SCRS/2021/023 Collect. Vol. Sci. Pap. ICCAT, 78(3): 198-211 (2021)

REPORT OF THE 2021 ICCAT GBYP WORKSHOP ON CLOSE-KIN MARK RECAPTURE FOR EASTERN ATLANTIC BLUEFIN TUNA

(Online, 8-9 February 2021)

Anonymous

SUMMARY

The online GBYP Workshop on Close-Kin Mark Recapture was held from 8 to 9 February 2021 with the specific objectives to evaluate the financial, logistic and scientific feasibility of implementing a CKMR study for Eastern Atlantic bluefin tuna. The requirements for the proper development of such a CKMR study were reviewed and examples of application of CKMR methodology in tuna stocks were provided. Genetic analyses and sampling issues derived from the necessity of getting well-mixed samples were discussed. As a result, a list of recommendations about further steps aiming at the implementation of a CKMR study for Eastern Atlantic bluefin tuna, in the case that it be decided to go on with this initiative, was elaborated.

RÉSUMÉ

L'atelier du GBYP sur le marquage et la récupération des marques sur des spécimens étroitement apparentés s'est tenu en ligne du 8 au 9 février 2021 avec pour objectifs spécifiques d'évaluer la faisabilité financière, logistique et scientifique de la mise en œuvre d'une étude CKMR pour le thon rouge de l'Atlantique Est. Les exigences pour le développement correct d'une telle étude CKMR ont été examinées et des exemples d'application de la méthodologie CKMR aux stocks de thonidés ont été fournis. Les analyses génétiques et les problèmes d'échantillonnage découlant de la nécessité d'obtenir des échantillons bien mélangés ont été discutés. En conséquence, une liste de recommandations sur les étapes ultérieures visant à la mise en œuvre d'une étude CKMR pour le thon rouge de l'Atlantique Est, dans le cas où il serait décidé de poursuivre cette initiative, a été élaborée.

RESUMEN

El Taller en línea del GBYP sobre marcado-recaptura de ejemplares estrechamente emparentados (CKMR) se celebró del 8 al 9 de febrero de 2021, con los objetivos específicos de evaluar la viabilidad financiera, logística y científica de implementar un estudio CKMR para el atún rojo del Atlántico oriental. Se revisaron los requisitos para el desarrollo adecuado de dicho estudio CKMR y se facilitaron ejemplos de la aplicación de la metodología CKMR en stocks de túnidos. Se discutieron los análisis genéticos y temas de muestreo derivados de la necesidad de obtener muestras bien mezcladas. Como resultado, se elaboró una lista de recomendaciones acerca de los siguientes pasos destinados a la implementación de un estudio CKMR para el atún rojo del Atlántico oriental, en el caso de que se decida continuar con esta iniciativa.

KEYWORDS

Close-kin mark–recapture (CKMR), Atlantic Bluefin Eastern stock,
Atlantic Bluefin Western stock, DNAging

1. Opening and meeting arrangements

The workshop was held on-line on 8-9 February 2021. The East and West Atlantic rapporteurs of the SCRS BFT species groups co-chaired the workshop. On behalf of the Executive Secretary, the Assistant Executive Secretary opened the workshop and welcomed the participants.

The GBYP Coordinator recalled the background, objectives and agenda of the workshop, which are included as **Appendix 1**. The List of Participants is included in **Appendix 2**. The attendees agreed that, as tangible output of the workshop, a short report focused on providing a series of recommendations on the eventual implementation of the Close-kin methodology to the Atlantic Bluefin Eastern stock would be elaborated as a SCRS paper to be presented for the consideration of SCRS BFT Group during the April 2021 intersessional meeting. Stasa Tensek and Nathan G. Taylor served as the workshop rapporteurs.

2. Introductory talk on CKMR methodology

Dr. Mark Bravington, member of the Commonwealth Scientific and Industrial Research Organization of Australia (CSIRO) research team that developed and first applied the CKMR methodology, gave an introductory talk to set the basis for further discussions. It included a brief overview of progress on the technique since 2017, when CSIRO specialists finalized a scoping study carried out under GBYP contracts to advise on the feasibility of the Close-Kin Mark-Recapture for estimating the abundance of the eastern Atlantic bluefin tuna stock (Davies et al., 2016; Bravington et al., 2017), noting that recent developments might have resolved some of the stumbling-blocks identified in that scoping study. The presentation included first an overview of the conceptual basis for CKMR, explaining how it works and what type of information it provides, and describing how different life-history characteristics of the population of interest lead to different sampling strategies. It was recalled that the detailed description of the methods was provided in the background documents made available for this workshop (Bravington et al. 2016 a and b; Davies et al. 2020).

