**Report of the 2024 ICCAT Intersessional Meeting of Bluefin Tuna Species Group (BFTSG)**
*(hybrid/ Sliema, Malta, 15-18 April 2024)*

*The results, conclusions and recommendations contained in this report only reflect the view of the Atlantic Bluefin Tuna Species Group (BFTSG). Therefore, these should be considered preliminary until the SCRS adopts them at its annual Plenary meeting and the Commission revises them at its annual meeting. Accordingly, ICCAT reserves the right to comment, object and endorse this report, until it is finally adopted by the Commission.*

## 1. Opening, adoption of agenda and meeting arrangements

The hybrid meeting was held in person at the Waterfront Hotel in Sliema Malta, and online, from 15 to 18 April 2024. Drs. Enrique Rodríguez-Marín (EU-Spain) and John Walter (U.S.), the Species Group ("the Group") rapporteurs and meeting Chairs, opened the meeting and welcomed participants. On behalf of the Executive Secretary, Dr. Miguel Neves dos Santos, Assistant Executive Secretary, welcomed the participants and wished them success in their meeting.

The Chairs proceeded to review the Agenda which was adopted with some changes (**Appendix 1**). The List of Participants is included in **Appendix 2**. The List of papers and presentations presented at the meeting is attached as **Appendix 3**. The abstracts of all SCRS documents and presentations presented at the meeting are included in **Appendix 4**. The following participants served as rapporteurs:

| *Sections* | *Rapporteur* |
|---|---|
| Items 1, 9, 10 | A. Kimoto |
| Item 2 | M. Lauretta, T. Rouyer |
| Item 3 | N. Rodriguez-Ezpeleta, J. Walter |
| Item 4 | C. Bridges, D. Álvarez-Berastegui, N. Duprey, E. Rodriguez-Marín |
| Item 5 | H. Arrizabalaga, M.N. Santos |
| Item 6 | A. Kimoto, N. Duprey |
| Item 7 | M.N. Santos, F. Alemany |
| Item 8 | J. Walter |

## 2. Close-kin Mark Recapture (CKMR) modeling

An age-structured close-kin mark-recapture model was developed to evaluate study design considerations for implementing an East Atlantic and Mediterranean bluefin tuna (BFT-E) close-kin pilot study, including possible spatial sampling locations and sample sizes (SCRS/2024/053). In general, the study design would provide an absolute abundance estimate of the spawning stock, while allowing for the possibility (and testing) of individual fidelity over time to a particular spawning ground within the Mediterranean. The Chairs thanked the analytical team for the excellent work, and highlighted the value of the estimation to better understand the study requirements, sampling strategy, and level of sampling effort.

Several important clarifications were provided in response to the Group comments. Initial clarification provided was on what comprises a pure, impure, or well-mixed sample. It was clarified that "pure" refers to samples representing fish from a single spawning ground in the year the sample is taken (even though the individual fish in the population may not use the same spawning ground in all years). By contrast, a "well-mixed" sample is one where all fish in the population (in the year the sample is taken and for the ages represented in the sample) are equally represented. An "impure" or "partly mixed" sample represents a situation in between the two previous cases (i.e. not pure, but not well-mixed). It was also clarified that the concept of "faithfulness" corresponds to a situation where the individual fish always spawn in the same ground, year after year, although this may not be their ground of birth. If, in addition to being faithful, the single ground where the individual fish chose to spawn is their ground of birth, then heritability occurs (in addition to faithfulness). The concept of heritability is important if it occurs together with faithfulness, but not particularly relevant if faithfulness does not occur. Faithfulness is important for CKMR regardless of heritability; faithfulness will not lead to any genetic differentiation between spawning grounds unless both it and heritability are very strong. No genetic study has ever detected such differences inside the Mediterranean (whereas there is a clear differentiation between BFT-E and West Atlantic bluefin tuna

(BFT-W)), but that only rules out the most extreme combination. The CKMR study design would allow for testing faithfulness and heritability, but this requires having enough sampling so that evidence from the observed close-kin pairs can be statistically meaningful.

A question was raised about the assumption that the Western Mediterranean subpopulation (i.e. the fish using that spawning ground in any year) was larger in scale than the Central Mediterranean. It was stated that there is no evidence of genetic separation within the Mediterranean (see above), and that individual bluefin tunas are known to have moved through (although not necessarily spawned at) more than one spawning ground in a single year. The authors clarified that there was no specific reason why the Western Mediterranean subpopulation was assumed bigger, but that different assumptions about the breakdown of overall BFT-E biomass between subpopulations would not be expected to have much effect on the precision of aggregate biomass estimates, although it might affect the precision of "movement" (i.e. faithfulness and heritability) parameters. By developing a reasonably complex model that allows the movement parameters to be estimated (rather than making a priori assumptions about faithful-or-not, etc.), the model should give unbiased estimates regardless. Additionally, if adults frequently do spawn at multiple grounds within a year, then the faithfulness aspect will not be an issue, and the lack of faithfulness will be clear from the CKMR results. If the data show that faithfulness is low, then the model could subsequently be simplified, and more importantly the options for cost-effective sampling from different fisheries would be expanded. The exclusion of within cohort comparisons, which is inherent in the sample design, also avoids some potential limited-mixing complexities within a single spawning season.

The analysts indicated that it is possible to consider alternative model configurations to incorporate into the modeling, but that the proposed revisions would need to be outlined during this meeting, in order to complete model revisions by July 2024.

The concept of "super-sibship" was discussed, which refers to the fact that larval samples typically exhibit a much higher proportion of same-cohort siblings than is seen when sampling ages 1 or older juveniles. Super-sibship does not cause bias in CKMR, but it certainly reduces precision compared to an equivalent sample size of older juveniles. To get the maximum statistical information from a CKMR study where super-sibship is present (e.g. the Gulf of Mexico larval samples for BFT-W), an alternative parameterization of CKMR models is required, since individual pairwise comparisons between larvae and other samples cannot be considered statistically independent. Aside from modelling complexity, the practical impact of super-sibship is that each larval sample contributes less statistical precision to the overall result, than a sample from age 1 or 2 fish. Nevertheless, larvae may still be an efficient data source for CKMR if they are easy to collect in large numbers.

The number of siblings in larval samples can increase rapidly with larval sampling intensity, and also depends on sampling strategy (e.g. if deliberately targeting larval aggregations, vs collecting across a larger number of spawning locations). For CKMR design purposes it is important to understand this impact, and some careful work is needed to predict the level of super-sibship based on existing samples.

It was noted that the total sample sizes investigated in the SCRS/2024/053 are considerably larger than those considered in the pilot design study from 2017. There are a couple of reasons for the increased sample requirements. First, the initial observations of sibship in the larval collection indicated higher sample sizes are required to allow for super-sibship. Second, the Chairs highlighted that the population has increased notably according to indices and the assessment, and therefore the increased sample size follows suit. A suggestion was made to focus the initial effort on the East Atlantic where few of the samples would be BFT-W (and therefore not useful for BFT-E CKMR). Regarding the use of fish caught in the northwest Atlantic (of which a substantial proportion are Mediterranean spawners and therefore useful for BFT-E CKMR), it was pointed out that the sampling for genetics is already underway and standardized as part of the BFT-W CKMR, and these fish represent freely available samples with complete metadata, while BFT-E collection programs still need to be initiated.

Regarding testing hypotheses about spatial structure, the Western and Central Mediterranean adult samples are specifically to test for faithfulness. If faithfulness is low, then those adults can contribute to an overall abundance estimate for BFT-E. However, if faithfulness is high, adult samples in the Mediterranean will not be well-mixed, and contribute more. It was pointed out that faithfulness has been observed in the Tunisian purse seines with cross-cohort half-siblings detected. This highlights the need for sampling Atlantic adults, which can be initially assumed to represent well-mixed spawners from the entire

population, at least for the older/larger animals. As long as some adult samples can be assumed well-mixed, it does not matter whether the juvenile samples are well-mixed (and indeed they will not be, since e.g. Balearic larvae obviously come from the Balearic spawning ground). With respect to Mediterranean adult samples, concern was raised about the capture of fish during migration from the Central or Eastern Mediterranean, which could result in false conclusions about mixed spawning. The authors responded that preferential targeting of fish actively spawning is a good point to avoid false conclusions. This point should be considered in the discussion of sampling logistics (see Section 4).

A point was raised on whether annual variation in spawner mixing in the Atlantic fisheries matters. The analysts responded that this is not expected to be a big issue due to the retrospective comparison of adults to larvae. That is, adults will only be compared to juveniles born in previous years, but not in the same year the adult is collected, to minimize non-mixing bias. Furthermore, the main expectation is that big fish will eventually migrate to the Atlantic, regardless of where in the Mediterranean they prefer to spawn, and the model assumptions don't require all spawners to migrate each year. It was clarified that the model currently assumes equal migration across years and age, and that it is not straightforward how this assumption might be tested initially.

An important consideration was raised, highlighting how managers and decision makers need to be informed, in plain language discussions on CKMR, particularly the advantages of the approach and how it will improve the stock assessment and Management Strategy Evaluation (MSE). For example, it should be communicated that CKMR might solve a major problem with the stock assessment/MSE related to the estimation of absolute spawner abundance.

There were questions on why the focus of juvenile sampling was on larvae, given the super-sibship complication, instead of using individuals of ages 1 and 2, which have already dispersed from the spawning areas. The simple answer is that large numbers of larvae are already archived back to 2019, and these are readily available to start exploring the project feasibility. Additionally, the use of larvae gives a clear, real-time genetic signal of adults using the different spawning grounds.

