Petersen estimator would estimate N = Rn/r = 4*3/2 = 6 parents (mothers) at the time of birth of the offspring.

Figura 1. Ejemplo de pares de progenitores y crías para mostrar cómo el muestreo de crías "marca" a los progenitores en el momento del nacimiento. El diagrama está simplificado y solo muestra a las madres. Los peces de color sólido indican los peces que fueron muestreados. Las líneas azules indican las relaciones entre progenitores y crías. Las líneas rojas continuas indican el marcado y la recaptura. Las líneas rojas punteadas indican el marcado sin recaptura. Los progenitores y las crías pueden morir entre el momento del nacimiento y el del muestreo (indicado por los esqueletos). Las crías y los progenitores que mueren entre el momento del nacimiento y el del muestreo no pueden ser muestreados. 1) La cría fue muestreada (marcada) y el progenitor fue muestreado (recapturado); 2) La cría fue muestreada (marcada) y el progenitor estaba vivo en el momento del muestreo, pero no fue muestreado (recapturado); 3) La cría

fue muestreada (marcada), pero el progenitor estaba muerto en el momento del muestreo y, por lo tanto, no pudo ser muestreado (recapturado); 4) El progenitor fue muestreado, pero su cría no fue muestreada y, por lo tanto, el progenitor no fue un pez marcado; 5) La cría estaba muerta, por lo que no pudo ser muestreada. (Nótese que con poblaciones grandes la probabilidad de muestrear dos crías del mismo progenitor es poco frecuente). En este ejemplo, R=4 peces son marcados, n=3 peces son muestreados para marcas (los progenitores muestreados), y r=2 peces que tenían marcas y fueron recapturados (los progenitores muestreados que tenían crías muestreadas). Así, un estimador de Petersen simple estimaría N = Rn/r = 4*3/2 = 6 progenitores (madres) en el momento del nacimiento de la cría.

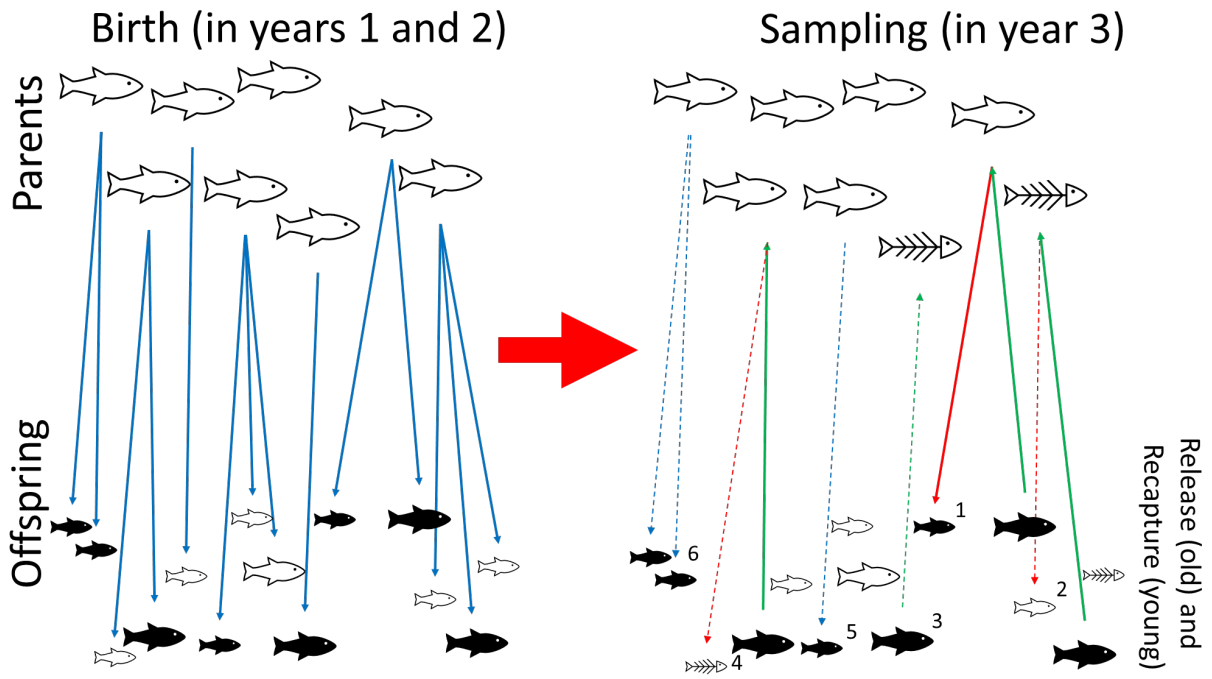

Figure 2. Example of half sibling pairs to show how sampling of the older offspring "tags" the parent and sampling the younger offspring "recaptures" the parent through the parental relationship. However, it may be easier to conceptualize the process if viewed through the sampling of the older offspring as tagging the young offspring. The diagram is simplified by showing only mothers. Large fish represent parents. The medium sized fish (older offspring) represent fish born in the first year and are one year older than the small fish (younger offspring) that were born in the second year. The sampling is conducted in the third year when the small fish are one year old and the medium fish are two years old. The solid fish indicate individuals that were sampled. The blue lines indicate parent offspring relationships. The solid redlines indicate recapture of the tagged young offspring. The solid green lines represent tagging of the young offspring through sampling the older offspring. The dashed green lines indicate tagging without recapture. The red dashed lines represent a possible recapture that did not occur. The dashed blue lines represent parent offspring relationships where the younger offspring was sampled, but the older offspring was not, so the young offspring was not tagged (i.e. sampling of an untagged individual). The parents and offspring can die between the time of birth and the time of sampling (indicated by skeleton fish). Offspring that die between the time of birth and the time of sampling cannot be sampled. In this case, if an old offspring is sampled, then the parent was alive at the time of birth of that offspring (year 1) and was tagged. If the parent survived to year two and had a young offspring and that offspring survived to year 3, it could have been sampled (recaptured). 1) The older offspring was sampled tagging the younger offspring which was sampled (recaptured); 2) The older offspring was sampled tagging the younger offspring which was not sampled and was therefore not recaptured. In this case the parent was not alive at the time of sampling, but obviously was at the time of birth of the two offspring; 3) The older offspring was sampled tagging any possible younger offspring, but the parent died before the second year so could not have any young offspring; 4) The older offspring was sampled tagging the younger offspring, but the younger offspring died before sampling and therefore could not be recaptured; 5) The younger offspring was sampled, but because the older offspring was not sampled, it was not tagged; 6) Two young offspring were sampled

