REPORT OF THE 2023 ICCAT GBYP WORKSHOP ON ATLANTIC BLUEFIN TUNA LARVAL INDICES

(hybrid/Palermo, 7-9 February 2023)

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SUMMARY

The hybrid GBYP Larval Indices Workshop was held from 7 to 9 February 2023 in Palermo, Italy, with the specific objectives of identifying potential sources of uncertainty or inaccuracy in tuna larval surveys, agreeing on a standard survey methodology to minimize potential sources of error or bias and exploring the possibilities for expanding surveys aiming at producing larval indices to other bluefin tuna spawning areas. The survey strategies and sampling methodologies, as well those applied to the analyses of biological samples and data, were presented by all the research teams currently involved in studies on Atlantic bluefin tuna larval stages and discussed by the Group. Finally, a series of specific points aiming at standardizing methodologies and exploring the possibilities for implementing new BFT larval index surveys were addressed by the Group, producing a list of action points toward the achievement of this objective.

RÉSUMÉ

L'Atelier hybride du GBYP sur les indices larvaires a été tenu du 7 au 9 février 2023 à Palerme, en Italie, et visait spécifiquement à identifier les potentielles sources d'incertitude ou d'inexactitude dans les prospections larvaires de thonidés, à convenir d'une méthodologie de prospection standard en vue de réduire les possibles sources d'erreur ou de biais et à étudier la possibilité d'élargir les prospections afin de produire des indices larvaires pour d'autres zones de reproduction de thon rouge. Les stratégies de prospection et les méthodologies d'échantillonnage, ainsi que celles appliquées aux analyses des échantillons et données biologiques, ont été présentées par toutes les équipes de recherche participant actuellement aux études sur les stades larvaires du thon rouge de l'Atlantique et ont été discutées par le Groupe. Finalement, le Groupe a traité d'un ensemble de points spécifiques visant à standardiser les méthodologies et à étudier la possibilité de mettre en œuvre de nouvelles prospections d'indices larvaires pour le thon rouge, produisant une liste d'actions pour atteindre cet objectif.

RESUMEN

El taller sobre índices larvarios del GBYP se celebró en formato híbrido en Palermo, Italia, del 7 al 9 de febrero de 2023, con los objetivos específicos de identificar las posibles fuentes de incertidumbre o imprecisión en los estudios sobre larvas de atún, acordar una metodología estándar de estudio para minimizar las posibles fuentes de error o sesgo y explorar las posibilidades de ampliar los estudios destinados a producir índices de larvas a otras zonas de desove del atún rojo. Las estrategias de prospección y las metodologías de muestreo, así como las aplicadas a los análisis de muestras y datos biológicos, fueron presentadas por todos los equipos de investigación que participan actualmente en estudios sobre las fases larvarias del atún rojo del Atlántico y debatidas por el Grupo. Por último, el Grupo abordó una serie de puntos específicos encaminados a normalizar las metodologías y explorar las posibilidades de realizar nuevas prospecciones sobre el índice de larvas de atún rojo, elaborando una lista de puntos de acción para la consecución de este objetivo.

KEYWORDS

Bluefin tuna, Fish larvae, Ichthyoplankton surveys, larval indices

1. Opening and meeting arrangements

The workshop was held on 7-9 February 2023 in a hybrid format, with some participants attending in person at LUMSA University in Palermo, Italy and others attending on-line. The workshop was opened by representatives of the hosting institution, LUMSA University, professors Gabriele Carapezza Figlia and Dr. De Angelis, who welcomed the participants to the campus. Dr. Francisco Alemany, the GBYP coordinator, and Dr. Angela Cuttita, head of the hosting CNR research team, chaired the meeting. After a round of introductions by all the attendants, Dr. Alemany spoke briefly about the need to coordinate larval survey activities for Eastern Bluefin Tuna (EBFT) and introduced the meeting objectives, which are as follows: to identify potential sources of uncertainty or inaccuracy in larval surveys; to agree on standard methodologies to minimize potential problems; and to explore the possibilities for expanding surveys to other BFT spawning areas. The agenda was reviewed and adopted with no changes (**Appendix 1**). The list of participants is included as **Appendix 2**. Drs. Christina Hernandez and Erin McClelland served as the workshop rapporteurs.

2. Presentations on the current status of larval index surveys

Need for collaboration to improve the larval indices used for Management Strategy Evaluation (MSE) (Dr. Francisco Alemany)

The presentation reviewed the two ongoing surveys used for elaboration of larval indices: the Southeast Area Monitoring and Assessment Program (SEAMAP), which started in the Gulf of Mexico (GOM) in the 70's, and the TUNIBAL Balearic Islands survey, which started in the Mediterranean Sea (MED) in 2001. These surveys cover the two historically recognized Atlantic Bluefin Tuna (BFT) spawning areas, the GOM and the MED. Other ichthyoplankton surveys, from decades ago, have confirmed the presence of BFT larvae in several areas of the MED where BFT spawners aggregate in summer, such as the Tyrrhenian and Ionian Seas in the Central MED and the Levantine Sea in Turkish waters. In addition, more recent ichthyoplankton surveys have identified BFT larvae in some regions of the Atlantic, including the Slope Sea and the Bay of Biscay. BFT spawning habitat models predict suitable spawning areas in other parts of the Central and Northeast Atlantic. However, despite ichthyoplankton surveys taking place during the BFT spawning period in some of these regions, such as the Canary Islands, Azores, and Gulf of Cadiz, BFT spawning activity has not yet been confirmed in these areas. A series of important questions were proposed by the GBYP Coordinator as a reference to guide further discussions:

- Is the survey strategy and sampling methodology adequate/optimal for sampling BFT larvae?
- Is the proportion of spawners in survey areas constant from year to year?
- Is the information on fecundity and population structure sufficient?
- Is the length distribution of the samples representative of that of the whole larval population?
- Are the current surveys representative of the targeted stocks?