A few notes were provided on sampling possibilities additional to those considered in the CKMR work done so far. First, juvenile (ages 2-3) fisheries exist in various areas (e.g. the Gulf of Lions, Gulf of Genoa, and Sicily) that are readily sampled in high numbers, if juvenile samples are desired. Second, there are active winter fisheries for adults in the Mediterranean, indicating not all mature fish migrate from the Mediterranean immediately after spawning. Currently, there is no evidence to determine whether those fish reside year-round or migrate later in the year.

It was also clarified that the concept of a well-mixed fishery (in the Atlantic) did not imply that all fish were migrating outside of the Mediterranean, but that a random proportion of them do, where the probability of migrating is independent of the Mediterranean area where the fish are. It was noted that the project could then provide future directions for satellite tagging.

Support was given to consider East Atlantic fisheries as important sources for the sampling, with the Atlantic traps, as they occur close to the Strait of Gibraltar and, therefore, are considered to be the most likely to be well-mixed. A clarification was given to where the samples in the Atlantic could be sourced, and that as long as the mixed sample assumption was met then other Atlantic fisheries could certainly be added. Furthermore, the analytical team noted that the working assumption they have made in their work so far is that all Atlantic fisheries are well-mixed as this seems a reasonable working assumption and the future observed CKMR pairs should provide evidence to the contrary if this assumption was not met. It was also noted that there might be ways of reducing the super-sibship aspect through adapting the sampling design of the larval collection.

As no stock structure has been evidenced within the Mediterranean yet, it was suggested that an ongoing small-scale pilot study could help to better understand the stock structure. It was noted that for Pacific bluefin tuna no stock structure could be found but that spawning location differed by age. However, the most important phenomenon to keep in mind, from the perspective of avoiding problems with CKMR, is faithfulness (not heritability), and faithfulness alone can never be detected with genetic stock structure analyses. Given sufficient sampling within the Mediterranean, the extent of faithfulness and heritability will be revealed directly by the CKMR data (i.e. by the locations of kin-pairs).

It was noted that opportunities where it was possible to sample 1000-2000 fish should be identified. It was noted that Japan had not been mentioned yet as a possibility, even though the Atlantic longline fishery could be an opportunity to sample well-mixed fish, and that sampling in the market could also be done. It was noted that COVID-19 had an impact on the sampling onboard, but that sampling in the market could be considered.

It was also noted that the Atlantic traps were an excellent location for efficient sampling, with 1000 fish per year being feasible if the otoliths don't have to be sampled. It was answered that for CKMR otoliths were not necessary, only length and tissue.

Regarding the use of potential mixed stock fisheries samples (i.e. from the BFT-W and the BFT-E stocks), some consideration needs to be given to how these will be screened to avoid potential bias in CKMR estimates. Two approaches were discussed, one which would run stock composition estimates prior to CKMR analysis and remove the BFT-W stock associated fish from consideration in the close-kin comparisons. The second approach is to run the close-kin comparison across the whole collection, and bias correct abundance estimates by the stock proportions. The latter approach is taken for the BFT-W close-kin study, in order to not exclude potential parents/siblings from different stocks, as collections of larvae in the West Atlantic (Slope Sea in particular) indicated mixed breeding, as well as the recently reported observation of Mediterranean-like adults collected on the spawning grounds in the Gulf of Mexico (GOM). It might be considered that observations of siblings across collections can provide some insight into the dynamics of spawning outside the GOM and the Mediterranean.

It was asked if the study design would allow for the estimation of the abundance of spawners using the Central versus Western Mediterranean spawning grounds. The analysts indicated that it would; the approach is described in more detail in the appendix of the report. It was pointed out that the question relates to the aspect of spawner faithfulness to a spawning ground. If no faithfulness exists, the relative size of Western versus Central Mediterranean spawner abundance doesn't matter (fish will choose different spawning areas within and across years), and the model assumptions/study design is greatly simplified. If faithfulness does exist, the CKMR study will provide insight into this aspect, for example through parent-offspring comparisons of Balearic larvae to the Western Mediterranean spawner samples versus Central Mediterranean spawner.

The expectation is that, if faithfulness occurs, it will result in different rates of parent-offspring pairs and cross-cohort half-sibling matches across juvenile-spawner collections. It was highlighted that, under the initial study design, the comparison of spawner faithfulness/mixing across spawning areas with the Eastern Mediterranean will be less informed/not available due to a lack of samples collected from the Eastern Mediterranean.

Overall, much of the uncertainties of stock structure and mixing, required sample sizes, and optimal design will be elucidated through the results of the actual CKMR sampling (i.e. patterns of observed close-kin pairs), as information on stock mixing by fishery/area, POPs, within cohort siblings, and cross-cohort siblings provide valuable insight into the population(s) dynamics.

SCRS/P/2024/016 presented the design and simulation of a next-generation, multi-stock assessment for Atlantic Bluefin tuna that incorporates close-kin mark-recapture data. The project is funded by the U.S. Bluefin Tuna Research Program (BTRP) with objectives to develop a spatial-temporal mixed stock assessment model (called MARS), include supporting diagnostics and documentation in an R package, and provide Markdown reporting of assessment output, model comparison, profiling, data weighting, and retrospectives. The model is mostly developed, with current preliminary fitting to bluefin tuna data types (MSE inputs plus CKMR data), and simulation testing of model complexity appropriate for bluefin tuna.

The Group commented on some of the difficulties with the spatial resolution of the MSE and available data to inform movement. It was replied that the current model has the spatial areas simplified from the MSE to four main areas, including the two main spawning areas and East and West foraging areas. It was also highlighted how the incorporation of CKMR data into the model could solve a major problem with prior assessments, which is data to inform population scale. One participant commented on a possible bias of estimated spawning stock biomass (SSB) in CKMR for their future consideration in the integrated model. In the case of POPs, the fishing gear selectivity for the adult sampling would affect the estimation, and the CKMR estimates from Half-Sibling Pairs (HSPs) might be biased due to the existence of the reproductively inactive adult population.

## 3. CKMR genetics

Results presented in SCRS/2024/057 are ongoing and some of them preliminary; but were shown so that the Group could provide feedback. It is mentioned that the arrays could be processed by different facilities, though the current arrays are manufactured by Thermo Fisher. The actual genotyping of samples can be performed later by any laboratory that has the necessary equipment; there are a number of commercial facilities that offer this service, at least four in Europe. Sample processing and data analysis with the array is straightforward: tissue or DNA samples are sent to the genotyping facility, and they send the genotypes per sample back. The array can provide information about kinship, sex, and also about population connectivity, allowing monitoring the ongoing recently discovered gene-flow from the eastern to the western Atlantic, including mixing in the Slope Sea, and the introgression from albacore.

Notwithstanding the genetic differences between BFT-W and BFT-E populations, the array and DArT processes are both suitable for kinship determination on either BFT-W or BFT-E samples. The question of which sex marker loci to use was discussed, since there are alternative sex markers; the authors commented that the five currently on the array have 95.8% accuracy of sex determination in samples (n=48) whose sex was determined using gonad information. The cost/sample of the array will depend on the number of samples to be analyzed, the more samples, the less expensive. In order to account for the potential Balearic super-sibship it is mentioned that the mitochondrial haplotypes from all larval samples should be needed. This implies that about half of the samples would need to be analyzed for mitochondrial haplotypes. One option might be to include this mitochondrial information in the chip, but this might not work, in which case sequencing would be needed. This is not a problem if mitochondrial haplotypes are only needed for the pairs of kin to be used (as is the case in many CKMR projects), but if it has to be done for almost all the larvae, it will increase the price significantly.

The Group discussed the Atlantic Wide Research Programme for Bluefin tuna (GBYP) pilot study on epigenetic age for Atlantic Bluefin tuna (Davies *et al*., 2024). The aim of the study is to assess the feasibility of epigenetic aging in view of the application of CKMR. The study used samples from the West Atlantic (provide by Fisheries and Oceans Canada (DFO), and National Oceanic and Atmospheric Administration (NOAA)) and the BFT-E data collection (provided by GBYP collection); they were aged according to standard ICCAT protocols. In total, 657 samples were processed, but some were lost due to contamination during the multiplex PCR. 361 samples remained for analysis. Markers whose methylation profiles "react" to aging were identified and a model combining all was developed. The best fitting version with the best combination of probes finds a good correlation between methylation and otolith age (both east and west combined and both sexes combined), certainly good enough for CKMR use. No sex or stock derived biases were observed. Cost (and the question of scalability) was discussed, and it could decrease if there are commercialization opportunities and enough demand. The model has been tuned using muscle tissue; it should also be checked whether any modification is required for tissue from fin clips.

***Breakout room for CKMR genetics***

A small group was tasked to discuss detailed specifications for genetic needs for CKMR, and the following list was provided to the Group.

a. High volume averaging 15-20 thousand individuals per year (for reference, Southern bluefin tuna (SBT) processes 25 thousand individuals for gene tagging);
b. Ability to detect cross-contamination;
c. High percentage of successful genotyping;
    - High yield of usable DNA from samples;
d. Kinship determination;
    - Capacity to determine kin-relationships (POPs, half-siblings, and full siblings);
e. Stock of origin determination;
f. Epigenetic aging;
    - CKMR modeling benefits from estimated fish ages to partition cross-cohort half-sibling relationships and to determine whether a fish could be a potential parent of a juvenile. Given the high cost and practical issues related to using otoliths, epigenetic aging will be the most effective means to age;

g. High capacity Mitochondrial DNA;
   - This is necessary to be able to address larval sibship due to high levels of within-cohort sibship;
h. Sex marker;
   - This is now quite inexpensive and allows the CKMR model to account for differential maternal and paternal reproductive contribution;
i. Dedicated project coordination and database curation;
j. Other needs.