(these may be full siblings if they share the same father, or half siblings from the same cohort), but no old offspring were sampled, so they were not tagged. Siblings from the same cohort are not used in the analysis. Other outcomes are possible but have not been shown. In this example, R=4 fish are tagged (the sampled old offspring), n=4 fish are sampled for tags (the sampled young offspring), and r=1 fish had tags (the sampled young offspring that had an old half sibling that was sampled). So, a simple Petersen estimator would estimate N = Rn/r = 4*4/1 = 16 parents (mothers). (note that this is a simple example and other processes such as survival of the parent or offspring were not taken into consideration and the actual implementation is through pseudo likelihoods based on probabilities of kinship [Bravington et al. 2016])

Figura 2. Ejemplo de pares de medios hermanos para mostrar cómo el muestreo de la cría mayor "marca" al progenitor y el muestreo de la cría menor " recaptura" al progenitor a través de la relación parental. Sin embargo, puede ser más fácil conceptualizar el proceso si se ve a través del muestreo de la cría mayor como el marcado de la cría menor. El diagrama está simplificado y solo muestra a las madres. Los peces grandes representan a los progenitores. Los peces medianos (crías mayores) representan a los peces nacidos en el primer año y son un año mayores que los peces pequeños (crías menores) que nacieron en el segundo año. El muestreo se realiza en el tercer año, cuando los peces pequeños tienen un año y los medianos dos. Los peces de color sólido indican los individuos que fueron muestreados. Las líneas azules indican las relaciones entre progenitores y crías. Las líneas rojas continuas indican la recaptura de las crías marcadas. Las líneas verdes continuas representan el marcado de las crías menores mediante el muestreo de las crías mayores. Las líneas verdes punteadas indican el marcado sin recaptura. Las líneas rojas punteadas representan una posible recaptura que no se produjo. Las líneas azules punteadas representan relaciones entre progenitores y crías en las que se muestreó a la cría menor, pero no a la mayor, por lo que no se marcó a la cría menor (es decir, un muestreo de un individuo no marcado). Los progenitores y las crías pueden morir entre el momento del nacimiento y el del muestreo (indicado por los esqueletos). Las crías que mueren entre el momento del nacimiento y el del muestreo no pueden ser muestreadas. En este caso, si se muestrea una cría mayor, el progenitor estaba vivo en el momento del nacimiento de esa cría (año 1) y fue marcado. Si el progenitor sobrevivió hasta el segundo año y tuvo una cría y ésta sobrevivió hasta el tercer año, podría haber sido muestreado (recapturado). 1) La cría mayor fue muestreada marcando a la cría menor que fue muestreada (recapturada); 2) La cría mayor fue muestreada marcando a la cría menor que no fue muestreada y por lo tanto no fue recapturada. En este caso, el progenitor no estaba vivo en el momento del muestreo, pero obviamente sí lo estaba en el momento del nacimiento de las dos crías; 3) La cría mayor fue muestreada marcando a cualquier posible cría menor, pero el progenitor murió antes del segundo año, por lo que no pudo tener ninguna cría menor; 4) La cría mayor fue muestreada marcando a la cría menor, pero la cría menor murió antes del muestreo y, por tanto, no pudo ser recapturada; 5) La cría menor fue muestreada, pero como la cría mayor no fue muestreada, no fue marcada; 6) Dos crías menores fueron muestreadas (pueden ser hermanos completos si comparten el mismo progenitor, o medios hermanos de la misma cohorte), pero ninguna cría mayor fue muestreada, por lo que no fueron marcadas. Los hermanos de la misma cohorte no se utilizan en el análisis. Otros resultados son posibles pero no se muestran. En este ejemplo, R=4 peces están marcados (las crías mayores muestreadas), n=4 peces están muestreados para marcas (las crías menores muestreadas), y r=1 peces tenían marcas (las crías menores muestreadas que tenían un medio hermano mayor muestreado). Así, un estimador de Petersen simple estimaría N = Rn/r = 4*4/1 = 16 progenitores (madres). (Nótese que se trata de un ejemplo sencillo y que no se han tenido en cuenta otros procesos

como la supervivencia del progenitor o de la cría, y que la implementación real se realiza mediante pseudoverosimilitudes basadas en las probabilidades de parentesco [Bravington et al. 2016]).

APPENDIX: length composition and spatial distribution of catch for the different fleets.

Silky shark

Male and female silky shark mature at about 150-220 cm (S. Soriano and L. Castillo, Mexico).

Purse seine

Silky sharks are caught throughout the purse seine fishery on floating objects in the EPO, but less frequently in the unassociated and dolphin associated purse seine fisheries (Figure A.1). The floating object fishery mostly captures silky sharks of sizes that are immature, but in some areas (e.g. the north costal area and south of the equator) mature sized silky sharks are caught (Figure A.2). The Unassociated (Figure A.3) and the dolphin associated (Figure A.4) purse seine fisheries take a mixture of silky sharks that are sizes of immature and mature individuals. Similar fishery and spatial patterns are seen in the western Pacific Ocean (Clark et al. 2018), but the individuals caught in the floating object fishery in the central Pacific Ocean are mainly sizes of juveniles (Figure A.5).

High seas longline

The spatial distribution of the longline effort in the western and central Pacific Ocean covers the majority of the region (Figure A1 in Clark et al. 2018), but the distribution in the EPO is limited in the equatorial and costal regions (Figure A.8). However, the spatial distribution of the longline observer data in the EPO (Figure A.10) and in the western and central Pacific Ocean (Figure A1 in Clark et al. 2018) does not well represent the distribution of the total effort. Longline vessels catch mainly silky shark of juvenile size (Figure 11 Clark et al. 2018), but also catch silky shark of mature size south of the equator in the western Pacific Ocean (Figure A5 in Clark et al. 2018).

Coastal longline

The coastal longliners of Central and South America do not go west of 95W (Figure A.11) and are general restricted by their base nation (Martínez-Ortiz et al., 2015; Pacheco Chaves et al., 2020). The vessels off Central America catch mainly silky shark of juvenile size, but do catch some of mature size (Pacheco Chaves et al., 2020).

Coastal gillnet

There is uncertainty about the information that is available for this fishery and further investigation of sources is required.

Bigeye Tuna

Female bigeye mature between around 120-150cm, but some mature at as small as just over 100cm (Schaefer et al 2005). Maturity at length for males is not known.

Purse seine

Bigeye are mainly caught in the purse seine fishery of floating objects (Figure A.6) and these are typically of a juvenile size in both the EPO (Figure A.7) and WCPO.

High seas longline

The spatial distribution of the longline effort in the western and central Pacific Ocean covers the majority of the region, but the distribution in the EPO is limited in the equatorial and costal regions (Figure A.8). However, the spatial distribution of the longline observer data in the EPO (Figure A.10) and in the western and central Pacific Ocean (Figure A1 in Clark et al. 2018) does not well represent the distribution of the total effort. Longline vessels catch mainly bigeye tuna of mature size but does catch some of juvenile size (Figure A.9).