Following this, some of the main problems that in 2017 had cast doubts on the feasibility of the application of CKMR on the Eastern BFT stock, as well the ways in which they might be solved, were addressed. These were:

- Poor quality of age (and sometimes even length) data. A potential solution would be the methylation-based epigenetic ageing (DNAge), which has shown great promise in producing reasonable age estimates, to such an extent that CSIRO recommends to use epigenetic ageing for all future close-kin studies. However, a one-time calibration exercise against otoliths is still required.

- Mixing of Eastern and Western ABFT stocks individuals. The demonstration of largely definitive genetic assignment to E or W-origin nowadays allows this problem to be overcome, since in a CKMR setting only fish that are clearly able to be assigned to one other population would be used in the modelling, whereas samples of ambiguous origin could simply be excluded from the analyses without inducing any bias to the results.

- Difficulties for sampling large numbers of juveniles. This problem can be solved by making use of the substantial BFT larval sampling carried out routinely in Balearic sea spawning area (which is probably the only realistic way to obtain large numbers of immature ABFT). This is because it has been demonstrated that the extent of within-cohort sib-ship is not overwhelming in Balearic or Gulf of Mexico larvae, although it remains important to check if this still holds true at larger sample sizes.

Next, the issue of incomplete mixing when samples are collected near discrete spawning sites was reviewed, since site-fidelity throughout adulthood, even if not heritable, can have an impact. This represents an important potential problem in the case of the Eastern BFT stock, since the most obvious potential sources for large juvenile and adult samples are associated with main spawning sites; under the presumption that BFT shows spawning site fidelity at this scale, samples taken in these areas would be not well-mixed. Comments were offered that most of the benefits of CKMR, i.e. the ability to estimate abundance and other key parameters, still apply even if only some of either the adult or the juvenile samples are well-mixed. However, if there are no well-mixed samples at all, the situation certainly becomes more complicated, because it may then not be possible to produce an unbiased abundance estimate.

In the Atlantic foraging areas, there is less reason for concern about imperfect mixing of adults outside the spawning season.

Mixing may not have to be perfect for the initial phases of CKMR implementation to still give useful (albeit provisional) results, as long as a sufficient number of well-mixed samples are obtained for verification in further phases. However, careful planning is still needed to explore different combinations of samples across different fisheries/surveys and size strata.

Finally, it was suggested that the prerequisites for a successful study would be to elaborate:

- A clear plan for getting 30K samples, including both adults and juveniles of known origin, within a few years (or some similar number, based on the 2017 study and depending on detailed sampling options, that is likely to yield meaningful numbers of kin). Samples do not need to come from everywhere; fewer larger sample-collections would be better. However, sampling adults from several sites allows cross-checks.

- A well-specified plan to, over the same period or longer, reach enough well-mixed adults or, less likely, enough well-mixed juveniles.

These sampling schemes should be re-planned once useful numbers of kin-pairs have been found, and mixing aspects thereby understood better.

The Group then initiated the discussion on how the sampling should be designed to get an absolute estimate of abundance for the Eastern stock in the Mediterranean, which was completed in the next session. Such discussions, and the resulting conclusions and recommendations are summarized in point 4.

3. Examples of application of CKMR methodology in tuna stocks

The use of CKMR for other tuna stocks was also reviewed in Bravington's talk. Special attention was paid to Southern Bluefin tuna CKMR study, which was the first one initiated, and has already been successfully incorporated in the stock assessment and management of this species. The key points were that with newer genotyping and HSPs (Half Sibling Pairs), estimates of both biomass and natural mortality have become possible. The combination of POPs and HSPs can largely eliminate the need to rely on fishery-derived data (e.g. catch rates), and provides additional power for verifying assumptions. Other tuna populations where CKMR has begun, or is being designed, include Pacific Bluefin tuna, Western Pacific albacore, Indian Ocean yellowfin tuna and Western Atlantic bluefin tuna.