## 4. Sampling to support implementation of CKMR

The activities of the larval group in the Western Mediterranean from 2023 to 2024 (SCRS/P/2024/019) were described with a number of tows averaging 106 with storage in ethanol and formalin. A table was presented of projected points of interest to CKMR sampling with an outline of the number of samples available from 2019 to 2023 in ethanol or formalin. Further plans for the eastern Mediterranean were presented for 2024 to 2028, which also include surveys along the Turkish coastline and around the eastern part of EU-Cyprus. Planned activities from the Strait of Sicily and the Western Ionian Sea for 2024 were also presented: Data for the Strait of Sicily and the Western Ionian Sea have been published (Russo *et al*., 2021, 2022).

SCRS/P/2024/019 also presented the SouthEast Area Monitoring and Assessment Program (SEAMAP) the GOM survey in 2023. Their protocols and standardisation were described with plans for 2024 with the number of additional days to conduct research on potential effects of Climate Change and sampling in larval hot spot areas. Finally, the activities at the BFT Technical Sub-group on early life history in each survey were listed with the various interactions and initiatives, and particularly the sharing of tools for standardization and sampling strategies (ethanol or formalin collections).

The Group was asked whether there would be a large number of samples for close kin. The response was that from 2019 the Western Mediterranean samples have been collected for the GBYP databank and other sample collections. The samples were split into formalin and ethanol for storage and the formalin samples have been analysed for abundance and taxonomy, but the ethanol samples remain to be processed. The question of whether historical samples exist for the Central Mediterranean, it does not appear that they are available. The programs also have only recently started sampling method standardization that might capture high numbers of larvae using the protocols outlined by the 2023 ICCAT GBYP Workshop on Atlantic Bluefin Tuna Larval Indices (hybrid/Palermo, 7-9 February 2023) (Anon., 2023). It was suggested that a good starting point would be the use of the Central Mediterranean larvae together with adults from the Central Mediterranean. For the Western Mediterranean it would be better to select what is already provided to the GBYP collection before using them for CKMR. The larval survey in the Central Mediterranean is for mixed species and also future sampling in the southern Ionian Sea should be investigated if funds can be found specifically for bluefin tuna.

Regarding samples from Sicily, the total number of larvae collected will be increased in the following campaigns by using the Balearic protocols and methodologies. The databank already has numerous samples in ethanol. It was stated that 1000 larvae are already ready for analysis from CKMR. A total of approximately 150 thousand larvae could be available for sorting and CKMR from the Western Mediterranean sampling campaigns from 2019 to 2022, though the processing of these samples would require specific funds.

Some worries were expressed about the use of larvae as their DNA content was small and the subsampling could affect cohort kin detection, and this requires some additional modeling. Also, high sibling relatedness reduces the effective sample size of independent parents (McDowell *et al*., 2023). However, it was pointed out that the larvae were one of the few opportunities to obtain large numbers of samples. It was also pointed out that the experience with the Gulf of Mexico larvae used in CKMR did not give any reason to suggest the presence of any bias in the use of larvae for CKMR, but it did require additional genotyping using mitochondrial DNA and additional modeling to address the added variance introduced by sibling relatedness within the larvae (SCRS/2024/053).

SCRS/P/2024/022 presented activities at the EU-Malta tuna farms and the availability of genetic material for CKMR studies. The definition of gene tagging was explained as the DNA fingerprinting of the parent fish and then the release of hatched larvae from these parents into the ocean for further development and catch as adults. This is the subject of "Tuna Ocean Restocking (TOR) pilot study - Sea-based hatching and release of Atlantic bluefin tuna larvae – theory and practice" (Bridges *et al.*, 2019). The question remains of the larval survival after release, as the farms are 6km offshore in deepwater and the food supply for larvae may be variable. Some information on the mitochondrial DNA analysis technology used in this study was requested and it was stated that by freezing the eggs before analysis a better DNA extraction could be obtained. A CPC scientist offered their help in giving information on their sex analysis technology to anybody interested. The Group pointed out that adult fish held in farms would be valuable for CKMR but that the eggs and larvae produced would not be needed for CKMR modeling as they are only progeny of the fish in farms and would not provide inference on the extant wild population, which is the focus of the CKMR project. Further the Group expressed some concerns regarding the potential of enhancement to homogenize extant genetic diversity and negatively affect the population structure.

Another question came from the Group concerning the development of tuna aquaculture and how one would be able to identify a wild fish from aquaculture produced fish. The gene tagging technology described in the present paper could be used to do this by DNA fingerprinting the broodstock used in full cycle aquaculture and therefore being able to identify the later progeny, either at the market or as escapees.

Since the paper pointed out that there was a large concentration of biomass in the EU-Malta farms of approximately 9,000 t of wild caught fish before fattening to 16,000 t after fattening and that this could constitute an artificial spawning area as a number of these fish do spawn in the cages. What is important for CKMR is that the origin of the fish in the cages can be determined (this will be dealt with later in Section 4) as this involves transfers from towing cages to grow out cages and mixing of the populations. Discussion then followed concerning the role of spawning in the farms or artificial enhancement of such spawning aggregations. The Group pointed out scientific concerns raised by moving fish from multiple spawning areas to one location and artificially enhancing spawning aggregations in farms. Such activities would homogenize any potential genetic diversity and alter patterns of natural spawning locations if there is spawning site fidelity. At the current level, artificial spawning aggregations would likely have limited impact, either negative or positive, on the population as a whole and would likely not affect the assumptions in CKMR model.

It was pointed out that through regulations and transfer the origin of the fish in the farms should be well documented which would support the capacity to use these fish in the CKMR modeling and assign them to a spawning location. It was also noted that since 2010 spawning in towing cages has been observed in EU-Spain and secondly in both EU-Spain and EU-Malta wild fish are attracted to the cages as well. The role of any eggs, which may be in large numbers in both EU-Spain and EU-Malta farming operations, in any recruitment into the general population is unknown and is assumed to be insignificant compared to the total spawning biomass when CKMR modelling is concerned. Further work is ongoing and/or required.

Tuna lifecycle alterations caused by farming are not known at present as fish may spawn on the spawning ground, during towing to the farms or in the farms themselves. Whether this may create fidelity to a spawning site outside of where a fish would normally spawn is uncertain and would depend upon the degree of and mechanism for spawning site fidelity. While there are varying degrees of opinions on the degree of spawning site fidelity, one of the benefits of the CKMR approach outlined in SCRS/2024/053 is that it would be able to estimate this.

SCRS/P/2024/013 introduced the sampling in the harvesting process of farmed Atlantic bluefin tuna in the Maltese Islands, in the GBYP sampling program frame. Farming operations are carried out from May to December with the fattening of the wild caught fish on the farms. The sampling procedure is extensive including otoliths, gonads, fin spines, muscle, length and weight and initially only five fish per day may be achieved but after practice and experience 30-40 fish per day can be sampled. Adult tunas are captured from the wild towards the end of May and towed to the farm cages via purse seiners. These are fattened from June up to the harvest season and then they are slaughtered. The harvest commences in September/October and ends in January. During the harvest season, each farm will have its own processing ship and the farm selected for sampling will depend on the origin of fish they would be harvesting, therefore, regular contact with the farm manager takes place.

Discussions were started on where in the harvesting operation is the best opportunity to obtain fin clips or muscle sample without cross contamination and without interruption of the harvesting operation methodology to ensure that no contamination occurs may differ from site to site (further discussion, see below).

***Major sampling programs for BFT-E and eventual adaptation to CKMR modelling needs (round-robin)***

The Group discussed other major sampling programs for BFT-E and eventual adaptation to CKMR modelling needs. A CPC scientist noted that it might be possible to scale up sampling from fish captured in the Japanese longline fleet at the main auction market. Current sampling rates of approximately 10 fish twice per month could potentially be increased to provide large numbers of fish from the auction, but this would require additional staff time. Possibly 10 fish per day could be sampled with possibly 300 fish per month.

CPC scientists noted that enhanced sampling for CKMR in Atlantic traps would be possible with additional resources. While the temporal dynamics of fish moving into and out of the traps may be changing, Atlantic traps represent focal areas for sampling for the CKMR study as the fish are assumed to be well mixed, fisheries have existing sampling programs and the numbers of fish are high.

***Current sampling program for the BFT-W***

SCRS/P/2024/024 presented the Atlantic bluefin tuna biological sampling program in the Northwest Atlantic, United States. The work in the Gulf of Maine was described with their sampling program with BTRP for the last 14 years. Sampling is challenging but works quite well together with the fishing industry collaboration. Staff numbers are large and are required to cover a 1600 km range from the Canadian border to Florida. Fish normally larger than 185 cm and are aggregated by commercial dealers before processing. At fishing tournaments approximately 100 samples could be obtained over the past two years. In general, since 2010, 14,000 otoliths and 15,000 muscle samples have been obtained. This sampling has been extended to recreational fisheries supplying them with the necessary sampling kits and collection of material. It is now necessary to reduce storage size of collected material, some of which are over 500 g per sample.