Coastal longline

Few bigeye are caught by the coastal based longline vessels. The coastal longliners of Central and South America do not go west of 95W (Figure A.11) and are general restricted by their base nation (Martínez-Ortiz et al., 2015; Pacheco Chaves et al., 2020).

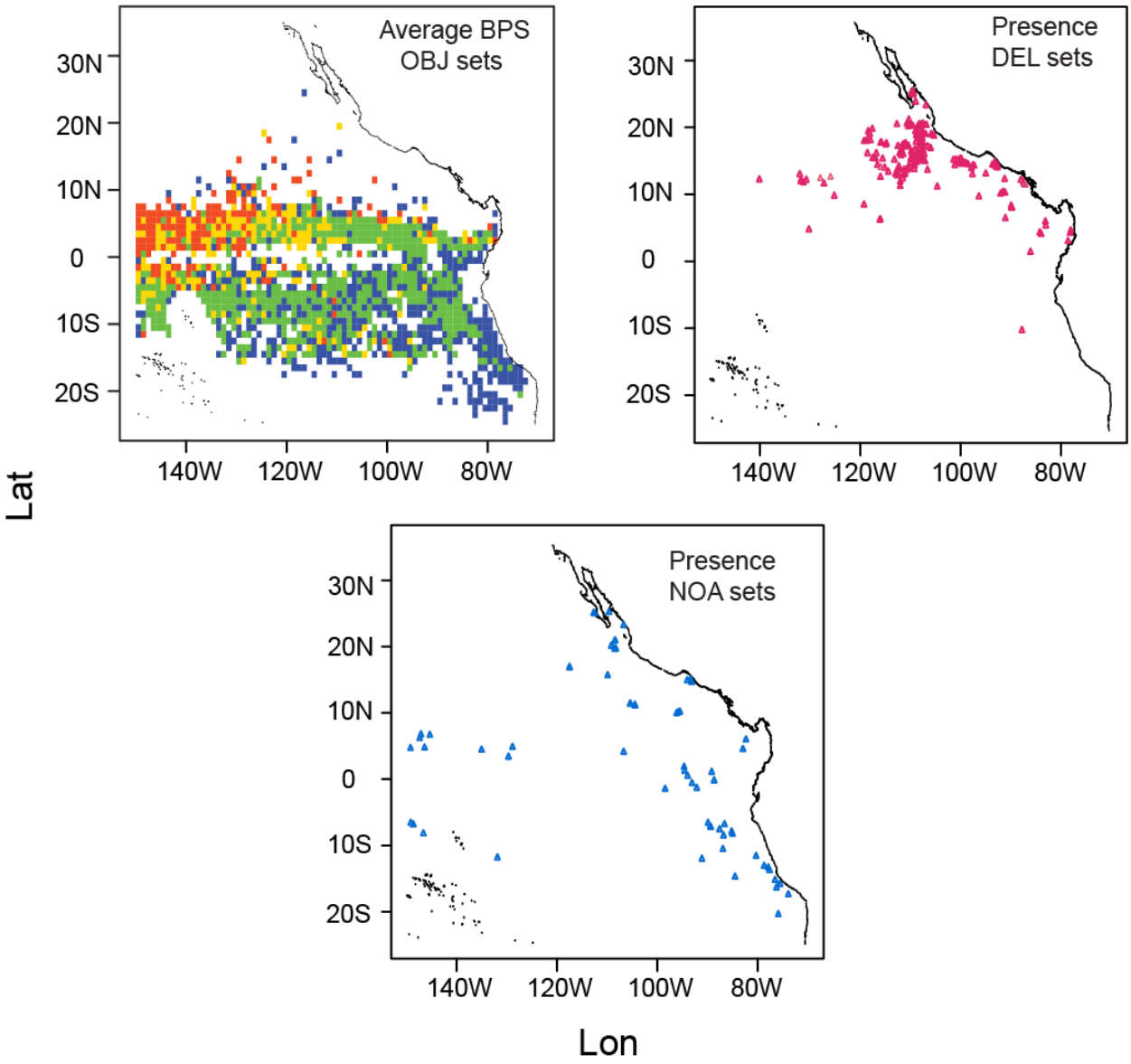

Figure A.1. Figure 3 from BYC-10 INF-A. Spatial distribution of silky shark bycatch in purse-seine sets, by set type, for 2019. For OBJ sets, the average silky shark bycatch-per-set (BPS; in numbers of sharks per set) is shown; blue: 0 sharks per set, green: ⩽ 2 sharks per set, yellow: 2-5 sharks per set, red: > 5 sharks per set. For DEL and NOA sets, the location of sets with silky shark bycatch are shown.