Regarding the last, a presentation was given by Dr. Matt Lauretta on that project to date. This reviewed the project's objective, which is to develop an estimate of the abundance of the Western stock breeding in the Gulf of Mexico. Comments were made on the sampling performed and the main results, as well as some of the advantages of the method. Unlike for E-ABT, there is no particular reason for concern about lack-of-mixing in W-ABT samples, given the biology and sampling arrangements in the Western Atlantic. Given the available electronic tagging data, it has been possible to document that the sampling is of a well-mixed state. The 2016-2017 pilot study showed that the mark efficiency 1.6 unique spawners per larvae successfully genotyped. In addition, two parent matches in the Canadian fishery in 2017 showed that the larvae could provide large-scale spawner genetic marking for close-kin mark-recapture. Furthermore, simulation modeling showed that larval sampling could provide a successful way to implement CKMR for Western bluefin tuna. The key messages from the pilot study were as follows:

- CKMR fits into existing WATL bluefin biological sampling programs.

- The path to an abundance estimate is now tested; this comprises larval sampling (to obtain the juveniles) coupled to fishery monitoring (to obtain adults).

- Larval aggregations in GoMex can be sampled efficiently, and "mark" enough distinct adults to provide a large number of kin-pairs discrete tagging events, enough to allow precise abundance estimation. Sampling larval aggregations will lead to a larger number of identified pairs, which will in turn increase precision of abundance estimates.

- For the Western Atlantic stock, the next steps are:

  1. Complete genotyping of the 2018 larvae and 2018-19 adults;
  2. Perform stock-of-origin analyses to define adult recapture samples; and

3. Implement CKMR for West Atlantic spawners abundance estimation.

4. Open discussion on the feasibility of the implementation of a CKRM study for EBFT stock

Given the preceding presentations, the Group discussed various aspects of implementing CKMR for EBFT, including general considerations of the use of products derived from CKMR studies for assessment, EBFT sampling strategies to fulfil CKMR requirements and methodologies and logistics for genetic analyses, as well as funding issues. To help structure the discussion, the Eastern co-chair provided an opening presentation showing spawning areas in the MED and known migration routes, leading to a first question of how to fulfill the requirements of mixing for the MED, given the position of spawning areas and apparent movement dynamics. Discussions and resulting conclusions are summarized in the following points:

4.1 General considerations on CKMR approach: objectives and products

It was first noted that there is a need to clarify what the objectives of a potential CKMR program would be (to support stock assessment? to replace the existing assessment?) and how CKMR could be integrated into the existing stock assessment process. The Group concluded that such issues will first need to be resolved by the BFT species Group and, given their strategic implications, later by the Commission.

The Group was interested in possible outputs of the CKMR method in relation to the assessment. Dr Bravington explained that, in theory, it provides the spawning stock biomass of the whole population and not only of the proportion of active spawners. He added that the information on absolute biomass would probably be available several years after initiating the project. At that time, the close-kin method could be integrated into the stock assessment or could even possibly substitute it completely. In any case, to monitor the spawning stock over the longer term, further sampling of spawning stock is needed on a yearly basis.

4.2 Sampling issues

Considering the heavy CKMR requirements as regards number of samples, attention was first drawn to the need for a reasonable number of sites, involving several CPCs, that are organized logistically for getting high number of useful samples (which requires qualified staff for sampling and fish measurements, appropriate and consistent use preservatives), as well the need of information about the relative stock composition in those areas.

To guide the discussion about a potential pilot study design, the Group reviewed the summary of biological sampling in 2019, noting that to reach the target of about 30000 samples, including around 15000 adults, the current sampling effort would need to be doubled over the next few years.

The Group discussed possible sites to sample in order to ensure a good level of mixing, given the complicated situation in the Mediterranean, as indicated by the existence of resident populations and apparent spawning site fidelity. The Group was also concerned as to whether the spawning areas in Bay of Biscay and Slope Sea needed to be considered. Dr Bravington explained that samples are not required from every spawning site.

Dr Bravington remarked that sampling does not have to be random, as long as the samples are well mixed. Adult samples do not need to represent the population in the similar proportions as on spawning sites. However, individuals need to be accurately assigned to a stock to define an appropriate adult sample to compare to the juveniles. In addition, it is important where a fish is born and where it spawns, but it doesn’t matter that much where it goes after spawning, as long as the marked fish with the unmarked population. The Group asked for a clear explanation of what “an appropriate level of mixing” implies, but it proved difficult to provide a single definition.