The Executive Director of the Bluefin Collaborative gave a verbal overview of the program. The Bluefin Collaborative is a collective of U.S. and Canadian fishermen to improve the management and sustainability of Atlantic bluefin tuna by sourcing data and promoting objective research. A committee member noted that this program of fishery-based tagging has similarities with work being conducted in the UK and recommended that the two programs could coordinate.

***Breakout room for CKMR sampling logistics and protocols***

A small group was tasked to discuss the feasibility of achieving the number of samples for CKMR using Table 3.2. from SCRS/2024/053 as a starting place for discussions.

**Table 3.2.**

| Number of samples per year | | | | | |
|---|---|---|---|---|---|
| | Larval survey | Juvenile fishery | Adult fisheries | | |
| | Balearics: *Wlar* | Croatia: *CROjuv* | West Med: *Wad* | Central Med: *Cad* | Atlantic: *ATLad* |
| 2019-2024 | 3000 (excluding 2021) | 0 | 0 | 0 | 0 |
| 2025-2030 | 8000 | 2000 | 2000 | 2000 | 2000 |

The Group reiterated that these figures are starting values in relation to the precision that could be achieved for obtaining certain parameters (mainly, total adult BFT-E abundance). For larval sampling those numbers were calculated based on an estimate of 50% of the collected samples being unusable with the intention of considering the possible super-sibship effect.

The breakout group leader began by encouraging the Group to look at what geographical areas we want to sample, then to look at what geographical areas we are already sampling in our existing biological sampling programs and if there are any synergies. Where synergies exist, this may provide significant savings for the CKMR sampling.

Discussions were broken down into five types of samples: Larvae; Juvenile fish (*CROjuv*); Western Mediterranean adults; Central Mediterranean adults; and Atlantic adults.

***Larval sampling***

Larval sampling in Balearic Islands is already established and in place as part of the EU data collection program for Balearic larval survey cruise, which is used to construct the bluefin tuna Western Larval Index. Since 2019, trial sampling has been carried out with two replicates, one preserved in formaldehyde and the other in ethanol. This has been used to provide larval samples to the GBYP tissue bank since 2019 for use in genetic studies. During the survey a mean of approximately 30,000 to 40,000 larvae are caught which could be preserved for use in CKMR and therefore this survey as a source of samples means no other sampling locations need to be considered.

Türkiye initiated a five-year sampling program, with the aim to develop a larval index. Therefore, this could provide a platform for the eastern Mediterranean to be incorporated into the sampling for CKMR. The Group has focused on the survey in the Balearics. Nevertheless, the sampling in this area of the Levantine Sea is potentially informative about faithfulness in the Mediterranean and will surely be possible to use it in the near future.

The survey platform captures more than enough bluefin tuna larvae each year and the increased costs would only be for increasing the sub-sampling and preparation of samples, but in the end the number of larvae retained for CKMR can easily be scaled up to satisfy the needs of the CKMR sampling. Funding for the Balearic larval survey cruise seems to be relatively secured via EU funding and this is good for the longevity of the platform as a collection method. There is still a need to clarify the methods-rules of selecting the 8,000 individual larvae across the ~100 stations surveyed on the Balearic larval survey cruise.

The collection of larvae from previous years may be able to help identify how big an issue the super-sibship is, and what the implications for CKMR program design would be.

***Juvenile and Adult Fish sampling***

The Breakout group first clarified what would be needed for each juvenile/adult sampled:

- Original catch location;
- The year the fish was caught (removed from the wild population);
- Date the fish was sampled;
- Length measurements of the sampled fish;
- The analysis of the samples will provide the other information that is needed for CKMR:
    - the age of each fish, this can be epigenetic aging and can come out of the analysis, and,
    - sex of the fish, this can be determined through the genetic analysis.

Sampling feasibility was then discussed for each of the geographical regions where adult/juvenile fish samples are needed: juveniles; Western Mediterranean adults; Central Mediterranean adults; and Atlantic adults.

*Western and Central Mediterranean adult sampling*

Adult fish from the Western Mediterranean can be sampled from existing fishery activities:

- EU-Malta farms – preferred,
- EU-Spain farms – preferred,
- EU-France longline fishery around Balearic Islands (~250t) – possible maybe not ideal, and
- EU-Spain longline fishery – possible although activity levels are variable.

*Maltese sampling*

The Maltese farming operations were extensively discussed as a potential platform where Western and Central adult bluefin tuna could be sampled. In particular, one of the farms is mainly supplied by specimens caught in the west of the Mediterranean.

Currently in the Maltese GBYP BFT sampling, the following are the rough processing steps:

- harvested fish are killed in the cages,
- then placed on an intermediate barge which moves the fish to the processing vessel, and
- then length and weight (not always individually if the specimens are not very large) measurements are done on the deck of the processing vessel, before starting processing. In the case of GBYP sampling, the specimens are tagged, before processing, on the head and under the dorsal fin. This allows the specimen to be identified for subsequent sampling of the head and dorsal fin.

A key issue for using the EU-Malta farms (and likely an issue with any farm that is used as a sampling platform) would be the ability to distinguish the original location and timing of the individual fishes' capture before moving to the farming location. In the EU-Malta cages there are currently fish from the Balearic Islands that are in isolated cages and are not mixed with fish that have been caught in other locations in the Mediterranean. In 2023 there was approximately 12,000 individual fish in the EU-Malta farms which originated from the Balearic Islands area (this represents about 10% of the EU-Malta farm capacity and would be variable year-to-year). There could also be the possibility that some EU-Malta cages might have mixed fish originating from different spawning areas within the central Mediterranean, however the cage records allow for this to be assessed pre-sampling and therefore these cages can easily be avoided if necessary. This means the Maltese farms could easily satisfy collecting up to 2,000 adult samples for the Western Mediterranean.

If farms are going to be used to obtain the CKMR samples then cross contamination needs to be carefully considered and taken into account where fish should be sampled in processing steps to reduce this.

A pilot study may allow for the best sampling methods for this platform might be needed. For example, the tagging of the fish has important information and since we are at the beginning of the new protocol it would be good to maintain as much information as possible.

*Juvenile sampling in EU-Croatia (CROjuv)*

It is proposed to use juveniles caught in June in the Adriatic Sea by EU-Croatia using purse seiners. The catches mostly correspond to juveniles between 2 and 3 years old. These specimens are transferred to fattening farms where they can remain for up to 18 months. Despite this long farming period, the 2 and 3-year-old cohorts are still easily distinguishable at the time of slaughter. Length at the time of capture is not possible to obtain, although measurements of cage transfers are obtained using stereoscopic cameras. Each year 40,000 fish are harvested, which is more than enough of a potential pool therefore other juvenile collection locations were not explored.

If the EU-Croatia farms were to incorporate sampling, they would have to increase sampling levels from current amounts that they are sampling under GBYP (currently sample about 250 fish). There are some uncertainties as tuna prices may impact availability of sampling as it could change practices on farming.

As for part of fish that is sampled there would be preference for collecting fin-clip. Protocol of how sampling is done was not discussed.

*Atlantic adult sampling*

In the Atlantic there are several fisheries that can provide CKMR samples from adult fish. The Group discussed all these potential fisheries and came up with a list of fisheries that should be further explored for collecting some or all of the needed samples. The following list are the fisheries the Group felt would be appropriate for CKMR Atlantic sampling:

- EU-Portugal/EU-Spain/Morocco traps, preferred, since good mixing is almost guaranteed;
- Canada + U.S. existing sampling as part of the BFT-W CKMR project;
- Japanese longline;
- EU-France – trawlers/rod and reel/longline, and
- Electronic tagging teams, complementary.

Generally, the Group would like to see samples collected from more than one location in the Atlantic and not have all the samples happening in one fishery. It was highlighted that there are already eastern bluefin tuna sampled within the Canada and U.S. BFT-W CKMR sampling project that may provide a good source of samples that have a clear collection method/platform in place already. The number of eastern bluefin tuna samples being collected each year are approximately 500 in Canada and approximately 700-800 in the U.S. These numbers vary each year depending on the proportional make up of the BFT-E and BFT-W stocks in the sampling effort.

Atlantic traps were favoured by several as a good platform to collect the needed samples. The fish in the EU-Portugal trap fishery are now mostly caught when they are entering into the Mediterranean and there is an estimate of about 300 fish processed each day, each fish is measured and weighted. In order to add CKMR sampling to their process the EU-Portugal trap sampling program would need more staff and the associated equipment, but it is possible.

Moroccan traps capture around 12-13,000 individuals each year and are kept in captivity for 3-4 months in cages for fattening. The current biological program, mainly based on size sampling, is not in place to collect genetic samples, but for the BFT-E CKMR project, it may be possible to collect genetic samples on land from biological scraps (heads) assuming financial assistance is available to cover the increased sampling effort. Lengths of sampled fish could be estimated using biometric relationships and date of capture of the sampled fish could likely also be recorded.

French fisheries (trawlers, longliners and rod and reel) operating in the Bay of Biscay, in 2023, landed about 330 t of bluefin tuna larger than 80 kg (age 7), representing about 3,000 individuals in 6 auction markets. Some of those locations are currently opportunistically covered and provide samples to GBYP and could be of help to CKMR.