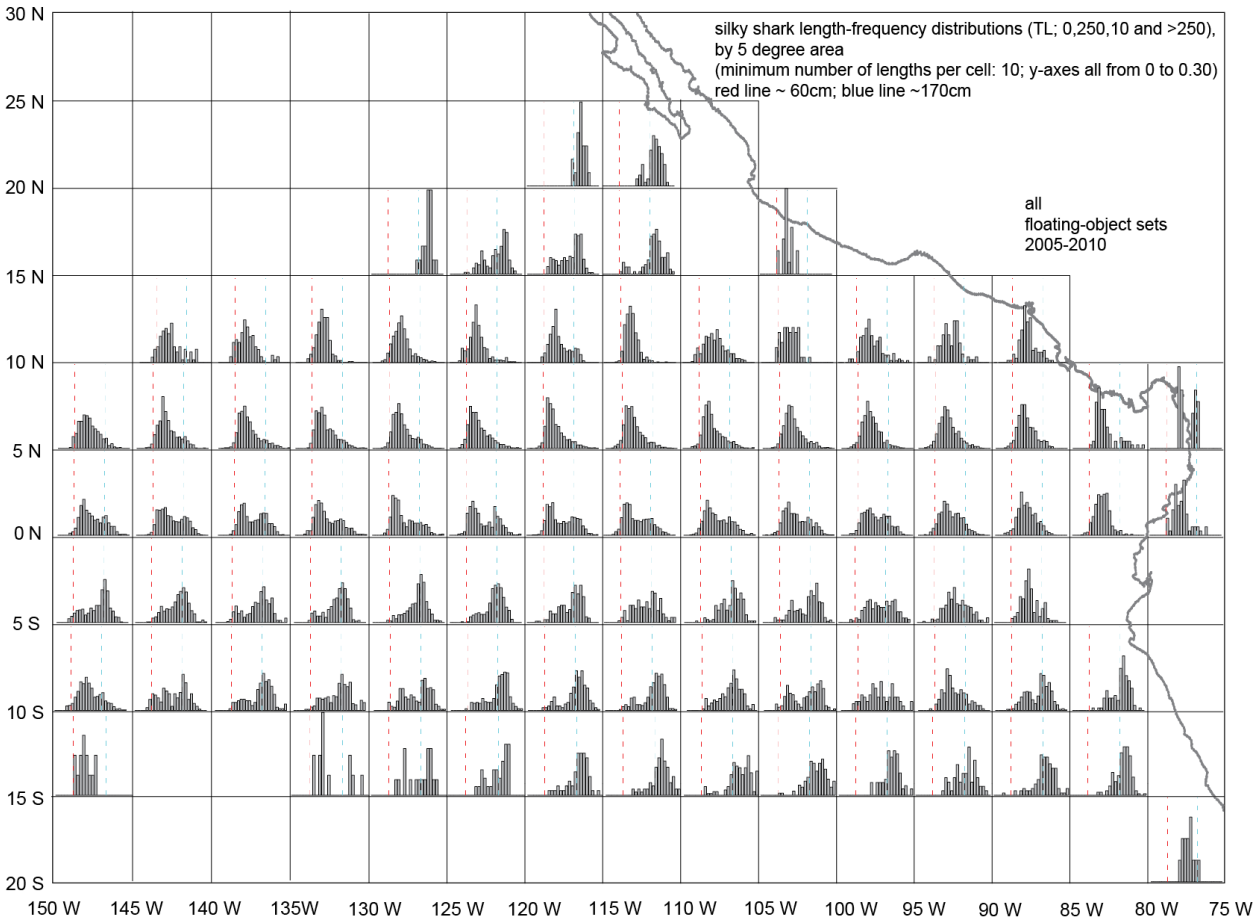

Figure A.2. Spatial distribution of length composition samples from purse-seine observer data for OBJ sets, 2005-2010.

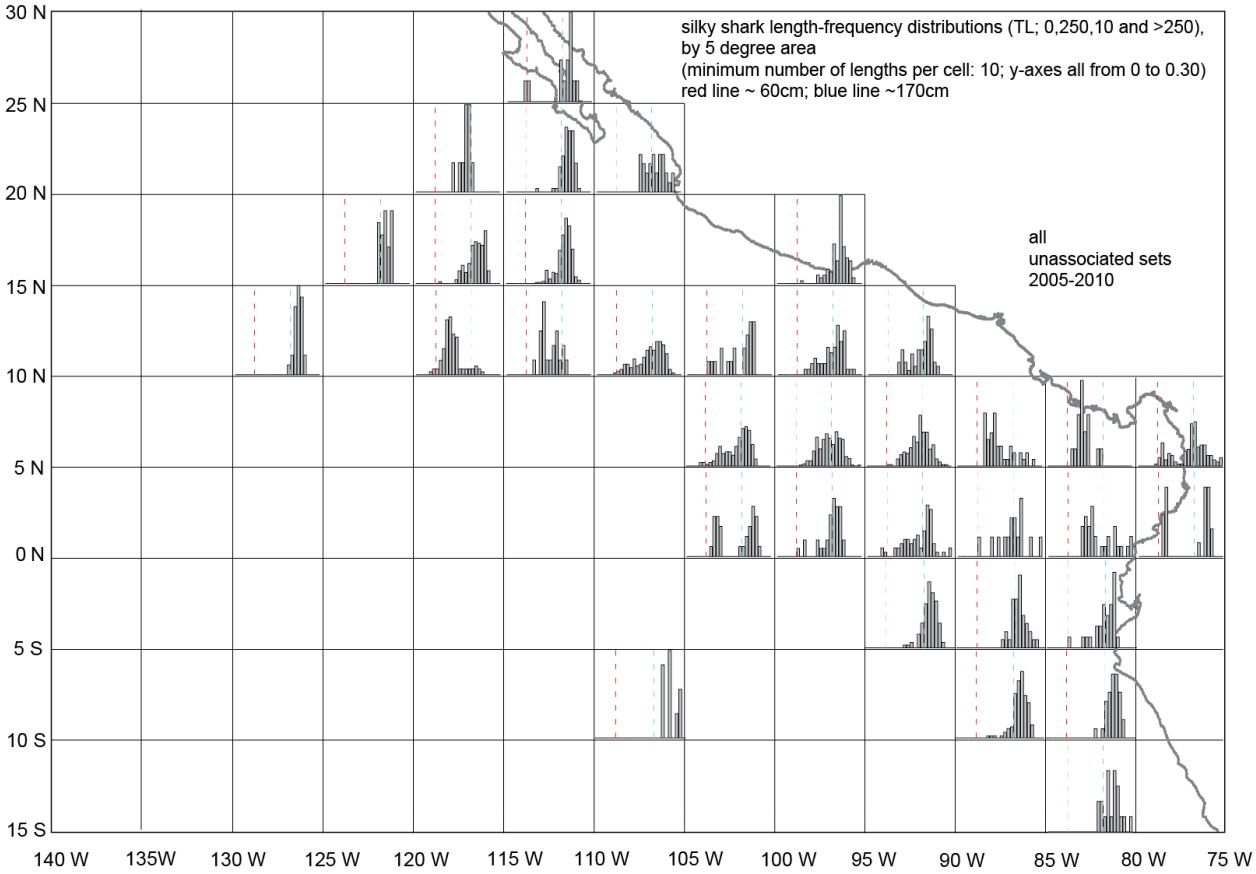

Figure A.3. Spatial distribution of length composition samples from purse-seine observer data for NOA sets, 2005-2010.