Given the aforementioned, the key requirement for CKMR is that samples from at least one component of the population (adult and/or juvenile) must be “well-mixed”. The group clarified further what is defined by well-mixed by considering a hypothetical situation with three spawning areas (W/C/E) in the Med, and two sampling sites (N/S) in the East Atlantic. In this scenario, if all fish from all three Med areas go to the Atlantic, all with the same relative probabilities of going to the N and the S sites, clearly the East Atlantic samples satisfy the “well-mixed” requirement, but for illustrative purposes the group considered the effect of different movement dynamics on alternative mixing scenarios as follows:

- a situation where none, or very few of the E are fish go to the Atlantic is problematic because the Atlantic samples are then not well mixed; at best the resultant abundance estimates would apply to the W+C MED components of the population only, and may be biased as the E marked fish would not be available for recapture, and therefore recapture rates for that component would be biased low;

- if appreciably different proportions from the W and C areas go to the Atlantic (ignoring E for the purposes of simplicity), then this is also problematic because the samples would not be well mixed and CKMR depends on these two proportions being the same on average over time;

- if fish from the W and C areas go to the Atlantic, but the relative proportions of the two in the N site and in the S site differ appreciably (ignoring E for the purposes of simplicity), then this is also problematic because the stocks are not well mixed and the CKMR approach would provide biased estimates.

The main conclusion from these discussions was that the selection of sampling sites needs to assess the probabilities of situations like those described above, and needs to make choices that intend to minimize these probabilities.

As regards the hypothetical permanent resident population in the Levantine Sea (i.e. a population in the MED whose link with Atlantic population is weak or inexistent), Dr Bravington explained that after getting enough samples, cross checking should be made to get indication if part of population had been missed, so that the selection of sampling sites could be revisited a posteriori.

Regarding the percentage of adults is known to remain in the Med all year round in any of the Mediterranean regions, clarification was provided that this does not present a problem if the proportion is similar between different sampling sites.

The Group recommended using electronic and conventional tagging data to get insight into the proportions of different groups of spawners in each potential sampling site. Comments were made that most of available PSAT data probably should not be used for that purpose, given the limited duration of deployments, which may lead to biased results. Archival tag data, which provide multi-year tracks, should provide more reliable information.

The Group also discussed appropriate sampling of juveniles in depth, given that there is some evidence for spawning site fidelity and therefore full mixing cannot be achieved. The use larval samples from the Balearic Sea was recommended, even if these are not well mixed, because these larvae are easily accessible and it is feasible to collect several thousands of larvae in that area each year. Lack of mixing would be not a problem, as long as the other samples (adults) are well mixed. The possibility of enhancing the probability of mixing in juveniles (larvae) sampling through sampling in different sites was considered, but it was pointed out that the number of sampling sites should be not too large, as scattered sampling across the MED would not be a good strategy.

In general, while one site is better to start the pilot project to get insight on feasibility, there are also some advantages to start sampling at different sites. Initial sampling in different sites, although more expensive, would provide insight into the population dynamics, and with time it would become more obvious where the sampling needs to be focused and redirected.

There was discussion whether samples already available in GBYP Tissue Bank could be used. Dr Bravington recommended not to use old samples, because they are scattered all over the Mediterranean, but rather to initiate anew sampling program for the purpose of close kin analyses only. Regarding the samples of dead fish which can be taken in markets, mention was made that these are probably fine from the genetic point of view, given that DNA degradation is unlikely to occur, but it is necessary to know where these fish were caught. In order to avoid contamination, the protocol for extracting samples from individual tuna should be followed strictly, including meticulously cleaning the desk before any new sample is taken.

As regards potential sampling sites of adults, a proposal was made to use Japanese LL fisheries, because apparently their catches are well mixed, *i.e.* represent Mediterranean spawning sites without many differences in proportions. Nevertheless, there still needs to be confirmation whether biological samples provided by Japanese LL can be used for close kin. The fact that there are no residents in the Atlantic samples does not matter if the residence proportion does not vary much depending on spawning site. Another possibility would be to get adults samples from the Mediterranean farms. Indeed, it would be more feasible to get the samples from the farms, but the level of mixing there is yet to be determined.

The Group concluded that, before going ahead, it is essential to plan different sampling options and to agree well defined criteria for selecting the spawning sites to be sampled.