Japanese longline fisheries currently collect approximately 100-300 samples for the GBYP biological study, and they are now looking to start sampling for the BFT-W CKMR but this has not started yet. There is not really a possibility to scale up the on-board sampling, however there is an opportunity to scale up sample collections of market fish. One issue with sampling market fish is that their tail has already been removed so fork length cannot be measured so preanal length is measured instead. Although other measurements can be made and then fork-length can be estimated using a conversion factor. The number of samples that could be collected through market sampling depends somewhat on human resources. At this time, they are getting about 20 samples per month via market sampling. The scale of available fish via market sampling appears to be very high with about 10-20 fish auctioned each day.

Initial studies done by the U.S. BTRP found that fish in poorer condition were still fine for genotyping.

*Tissue bank*

There appears to be two options on storing and managing the collected samples for CKMR work: a single central tissue bank, or various laboratories/hubs where tissue samples are stored. The Group felt that the best option would be to have all the samples maintained in a central location as it has several important advantages (improved organization, standardizations in storage methods, standardization in labeling and "banking", etc.). This does not remove the importance of having a strong system in place for the recording, labeling and storing of samples. A movement to a centralized tissue bank for CKMR also highlights the need for ICCAT to consider developing a tissue bank for all its biological samples and this is something the SCRS should be considering for recommendations with annual budget implications at this year's annual meeting. There are currently companies that already provide this type of centralized tissue bank and these would be ideal candidates to discuss their ability to take on a larger number of samples.

In summary:

- Preference is to have a centralized storage facility;
- Need to develop/agree on a master database (metadata) to cover all the collected samples;
- Need of Terms of Reference giving a clear description of what is needed for a tissue centralized storage facility (AZTI would be a good position to draft these as they have already provided this service for GBYP tissue bank);
    - Capability of storing 20-25 thousand samples per year with replicates;

  - Minimum total samples would be 100 thousand.
  - Need to know what is being stored and to what specifications.
- Energy and supply to keep collection in good quality;
- Costs will be estimated roughly by AZTI to include in September budget planning;
- This type of task should be part of the long-term research plan of the SCRS.

*Sampling methods and logistics*

Protocols for sampling and the type of samplers or devices, both for fin clipping or muscle samplers, used to capture the CKMR biological sample needs to be fully developed and this would be provided as soon as possible to guide how to conduct pilot sampling activities for CKMR this year. A small group will work on developing this protocol. Participants considered that the use of single-use sampling devices was advisable to avoid contamination.

## 5. Funding sources for CKMR

### *5.1 GBYP contribution to CKMR implementation*

GBYP has been providing substantial funding to many research lines of activities and could be a partial funder for CKMR for BFT-E, however other sources of funding are necessary, as GBYP alone is insufficient. It was highlighted that there is a general decrease in the available funds for GBYP and that funding CKMR would decrease funding for other research activities that has been funded by the program.

### *5.2 U.S. Bluefin Tuna Research Program (BTRP) contribution*

An overview was provided on the BTRP in the West Atlantic for the period 2015-2023 (SCRS/P/2024/014), which objective is to provide a basis for advancing science-based fisheries management. Research priorities for this funding opportunity include: representative sampling of hard and soft tissues, and associated analytical techniques for studies (genomics, age composition, growth and reproductive contribution by size and age); large-scale conventional, electronic and genetic tagging experiments; historical data mining; simulation modeling related to assessment models and management strategies; improving the quality of fishery data for stock assessments; developing novel fishery-independent techniques to estimate abundance, mortality or to implement novel management strategies; integration of satellite remote sensing, oceanographic modeling and other multidisciplinary scientific products to consider environmental effects upon biology, fishery operations or to resolve the uncertainties in current and historical recruitment. Finally, a summary of the BTRP research benefits since 2015 was provided. Annual funding of BTRP is US$600,000.

The Group highlighted the importance of BTRP to advance research and the provision of SCRS scientific advice to the Commission. The Group also discussed opportunities to improve coordination between BTRP and GBYP, a sentiment that was extended to include a call for greater coordination between all national scientific and data collection programs. There was a desire from the Group to be kept informed on other national science and research programs, however it was noted that meeting time and space is limited and that such presentations should be coordinated a priori to be most effective.

The Group asked whether BTRP funded the BFT-W CKMR and noted that BTRP supported some aspects of the pilot studies and part of the enhanced biological data collection program. The actual annual base funding of approximately US$150,000 for the genotyping and analytical support for the BFT-W CKMR was relatively low relative to the magnitude of leveraged support in the form of annual larval surveys, and fishery monitoring programs. The majority of the support that made the BFT-W CKMR possible, came from annual ongoing research surveys, in-kind labor and data contributions from within the U.S. and from Canada and substantially leveraged the annual investment (of approximately US$150,000) for genotyping and analytical support. This funding model has some insights into how the BFT-E CKMR program could succeed as it will need to similarly leverage ongoing surveys, fishery monitoring and in-kind participation from national CPCs to be successful, given the magnitude of the project.

It was noted that coordination between BTRP and GBYP has increased in recent years, but it should be further enhanced in the future as well as with any other bluefin tuna national program for the benefit of the provision of scientific advice and to avoid unnecessary duplication of research initiatives.

### *5.3 Other potential sources of funding*

The Group discussed possible development of a set aside to support bluefin tuna CKMR representing a small fraction of the overall Total Allowable Catch (TAC) to support CKMR funding needs. The Group revisited some of the issues raised by the Commission when this possibility was discussed in the past. The Group suggested the SCRS Chair to coordinate intersessionally with relevant Commission Officers, aiming for a discussion of possibility during the next annual meeting of the Commission.

Other external funding opportunities, whether provided by institutions or private funds (e.g. Horizon Europe, European Maritime, Fisheries and Aquaculture Fund (EMFF)) were identified as potential funding platforms to support activities and objectives of the CKMR for BFT-E.

Costs will be estimated roughly by the BFTSG to include in September 2024 budget planning considering the research activities that could be replaced/become obsolete if the CKMR starts to be implemented.

## 6. Abundance indices

SCRS/P/2024/017 provided the potential feeding and spawning habitat of the Atlantic bluefin tuna. The authors highlight the possibilities for use in the standardization of abundance indices and in the parameterization of stock assessment (growth, recruitment).

There was a lot of interest in the presentation and the potential use of such a data layer in index standardizations, informing new research areas, and informing assumptions about adult bluefin tuna movements, spawning aggregations, and seasonality of habitat use. Recent years data on bluefin tuna tagging and other observations would be useful to incorporate into the presented analysis and the author was open to and seeking bluefin tuna expertise and data holders to collaborate with on improving the modeling.

Questions were raised on what data were used in the presented analysis. For example, the author clarified that the upper layer representative of the mixed layer from operational Copernicus Marine Environment Monitoring Service (CMEMS) models was used to determine sea surface temperature (SST), and "potential habitat" for spawning, especially when these coincide with areas that do not have any records of larval presence, might be caused by similar oceanic conditions than identified from the data in known spawning areas. Comparing these results with other analysis would be helpful to see where there are differences and similarities and get a sense of the potential bigger picture. In the end it would be good to have clear objectives for this type of work, for example perhaps focusing on one area to get an abundance index for that area (e.g., main feeding grounds of the bluefin tuna adults in the North Atlantic excluding, as a first step, the main spawning grounds in the GOM and the Mediterranean), as opposed to the whole area. Looking forward to seeing continued work on this and perhaps an update of the work at the 2024 Species Group meeting.

SCRS/P/2024/020 provided information about the development of bluefin tuna larval abundance index in the Western Mediterranean. The authors have been working on improving the methodology of index standardization to reduce the potential bias in this index by including new environmental variables; moon phase at the larval catches (Ottmann *et al*., 2023).

It was pointed out that there is evidence of bluefin tuna spawning activities throughout the day, and the Group questioned if the suggested method accounts for daytime spawning. The authors indicated that the timing of spawning activities between day versus night is not well understood, and that further work will be done prior to providing the updated index in September.

SCRS/2024/058 proposed some points on how to improve the models used in the current bluefin tuna MSE. The authors suggested reconsidering the area stratification in the model, incorporating a more comprehensive CKMR approach and an updated bluefin tuna MSE system to reflect the best scientific knowledge.

The Group exchanged some ideas on the suggested points, and the Chairs noted that these points would be discussed at the next round of Operating Model (OM) revision in 2027/2028 by acknowledging the importance of discussion. At the 2024 September Species Group meeting, the Group would consider a date to begin upon which to consider planning the schedule for the OM revision.

SCRS/P/2024/021 provided the strict update of the U.S. rod and reel index for 66-144 cm that has been used in the Management Procedure (MP). The Group thanked the author for their quick presentation and update to the index. There was discussion on the increase seen in the index from 2018-2021 and the following drop in the index starting in 2022. It appears this could be caused by a strong cohort moving into the index and then dropping out as they age/size out of the index. The author indicated that the size frequency data will be provided at the 2024 September Species Group meeting, and it is possible to review the size binning data (as this index is made up of samples that are binned into 66-114 cm and 115-144 cm size categories).

The Group reopened the discussion on how to calculate a "strict update" of the indices used in the MP. Ideally, the "strict updates" to the indices are standardized using the latest data by fixing the covariates in General Linear Model (GLM) already estimated at the time of MP adoption (in 2022) to have the same index values prior to 2021. It was confirmed that the presented update to the index was a "strict update" in the sense that the GLM re-estimated parameters using the entire time-series, and there were no differences in the annual values pre-2023. The aim for the BFTSG is to have a clear methodology across all indices on how to fix the covariates. Although the re-estimation process is not an issue for this update it will be very important for some of the other bluefin tuna indices which still need to be updated and presented to the BFTSG. It was commented that it would be worth reexamining the method used in Lino *et al*. (2023). The authors noted that the R codes will be available to the index sub-group to coordinate their further work.