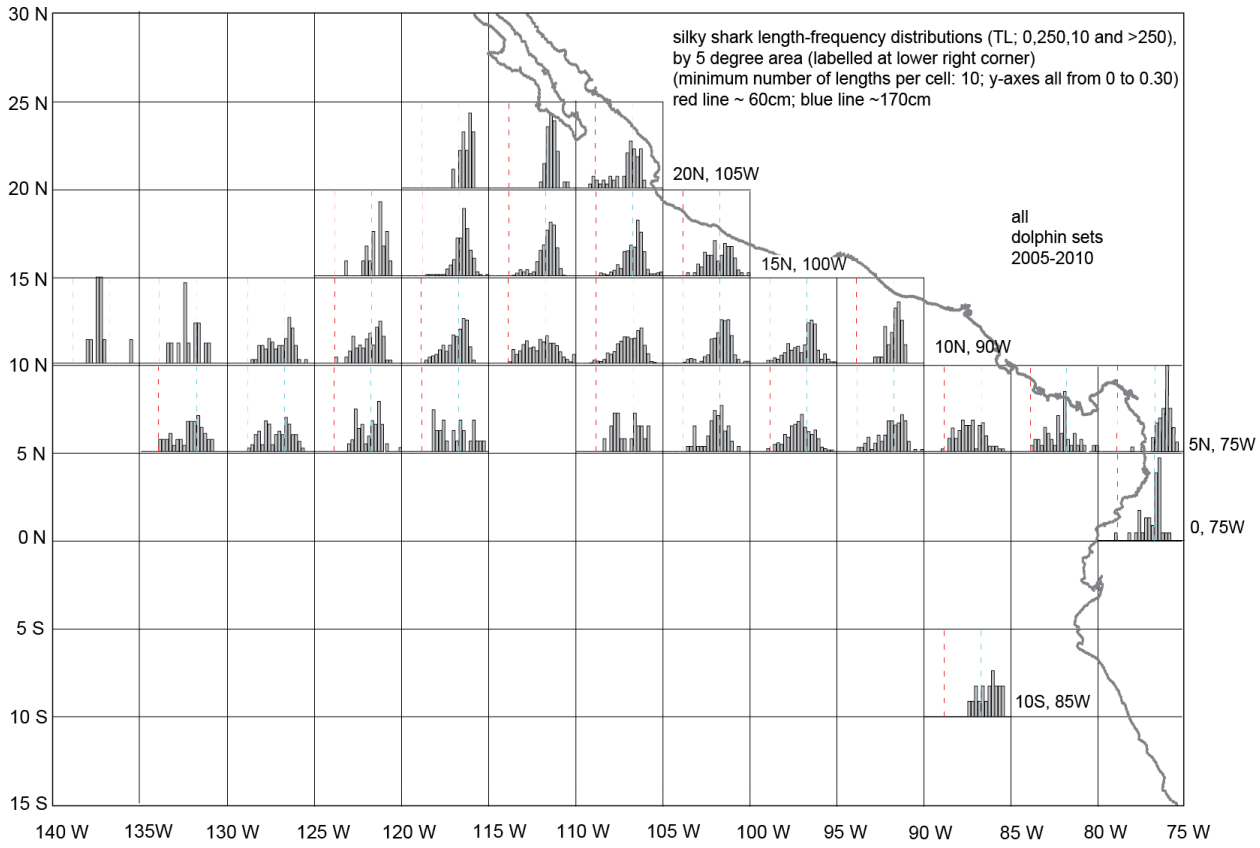

Figure A.4. Spatial distribution of length composition samples from purse-seine observer data for DEL sets, 2005-2010.

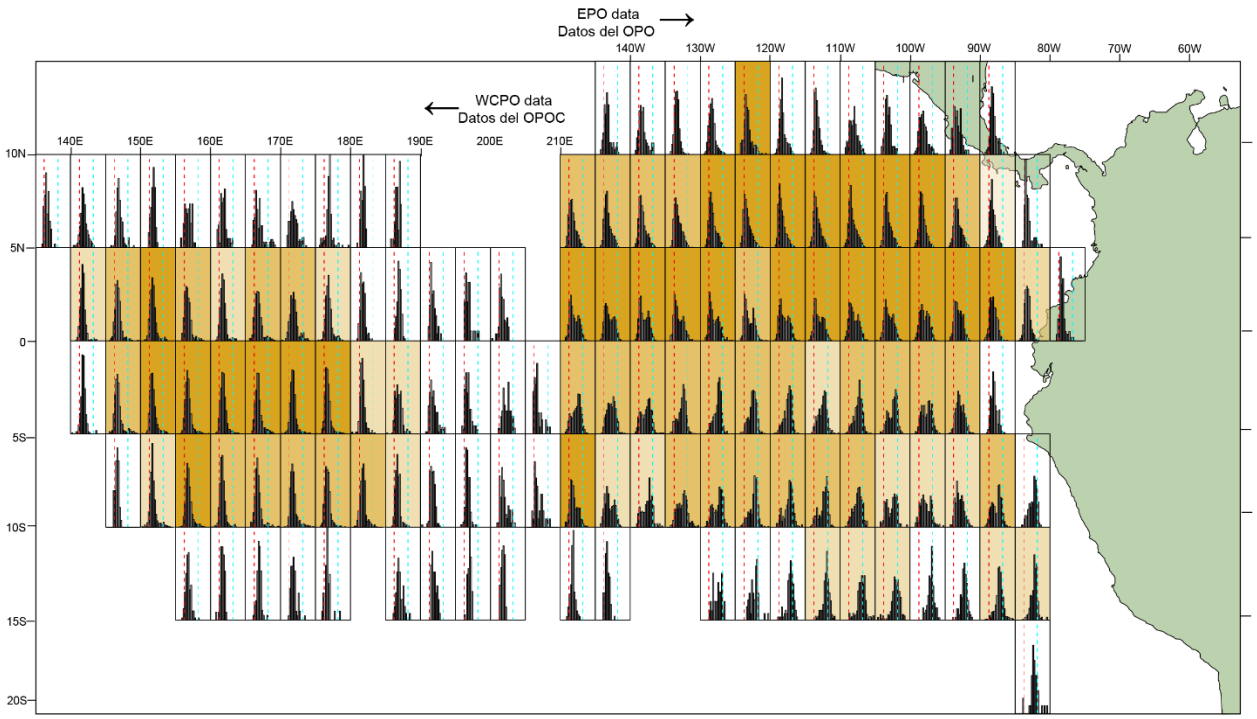

Figure A.5. Figure 7 from SAC-08-08a(i). Length frequency of silky sharks in the purse seine sets on floating objects.