4.3 Genetic analyses issues

The Group was also concerned about several issues regarding the logistics and methodologies associated to genetic analyses: consistency about where on the fish tissue samples are taken, the need for qualified and consistent genetic processing for samples, and the availability of facilities to properly store the samples until they can be analyzed.

The Group was worried about samples that could not be clearly assigned to one specific stock, but Dr Bravington explained that this does not represent a problem, since fish with ambiguous assignments could be discarded from the CKMR estimation.

The uncertainty in genotyping and choice of genetic markers was also addressed. The Group wanted to know if the set of SNPs markers chosen by the CSIRO team to be used for CKMR represents the best set possible. Dr Bravington explained that it seems to function well, but that ideally the choice could be improved.

The Group was informed that a full BFT genome will soon be available, as soon as a related scientific paper is published by Stanford team (Dr Barbara Block). When this paper is released, the application of long-read sequencing technologies to BFT larvae from different spawning areas should be a priority to evaluate current stock structure hypotheses.

It was also pointed out that should Balearic larvae be used as the main source of juvenile fish, a batch of at least 1000 larvae should be genotyped to determine kinship within and between tows, and that preliminary information should be considered to provide a basis to optimize the design. Mention was made that, if deemed necessary, larvae could probably be also obtained from the Central Med and the Levantine Sea, because there are some larval surveys in these areas. In the event that a collaboration is established with these teams, there will be a need to ensure that proper sample handling is instituted, and adequate preservatives are used.

4.4 Funding

Possible funding sources were discussed. In case SCRS BFT Group recommends implementing the Eastern stock CKMR project and the Commission ratifies it, it will be essential to ensure continuity before starting the project. The Group wondered whether the work could be done within national sampling programs, namely the EU Data Collection Framework. It was noted that DG Mare will not be able to provide additional funds, because it already supports GBYP and indirectly the larval sampling in the Balearics. GBYP might be an option to ensure funds if so decided. In any case, a recommendation by SCRS should be provided to Commission to ensure continued funding, through GBYP or otherwise.

5. Recommendations about further steps

To address the specific objective of the workshop, the attendees agreed to recommend that a CKMR pilot study include the following tasks (not in the order of priority) to be carried as a first step toward implementation of the CKMR approach, in the event that the SCRS and Commission decide to go further with this approach:

1. Perform an epigenetic ageing study for BFT (using a reference collection) in order to check if this method works for BFT. If it proves not to work, otoliths will be needed, which would drastically increase the cost of sampling; it is unsure whether it would even be possible to collect a sufficient number of otoliths.

2. Genotype at least 1000 Balearic larvae to determine kinship within and between tows. Use this preliminary information to evaluate an optimal design.

3. Develop a sampling design (identify high yield, high mixing areas) and protocols for CKMR. The project should not commence before confirming realistic and feasible sampling options. Sampling protocols also need to clearly identify methods to avoid DNA cross-contamination and ensure high quality samples.

4. Increase current sampling efforts. Rough simulations of necessary sample size indicate 30,000 adults and juveniles. Ideally this would include sampling of ~2% of the EBFT annual catch of 300,000 fish over several years (e.g. 5000 adults per year).

5. Archiving of larvae and adult samples in a GBYP tissue bank until funds can be obtained to genotype.

6. Explore the feasibility of getting additional larval collections in other spawning areas.

7. Use the newly available full genome with existing samples to evaluate current stock structure hypotheses.

8. At some point in the future, identify best practices for genomic methods for kinship and stock identification based on accuracy, precision and cost-effectiveness.

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Appendix 1

Workshop Objectives and Agenda

Objective

The specific objective is to evaluate the financial, logistic and scientific feasibility of implementing a CKMR study for EBFT. The outputs from this workshop will be presented and discussed within the Intersessional Meeting of the SCRS BFT Species Group, to be held in April 2021, with the aim of issuing research recommendations that could be considered by the SCRS and the Commission.

Agenda (tentative)

1. Opening
2. Introductory talk on CKMR methodology
3. Examples of application of CKMR methodology in bluefin tuna stocks
4. Open discussion on the feasibility of the implementation of a CKRM for EBFT stock
5. Workshop report and adoption
6. Closure

Appendix 2

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GBYB Close-Kin workshop (Online 8-9 February 2021)

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