The Group checked the status of updates to other indices used in the MP. Preliminary Japanese longline indices in the East and West Atlantic for 2023 have been already provided, and the authors will finalize the values in September 2024. Authors for some of the other indices confirmed that their strict update indices will be provided by the 2024 September Species Group meeting.

## 7. *GBYP Strategic directions*

### *7.1 Funding*

The ICCAT Secretariat provided a brief overview of the ICCAT Science funding in recent years, with a particular focus on the ability of the effective use of the available funds. It was highlighted that GBYP has been able to use most of the available funds in line with the activities included in the annual workplans, but not complying with the set time frame. The latter caused that by the end of 2023, GBYP had a positive balance of €695,144, while in the case of the other Research and Data Collection Programmes that balance amounted to €1,170,906. As a consequence, the Commission significantly reduced the Science funding through the regular budget for the year 2024 to €45,000, which is lower than the amount of funding provided back in 2018, and will review the 2025 Science budget during the 2024 Annual Commission meeting.

Based on the above the ICCAT Secretariat informed that the Science budget for 2024 shall be used strictly in line with the approved budget by the Commission, that is detailed in Table 1 of document "SCRS research activities requiring funding for 2024 and 2025" in Appendix 2 to ANNEX 7 of the to the *Report for Biennial Period 2022-2023, Part II (2023), Vol. 1*). Accordingly, no extensions will be granted, nor changes between chapters will be allowed.

The Group acknowledged the aspects highlighted by the ICCAT Secretariat and agreed that the financial requests should be based on thorough assessments. On the other hand, the Group agreed that is essential to have a good knowledge of the ability to effectively deliver in line with the workplan approved by the SCRS and endorsed by the Commission.

Accordingly, the Group agreed to develop its workplan for 2025 and to prepare the necessary Terms of Reference (ToRs) that might be required for the implementation of the GBYP activities for the 2024 September Group meeting. Pending on the SCRS plenary decision, the final developed ToRs will be made available by November 2024.

### *7.2 Program update*

The GBYP Coordinator provided SCRS/P/2024/011 with a program update. He informed the Group about relevant aspects related to program management, namely those related to the major funder platform the European Climate, Infrastructure and Environment Executive Agency (CINEA), and highlighted the need for aligning the annual workplan and GBYP activities with available annual funding as adopted by the Commission. In addition, he briefly presented progress by main lines of research (data management, abundance indices, tagging, biological studies, and modelling) of GBYP Phase 13, that will be closed in July 2024.

The Group requested some further information about the studies for the determination of stock of origin in individuals captured in the Bay of Biscay. Those responsible for the study explained that from some years some changes had been observed in the dynamics of juvenile individuals belonging to the Eastern stock. In parallel, the presence of large individuals that were not previously detected in the area, had been observed which justified carrying out an ad hoc study to determine their origin.

The possibility of resuming the bluefin tuna genetic sampling in the Canary Islands and Morocco, was also raised, considering that previous studies had detected the presence of BFT-W stock individuals in these areas. The Group was informed that the genetic sampling in the Canary Islands area is being carried out, and that the genetic sampling in Morocco could be resumed in 2025 if deemed necessary. Embarking on CKMR sampling would address many of these genetic stock of origin questions even more comprehensively. Finally, it was recalled that genetic samples from the Levantine Sea would be available beyond 2025.

### *7.3 Close-Kin Mark-Recapture (CKMR) external expert*

Dr Ruzzante, contracted as an external advisor for the GBYP Steering Committee for CKMR matters provided SCRS/P/2024/026, which summarized genomic approaches for CKMR estimation of population abundance of BFT-E. He presented a review and synthesis of the recent literature on the genetics of Atlantic Bluefin tuna, including the results by Diaz-Arce *et al*. (2023, 2024) outlining the characteristics of the microarray developed by AZTI. References were made to studies published between 2018 and 2022, most of which described genetic differences between BFT-W and BFT-E. This was followed by a description of progress achieved in the Atlantic halibut CKMR project, which uses an *Illumina* microarray comprising 4,000 single-nucleotide polymorphism (SNP) markers. Next, he discussed recent published work on BFT-W along with Davies *et al.*, 2024 on the epigenetics of aging and suggested that an important next step for this approach is to find a way to scale up the process in a way that it is economically feasible for it to be conducted routinely on a large-scale basis aiming management objectives. This was followed by a presentation of the genotypic data received from the microarray genotyping facility, of the various measures taken for quality control. It was suggested that a way forward for compatibility between BFT-E and BFT-W is for the two groups and institutions involved to share a subset of the SNPs examined in the two different platforms. Different forms of quality control and the need for a clear sampling protocol were also discussed.

The Group acknowledged that the presentation constituted an excellent summary of the current status of the CKMR Atlantic bluefin tuna stock related initiatives. The presentation was followed by a discussion largely focused on the steps needed for the sharing of SNPs to be made effective.

A question was raised regarding the identification of POPs in the halibut study, specifically why there was a range estimated when the likelihood ratio indicated a good distinction of POPs from other kin pairs. The expert clarified that until fish can be aged, the observed distribution may include both full-siblings and POPs, and that the age separation would allow for determining which of the kin pairs were specifically parent-offsprings.

In the discussion, one participant with experience in CKMR using both microarrays and DArT, noted that, in his experience, both approaches could be successful for kin-finding in CKMR, and that DArT's sequencing approaches also scaled up well to large projects (e.g. BFT-W and SBT). The presenter agreed that both approaches could be effective for kin-finding. However, DArT's approaches are proprietary; it was noted that although it is possible to implement similar approaches in an independent lab, it is challenging to do so efficiently, especially at large sample sizes.

With respect to the scaling-up of epigenetic age, a participant noted that epigenetic ageing in general is apparently becoming available as a commercial service offered by at least two companies, which would imply that the issue of scaling-up can be addressed. Costs are not yet known, although a likely maximum limit is suggested in Davies *et al.*, 2024.

The context that motivated developing a halibut CKMR program was also discussed, and a question was asked regarding what the ultimate abundance of halibut was and how long it would take to obtain the estimate. The author responded that the context was that the stock assessment estimates were not precise and there was interest in exploring the application of advanced methods for other species of conservation concern or exploitation including marine mammals. The CKMR abundance estimate is not yet available but is expected within the next year, noting that this was a 5-year project. For context for bluefin tuna, the halibut stock assessment estimate of the population is four million adults and the 2024 quota is 4,927 t.

The external advisor noted that the genotyping and kin-finding steps for halibut were expected to be completed within the next 12 months. The CKMR model itself, which is required to analyse the kin-finding outputs and to produce abundance estimates, is still being developed.

Regarding bluefin tuna, it was recalled that if the markers are shared between different kinship identification methods the results would be comparable. It was also stressed that to implement in the future a pan-Atlantic CKMR study it is not strictly necessary to use now the same genotyping methods in the BFT-W and BFT-E CKMR studies, but that it is crucial to develop standardized protocols and compatible databases from the very beginning.

A question was raised about the availability of the array developed by the GBYP Consortium led by AZTI. It was clarified that this array was not developed for commercial purposes, and it is possible to share it, but since a variety of companies and institutions have participated in its development it is still required to discuss amongst the developers its use by third parties. Nonetheless, the Group agreed to find ways to share the array developed under GBYP with other teams.

The difference between modifying the existing array versus developing new versions was discussed. It was mentioned that minor modifications of the array are possible, e.g. adding mitochondrial DNA. However, more extensive modifications such as adding many of the SNPs used in the BFT-W CKMR study would require the development of a new array, which would have associated costs and would require substantial time.

The potential to integrate the SNPs used for BFT-E CKMR into the BFT-W CKMR genotyping platform was also discussed. This appears to be technically feasible but the consistency of genotypes from the two platforms would need to be tested. It was stated that the BFT-W CKMR team is open to and willing to integrate those SNPs, in order to make both studies fully compatible, and advance the pan-Atlantic CKMR approach. The external advisor recommended this approach as one possible solution to achieve compatibility between the BFT-E and BFT-W CKMR programs in the future. While participants expressed a desire to find ways to share the SNPs, this may require consideration of confidentiality agreements between institutions. The Group noted that they would like to see a process to facilitate the sharing of information and hoped that this matter can be reconciled.

## 8. Path forward

The Group created the list of BFTSG tasks in 2024.