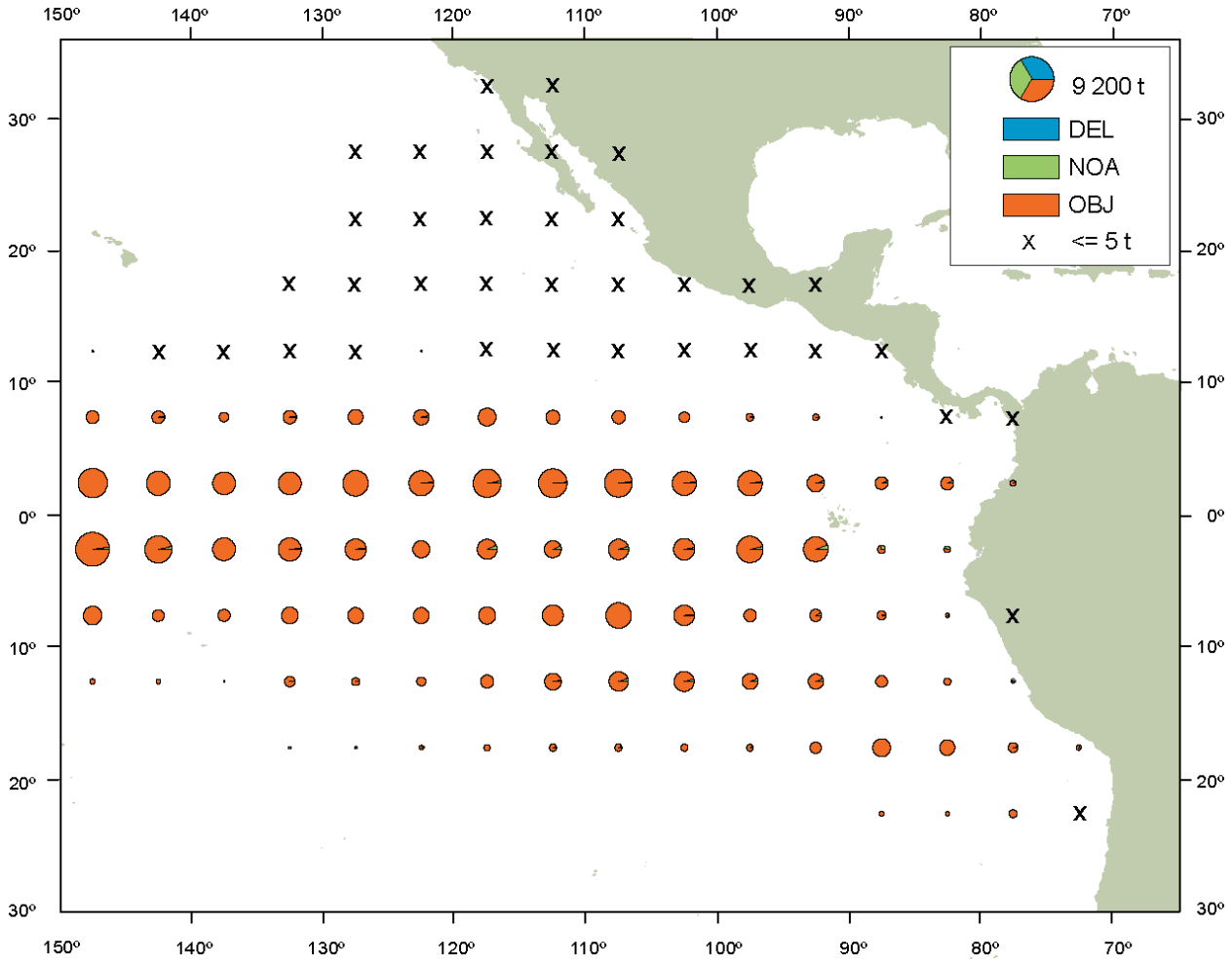

Figure A.6. Figure A-3a from IATTC 2019b. Spatial distribution of purse seine catch of bigeye tuna in the EPO. From FSR figure A-3a

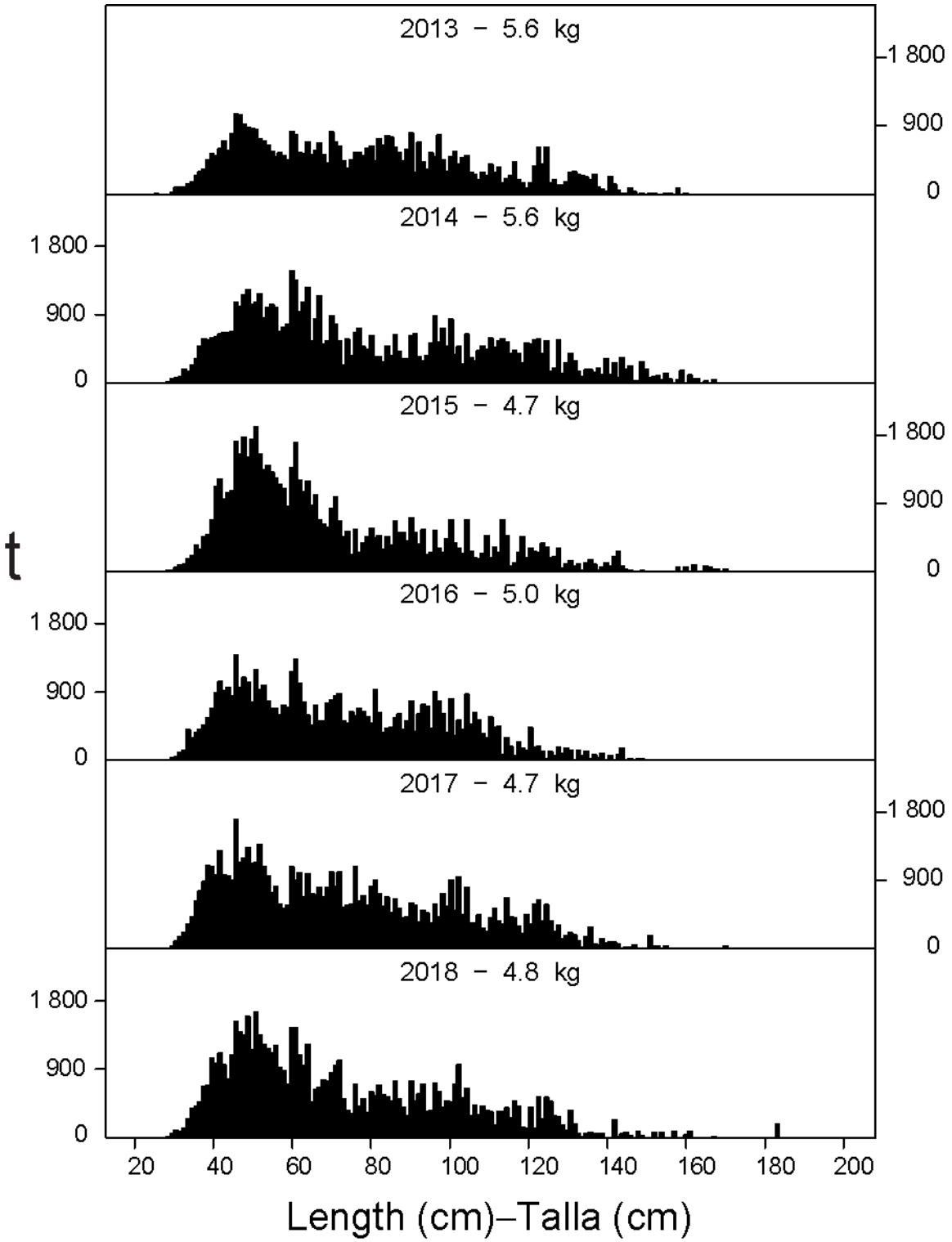

Figure A.7. Figure A-8b from IATTC 2019b. Bigeye length composition of the purse seine fishery. From FSR figure A-8b.

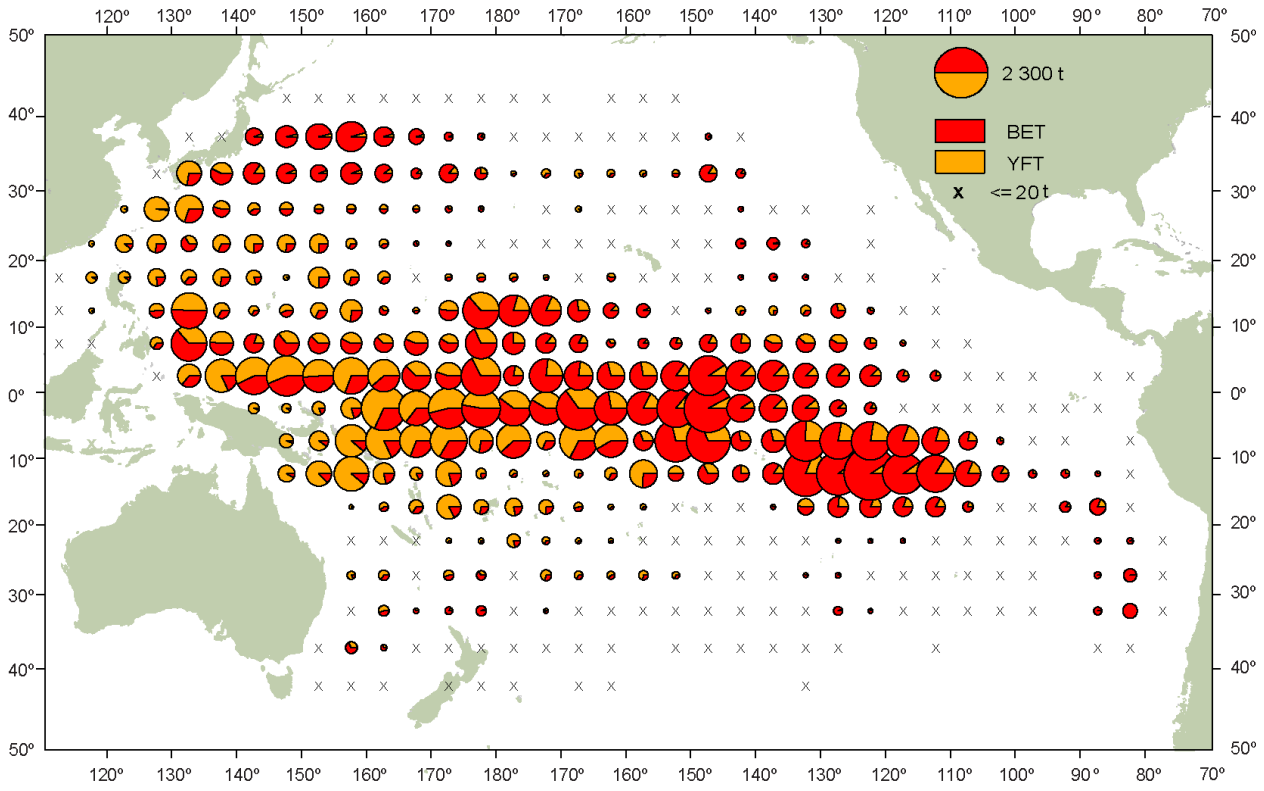

Figure A.8. Figure A-4 from IATTC 2019b. Spatial distribution of bigeye (and yellowfin) high seas longline catch in the Pacific Ocean. From FSR figure A-4

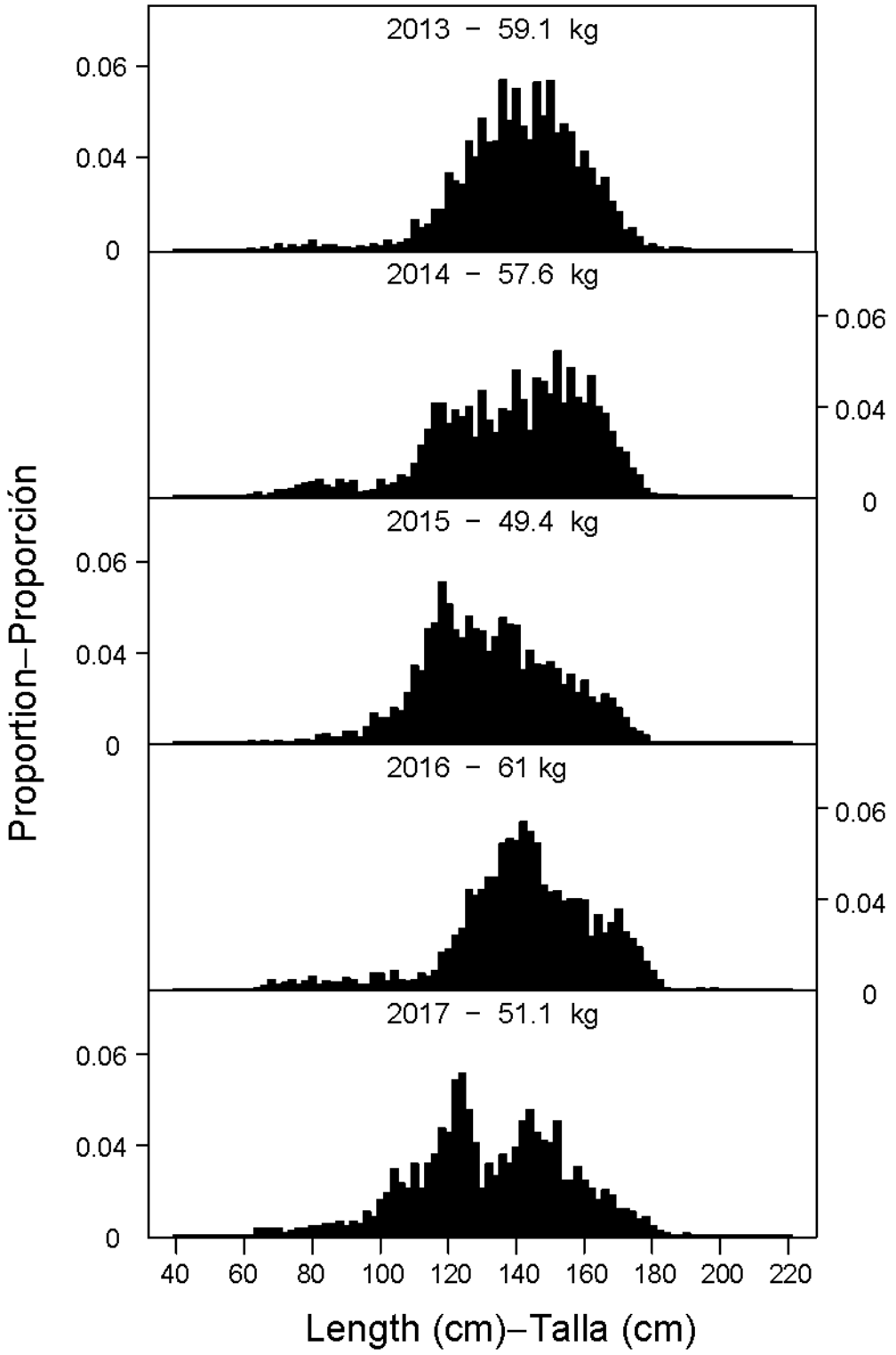

Figure A.9. Figure A-11 from IATTC 2019b. Bigeye length composition of the longline fishery. From FSR figure A-11.