***2024 tasks***

- 1-pager on CKMR benefits and opportunities. *Responsibility:* BFTSG Rapporteurs;

- Potential GBYP biological studies for 2024. *Responsibility:* GBYP, *Deadline:* December 2024;

    1. Adapting existing biological sampling for CKMR and possible CKMR protocol trials to include collecting fin clips, muscle, and otoliths (check ranges of existing ages);
    2. (Evaluation of sibship from Balearic larvae & genotyping) and Preparation of larvae from 2024 possible genotyping (funding dependent);

    3. Evaluate if an epigenetic age clock derived from muscle tissue will work with fin clips or whether a new clock will need to be derived;

- Document on technical specifications for sampling protocols. *Responsibility:* CKMR Coordinator, *Deadline*: Draft of draft by 15 May 2024, Draft by July 2024;

- SCRS document on completed statistical design plan for presentation to BFTSG and SCRS taking into account discussions at the meeting to include any additional modeling runs. *Responsibility:* Contractor, *Deadline*: July 2024;

- SCRS document on design specifications for logistics and analytics for BFT-E CKMR program. *Responsibility:* BFTSG Rapporteurs, CKMR Coordinators, Contractors and External experts, GBYP Steering Committee. *Deadline*: September 2024;

    1. Project management
    2. Sampling
    3. Genotyping
    4. Sample curation and tissue bank
    5. Database management
    6. Statistical Analysis and Modeling
    7. Future compatibility with existing close-kin development work (both BFT-E and BFT-W CKMR)
    8. Cost estimation

- TOR for call for tenders. *Responsibility:* GBYP[1], *Deadline*: September 2024;

    a) Full multi-year CKMR with intermediary products to inform 2027 MSE reconditioning.
    b) Full multi-year CKMR on an extended time frame not intended to inform 2027 reconditioning.
    c) Scalable individual projects to support (a) or (b).

- Issue 2025 call for tenders. *Responsibility:* Secretariat[2];

- Initiate sampling in 2025.

***Compatibility with existing genomic programs***

To date, two groups have presented development work on building capacity for CKMR, developing the methods for stock identification and kin finding for BFT-E and BFT-W CKMR and CKMR model development, but these are not the only groups who could conduct CKMR or who have expressed interest. Should ICCAT embark upon CKMR it would likely be through issuing an open Call for Tenders for which *compatibility* with existing and ongoing efforts would be a requirement to maintain continuity of information and to build on the extensive developmental work conducted to date.

[1] Pending recommendation of SCRS to move forward.
[2] Pending approval by Commission and successful acquisition of necessary funding.

The focus of this meeting has been on CKMR for the BFT-E, so compatibility with existing efforts conducted by the GBYP Consortium and funded by GBYP is essential. This would mean being able to use the existing markers and samples so that there would not be a loss of information, data or insights learned through several years of development work.

With the movement to a MP approach that considers both BFT-E and BFT-W stock dynamics a pan-Atlantic close-kin program could also support future genomic-based MPs similar to how these methods have been incorporated into the Commission for the Conservation of Southern Bluefin Tuna (CCSBT) MP. A Pan-Atlantic approach, e.g. one that considers both BFT-E and BFT-W, would be desirable both to support future MP development and MSE reconditioning, to realize substantial economies of scale as well as and to provide the capacity to use these inferences to address emerging scientific questions.

While there was consensus in the above sentiment, it is beneficial to define what 'compatibility' of BFT-E and BFT-W CKMR would look like. As the BFT-W CKMR is at an inflection point where it will be moving from the pilot phase to an operational phase and moving from DArT-CAP to possibly a sequencing approach that is more efficient and similar to a chip or array, there are a number of decisions before the BFT-W CKMR. A participant involved with BFT-W CKMR noted that the decision has not been made as to what the 'operational' phase looks like, but given this transition period, it would be optimal to achieve compatibility with BFT-E close-kin genotyping so that they could be a single integrated program in the future.

Note that an "integrated program" and "compatibility" do not necessarily imply an "integrated analysis model" that uses all BFT-E and BFT-W data together inside a single model. Separate BFT-E and BFT-W CKMR models are perfectly capable of delivering absolute abundance estimates. Experience elsewhere has shown that it is better to start simply with CKMR, to gain experience and learn from qualitative insights it gives e.g. about spatial issues, and only later to move to incorporating more data into an integrated assessment-type model.

"Compatible" here means that the genotype of an individual sample which is collected and genotyped for use in a BFT-W CKMR model can instead be used directly in an BFT-E CKMR model if that sample turns out to be from a BFT-E stock fish. Note that compatibility of genotyping methods is desirable for efficiency, but not absolutely essential, since in the worst case a sample could simply be genotyped again using the "other" method if it turns out to be from the "other" population, incurring extra expense; however, the proportion of such samples, and thus the extra cost, would not be large in the context of a full-scale BFT-E CKMR program.

Achieving compatibility of BFT-E and BFT-W CKMR:

1. Separate genotyping but with the capability to share genetic markers, and separate modeling

    a. Adding DArT markers to the chip;
    b. Adding chip markers to a new DArTprocess.

2. Joint genotyping, separate modeling

    a. This could involve BFT-W CKMR moving to use the chip and re-running previous larvae only through the chip;
    b. Development of a new chip or array with both sets of markers. (note that this would be desirable should a separate entity take on the project).

3. Joint modeling (future option)

    a. This could involve a joint BFT-E and BFT-W CKMR model;
    b. All existing and prior genotype data would still exist allowing for this to be a future, longer-term task.

## 9. Other matters

Due to time constraints, SCRS/2024/059 or the MSE participant questionnaire, was not presented at the meeting. The discussion of this document is postponed to the 2024 September Species Group meeting. This document proposes to evaluate what has worked and how we could improve the process moving forward through a poll of MSE participants.

## 10. Adoption of the Report and closure

The report was mostly adopted during the meeting, and a part of Section 2 was adopted by correspondence. The Chairs of the Group thanked all the participants and external experts for their efforts and also thanked to Department of Fisheries and Aquaculture of the Maltese Ministry of Agriculture, Fisheries, Food and Animal Rights for hosting the meeting and providing support to our work. The meeting was adjourned.

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**Appendix 1**

## Agenda

1. Opening, adoption of agenda and meeting arrangements
2. Close-kin Mark Recapture (CKMR) modeling
3. CKMR genetics
4. Sampling to support implementation of CKMR
5. Funding sources for CKMR
6. Abundance indices
7. GBYP Strategic directions
8. Pathforwad
9. Other matters
10. Adoption of the report and closure

**Appendix 2**

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**Appendix 3**

## List of papers and presentations

| Doc Ref | Title | Authors |
|---|---|---|
| SCRS/2024/053 | Model-based sampling design for eastern bluefin tuna close-kin mark recapture | Bravington M., Fernandez C. |
| SCRS/2024/057 | ABFT SNP array: A new genomic resource for Atlantic Bluefin tuna connectivity and CKMR studies | Diaz-Arce N., Rodriguez-Ezpeleta N. |
| SCRS/2024/058 | Planning necessary revisions for updating some of the current CPUE data set aggregations and areas for the bluefin tuna (*Thunnus thynnus*) | Di Natale A., Garibaldi F. |
| SCRS/2024/059 | MSE Poll regarding the MSE process | Walter J. |
| SCRS/P/2024/011 | Updating on GBYP | Alemany F., |
| SCRS/P/2024/013 | Harvesting process of farmed atlantic bluefin tuna in the Maltese islands | Galea J. |
| SCRS/P/2024/014 | A summary of research activities conducted under the U.S. Bluefin Tuna Research Program (BTRP), 2015-2023 | Ruiz D. |
| SCRS/P/2024/016 | Design of a next-generation, multi-stock assessment for Atlantic bluefin tuna that incorporates close-kin mark recapture | Huynh Q., Carruthers T., Lauretta M., Walter J. |
| SCRS/P/2024/017 | ABFT potential habitat: Monitoring the distribution of a healthy population at all time scales for management | Druon N. |
| SCRS/P/2024/019 | ICCAT area tuna larval sampling update activities in 2023-2024 | Alvarez-Berastegui D., Ingram G.W. |
| SCRS/P/2024/020 | Western Med: Larval abundance indices and advances on the integration of environmental variability on monitoring bluefin tuna | Alvarez-Berastegui D., Martin-Quetglas M., Perez-Torres A., Tugores P., Casaucao A., Ottmann D., Reglero P. |
| SCRS/P/2024/021 | Updated index of abundance, U.S. rod and reel 66-144cm (NOAA large pelagics survey) | Lauretta M. |
| SCRS/P/2024/022 | Maltese tuna farms and the availability of genetic material for CKMR studies - An overview | Bridges C.R., Borutta F., Schulz S., Na'amnieh S., Vassallo-Agius R., Psaila M., Ellul S. |
| SCRS/P/2024/024 | Atlantic bluefin tuna biological sampling program northwest Atlantic USA | Golet W. |
| SCRS/P/2024/026 | Genomic approaches for CKMR estimation of population abundance of East Atlantic bluefin tuna | Ruzzante D. |

**Appendix 4**

**SCRS Documents and Presentations Abstracts as provided by the authors**

SCRS/2024/053 - This report develops a spatially-explicit Close-Kin Mark-Recapture (CKMR) model suitable for Eastern Bluefin Tuna (EBFT), and uses it to investigate some sampling options (e.g., sample sizes by fishery, number of years, whether to preferentially subsample bigger or smaller fish, etc), to check what kind of precision might be achievable for quantities-of-interest (mainly, total abundance of adult EBFT) and by when.

SCRS/2024/057 - Studies on the Atlantic bluefin tuna population structure reject the previous assumed paradigm of two non-mixing genetically isolated populations, challenging the development of an infallible genetic stock identification method. Responding to the need for a tool that allow for cost-effective and time and space comprehensive monitoring of mixing and ecological dynamics of ABFT, we have developed a genotyping array including a total of 7K genomic markers, hereafter called ABFT-Array. This array is also a key tool for future Close Kin Mark Recapture studies as it also provides sex and kinship relationships. Applied to >1,700 samples, including replicates, fin and tissue samples as well as mock contaminations, we show the robustness of this newly developed genotyping tool which will be key for gathering further knowledge about ABFT population dynamics, as well as for imminent CKMR studies as it can provide sex and kinship information.

SCRS/2024/058 - After the progressive improvements and developments of BFT population studies (such as the CKMR proposal) and management tools (the first cycle of the MSE) made after many years of SCRS and GBYP meetings, it is now the right time for enhancing the data and the system, as it was discussed and agreed in previous BFT SG and MSE meetings. In particular, there are some combined data sets that should be disentangled and reassembled in a different manner by the Secretariat, the areas should be rearranged according to the existing scientific knowledge and the "one stock" approach should be explored and simulated. Some of these changes need time and effort and this should be duly planned. The purpose is to have a more comprehensive CKMR approach and an updated BFT MSE system, including at the best the scientific knowledge, taking into account all possible components.

SCRS/2024/059 - ICCAT's SCRS has been tasked by the Commission to develop management procedures (MPs) through Management Strategy Evaluation (MSE) for many of the ICCAT-managed stocks. With the recent adoption of MPs for Northern Albacore and Bluefin tuna and ongoing MSEs for several other stocks, now is an ideal time to evaluate what has worked and how we could improve the process moving forward. To assist in this, the SCRS is embarking upon a poll of managers on three key aspects of MSE: Process, communication and stakeholder outreach. The SCRS would like to collect information to better inform how we carry out future MSEs and MP related processes. We hope to be able to identify effective approaches to stakeholder engagement to improve the overall degree of MSE-literacy for all participants in the process. All responses will be kept confidential and aggregated by region, not by CPC.

SCRS/P/2024/011 - GBYP coordinator informed the Group about relevant issues affecting the program management, focusing on the need of adapting it to the new scenario derived from the funding through European Climate, Infrastructure and Environment Executive Agency (CINEA), and stressing the importance of elaborating detailed annual work-plans and its corresponding budgets, as well of following them strictly once approved by the Commission. In addition, the recent progress in each of GBYP main lines of research (data management, abundance indices, tagging, biological studies and modelling), was presented.

SCRS/P/2024/013 - The annual GBYP sampling of Atlantic Bluefin tuna which takes place during the annual harvest of tuna farms, is crucial to obtain a sufficient sample size representative of the adult population for reliable stock assessments. The Maltese Islands represent a suitable location for sampling due to a relatively high density of farms which are located in the vicinity of major spawning grounds including the South-Central Mediterranean and South Tyrrhenian Sea, were the majority of adult individuals in Maltese farms are captured from. Harvesting takes place on a daily basis from October till January onboard reefer ships capable of harvesting approximately 30 to 70 tons daily, depending on the ship's capacity. Biological sampling takes place simultaneously, where field scientists must quickly adapt to the swift pace of the harvesting crew, sequence of the processing line, deck layout and size; factors which vary between ships. This presentation provides insight into the fundamental steps taken during biological sampling onboard any harvesting ship, the challenges faced and the general field requirements for successful sampling.

SCRS/P/2024/014 - A review of best practices for natural mortality assumptions in tuna stock assessments was presented (SCRS_P_2024_012). To best align the natural mortality assumptions for Atlantic yellowfin tuna in the 2024 stock assessment, it was recommended to assume a maximum age estimate of 18 years old, with a commensurate estimate of base natural mortality equal to 0.3, based on the Hamel and Cope (2022) longevity estimator. The base estimate of 0.3 M was recommended as the median across fully selected ages, which can be considered age 2, 3, and 6-10 years old. To incorporate uncertainty around the base M, it was suggested to model M using a lognormal prior distribution with a CV=0.31, and potentially incorporate the full distribution in the stock assessment using Monte Carlo resampling. A Lorenzen function of M-at-age can be assumed to account for higher mortality at smaller sizes, modeled directly in SS3 to allow for model flexibility to alternative assumptions and consistent parameterization of M across trials.

SCRS/P/2024/016 - Previous stock assessments of Atlantic bluefin tuna have failed peer review due to the challenges of accounting for seasonal and spatial mixing of the Eastern and Western stock in separate models. We present a prototype of a multi-stock assessment (MARS) that integrates assumptions of stock composition and relative scale within a single model. The age-structured model fits to fishery catch, CPUE, and length composition similar to many tuna assessments, with additional requirements for stock composition and tag data to estimate spatial distribution of the two stocks. When available, close kin mark recapture data have the potential to inform stock size, natural mortality, and fecundity at age schedules. The MARS assessment R package will contain diagnostic functions, such as profiling and data weighting procedures, to facilitate review. Simulation testing is planned to evaluate simpler models, e.g., annual time-step model with fewer spatial strata than in the operating models used for the MSE.

SCRS/P/2024/017 - The potential habitat of bluefin tuna developed at the JRC (juveniles/adults, feeding centered on productivity fronts/spawning depending on mesoscale activity, relatively warm waters and low chlorophyll-a levels and stratification build up) was presented highlighting the possibilities for use in the standardization of abundance indices and in the parameterization of stock assessment (growth, recruitment). Differences between the potential and realized ecological niche were emphasized notably through the return of large ABFTs in the European Nordic Seas during 2012-2022 period and where no substantial change in potential habitat was observed compared to the period 2003-2011, advocating for a return associated to an increase of population size and a larger realized habitat due to an inter-species competition for food. Multi-decadal northward trends were, however, observed for the potential feeding habitat as well as regional longitudinal gains and losses in the Gulf Stream area. Similar northward changes in potential spawning habitat were also observed with decreasing occurrences in the South of GoM and Eastern Mediterranean Sea. Additional observation data (e-tagging and others) from the recent years will allow confronting the habitat model with increased population distribution (closer to the potential niche) and with possible improvement of the parameterization, notably of the spawning habitat in the three main areas (including the Slope Sea). This will lead to present actualized results in the next meeting in September 2024 pending the observation data availability. Updated information are available at: https://sustainable-fisheries.ec.europa.eu/spatial-fish-habitat-and-fishing-effort/fish-habitat/

SCRS/P/2024/019 - The presentation shows the state of the art related to the development of Bluefin tuna larval abundance index in the Western Mediterranean. The last update was presented in September 2023, which includes data up to 2022. Current research to reduce the uncertainties of the index are focusing on the role of hydrodynamics in the retention dispersion patterns in the Western Mediterranean and inclusion of new environmental variables in the standardization processes.

SCRS/P/2024/020 - The presentation reviews the activities carried on by the research groups monitoring tuna early life stages in the Mediterranean and in the West Atlantic. In the Mediterranean, the sampling programs are being reinforced from 2024 with samplings planned for Western, Central and Eastern Med. The groups are implementing standard methodologies and common strategies to increase the catchability, keeping also methods to monitor changes in abundance with methods applied traditionally in each spawning ground. The preservation of larvae in the different areas will consider using both ethanol and formalin, to ensure the possibility to develop genetic studies on the samples.

SCRS/P/2024/021 - Provided the updated index of abundance up to 2022 for the U.S. Rod and Reel for 66-144cm using the data by NOAA Large Pelagics Survey.

SCRS/P/2024/022 - Maltese Bluefin Tuna Farms represent a unique opportunity for sampling genetic material since they represent the concentration of a large biomass (> 12,000 tons) of adult bluefin tuna within a relatively small area defined by the numerous production sea cages placed into 2 major designated zones around Malta approximately 6 km offshore. The origin of catch of these fish, which is throughout the Mediterranean, is widely known through ICCAT documentation. This large biomass also represents a large spawning biomass that has an unknown influence on the population geneticsof this species. Throughout numerous projects on the domestication of bluefin tuna aquaculture it has been possible to collect large quantities of both eggs and larvae from around these production cages with samples available since 2019 ungtil the present day. Recent experiments carried out in our laboratory have shown it possible to identify tuna sex even in the egg stage thereby demonstrating the ability to extract enough DNA from a single egg. This has been done in 96 well plates using simple extraction techniques. Since the hatching time of tuna eggs can be around 32 hours,there is no problem to obtain yolk sac larvae in this species. Obviously at harvesting of the mature adults, tissue samples / fin clips are available and we describe some of the methods which have been used successfully to provide uncontaminated samples that could be used for CKMR studies.

SCRS/P/2024/024 - The presentation introduced the fisheriens dependent Atlantic Bluefin Tuna Biological Sampling Program (USA) in the northwest Atlantic. This research program has been supported by the US Bluefin Tuna Research Program, a competitive grants program designed to fill in critical information gaps for Atlantic bluefin tuna. The program focuses on sampling tissues that fill in life history gaps related to vital processes of this species including age, growth, stock structure, foraging ecology, reproductive biology, and the necessary samples for the close kin mark and recapture pilot program for the western ABFT stock. The sampling program focuses on the US commercial fishery, (>185 cm CFL) that targets larger individuals. Since it's inception in 2010, the program has sampled and archived over 14,000 otoliths and ~15,000 muscle samples from fish landed between Maine and North Carolina. This includes rod and reel, purse seine, pelagic longline and harpoon fisheries. The program, on average, samples between 1,400->1,800 fish per year, about 20-25% of the commercial ABFT landings.

SCRS/P/2024/026 - This presentation summarized genomic approaches for CKMR estimation of population abundance of Eastern Atlantic Bluefin tuna, and contained a review and synthesis of the recent literature on the genetics of Atlantic Bluefin tuna. The author suggested that an important next step for this approach is to find a way to scale up the process in a way that it is economically feasible for it to be conducted routinely on a large-scale basis aiming management objectives.