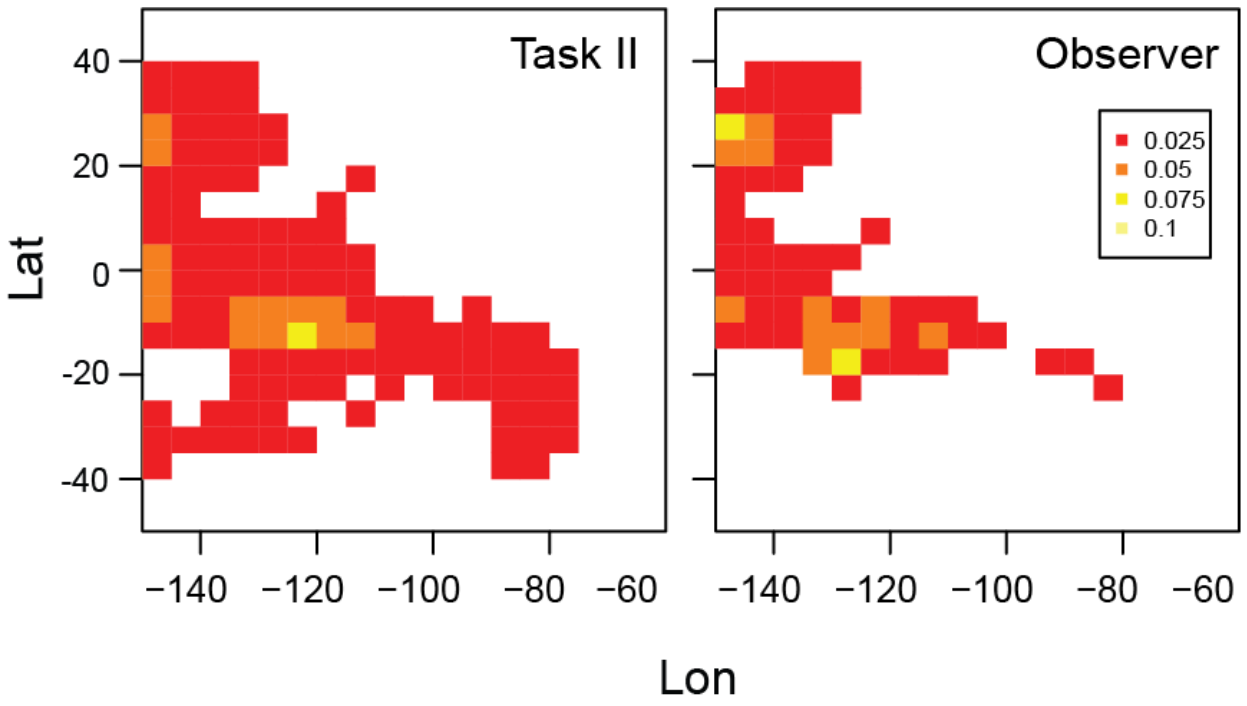

Figure A.10 Proportion effort (from numbers of hooks) for the high-seas longline fisheries of Japan, Korea, Chinese Taipei and United States, combined, for 2016-2018, from the Task II and observer data provided to IATTC. Data have been limited to those 5° x 5° areas with effort from at least three vessels during the three-year period.

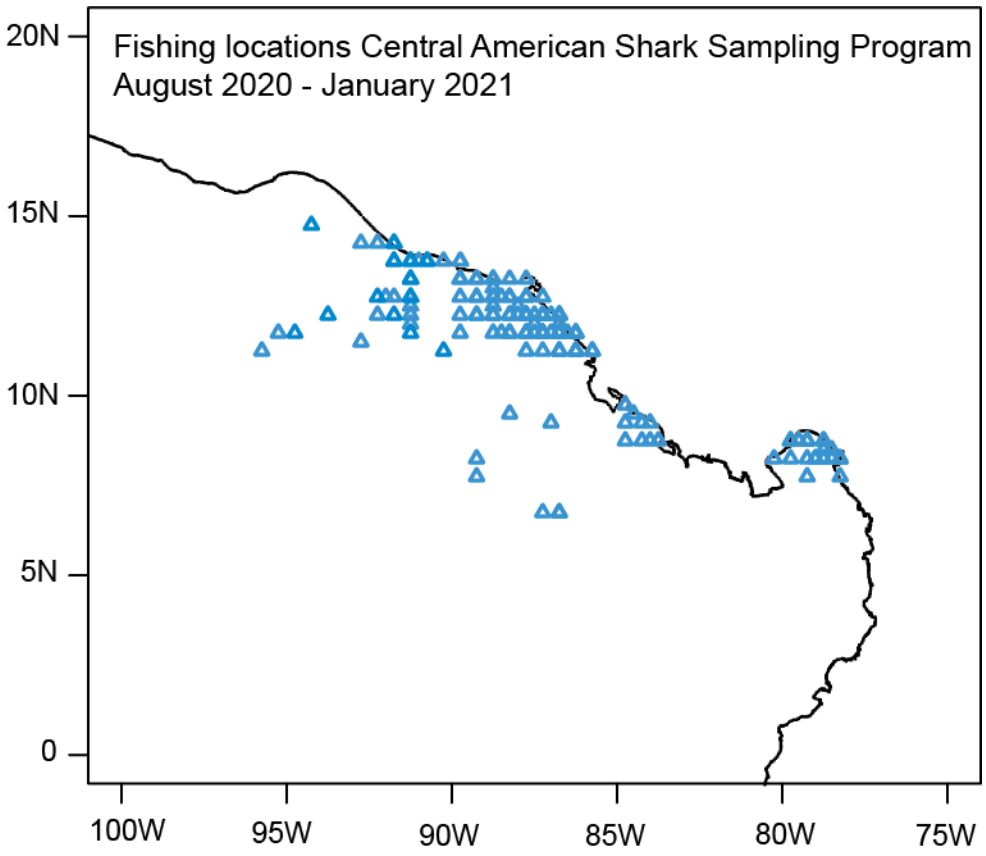

Figure A.11 Sampling locations in the Central American shark sampling program.