It was noted that the immediate goal of the SEAMAP and TUNIBAL surveys is to develop larval indices to estimate abundance of spawners and to provide input for stock assessments; however, they can be used for other complementary purposes, including collecting biological samples for close-kin mark recapture (CKMR) studies. In general, the surveys constitute a good platform to characterize the larval habitat, considering both biotic and abiotic variables, and contribute to a deeper knowledge of BFT early life stages, and hence in the understanding of the recruitment processes that constitute one of the main drivers of the dynamics of the stocks.

Using larval fish abundance to index spawning stock biomass (Dr. Walter Ingram, NOAA)

This presentation addressed the use of larval fish in stock assessment. Life-history characteristics, geographic boundaries, habitat preference, and environmental factors all provide data for stock assessment. These data include fishery dependent data (e.g., derived from observers, self-reporting, portside surveys, monitoring systems, etc.) and fishery independent data (e.g., from research surveys throughout a spatial and temporal range, tagging experiments, etc.). Larval survey data is used to generate indices of spawning stock biomass (assuming a positive relationship between larvae in the plankton and the adults that produced them) and can potentially also be used to develop recruitment indices, combining larval abundances and the knowledge and data on the main drivers of larval mortality. In order to develop a larval index of spawning stock biomass it is necessary to standardize larval sampling, cover the entire potential spawning area, conduct surveys during the spawning season, and ensure correct species identification. The western stock has historically only been sampled in the GOM but more recently evidence has been found for spawning in the Slope Sea, the southern GOM/Yucatan Strait, and the US South Atlantic. Catch data must be standardized by back calculating larval number at size (or age) for each year using

mortality curves. However, it should be noted that different sampling gears can generate different mortality curves. To model the abundance indices, it is important to choose the appropriate model to account for zero inflation (whether due to true zeros, false zeros, or both), as well as appropriate variables for inclusion (such as year, date, time of day, and environmental and geographic variables). The GOM Larval index is the second most influential index in the model used for estimating BFT spawning stock biomass in the western Atlantic. To combine data from multiple surveys, as has been proposed for the MED, it is important to think carefully about how to combine data to provide an overall index. The Southeast Data Assessment Review Procedural Workshop in the United States is currently looking at methods for combining abundance data surveys for index development; they will complete a report with recommendations, software and code that could potentially be useful for the current topic.

It was noted that it is important to define sources of error/uncertainty. Mortality increases at younger ages, and in particular, mortality of eggs is too great to use in the index. It is assumed that there are no density dependence factors at early stages.

It was also noted that there may be problems with auto correlation such that inclusion of a correction could potentially benefit the model; however, in reality, there is often only one value for an area, so autocorrelation isn't a problem. Environmental/oceanographic variables that may have a direct impact on survival rates should be included in the model. Additionally, when deciding to include an index in the assessment, the team evaluates the distribution of survey data and uses a series of diagnostics to decide if the index should be included. These diagnostics include hindcasting to see if the predictive ability of an index merits its inclusion in the assessment. Something to consider when developing new indices is how to present that information to assessors and to determine if the index has predictive power. Along this line, it was pointed out that in the case of skipjack, for example, the ICCAT assessment does not include a larval index because it was found that the available data were not spatially and temporally representative of the entire population.

History and methods of the larval survey for Pacific Bluefin tuna (Dr. Atsushi Tawa and Dr. Yohei Tsukahara)

The authors provided a summary of the Japanese survey for Pacific BFT larvae, which has been operating since 1956. Current goals of the project are to understand growth and survival of early juvenile stages and to locate spawning grounds. Larval samples are used to generate size curves for the Sea of Japan and the Nansei area. The annual variability in density has increased since 2015, and the overall abundance of larvae appears to be increasing in both locations.

Because this larval index is in development, these data are not yet incorporated into the assessment. However, Japan does have another index used to estimate spawning stock biomass (SSB). They are still looking at whether the larval index correctly tracks changes in SSB.

It was noted that a similar increase in the number of positive stations and proportion of larvae is seen in the MED as compared with the Pacific.

There was some discussion of specific methodological issues, such as the depth of the tow (horizontal tow at the surface) and preservation method (in ethanol). It was noted that, in the MED, BFT larvae are found only above the thermocline (typically in the top 30m) but that this is not the case in the GOM and that it would be interesting to determine the vertical distribution in the Pacific, as it is currently unknown.

Bluefin tuna larval abundance indices in the Balearic Sea: History, methods, results, impacts (Dr. Diego Alvarez)

The presentation focused on the BFT survey for larval abundance in the Balearic Sea. It was pointed out that one advantage of using a larval index is that larvae remain in the sampling area longer than the adults and that their distribution is directly influenced by oceanographic processes, thus, the spatial distribution can be modelled better than that of the adults. Ocean dynamics models were used to understand how particles would concentrate around the Balearic Islands to provide information on spawning locations and where larvae are likely to be concentrated (for more efficient sampling of the whole population). Methods have shifted over time, but new methods are calibrated to show that data can reliably be combined, following the methods presented by Dr. Ingram. Model outputs were presented. Factors included in the model were fishing depth, gear, time, date, and oceanographic changes. The model needs to account for factors that affect catchability but not abundance. One disadvantage of the larval index is that larvae are subject to high mortality rates that are influenced by numerous environmental factors and therefore numbers are not directly related to SSB since this relationship can vary among years and regions depending on the environmental variability. However, it should be noted that estimates of population size from the aerial GBYP survey in the western MED are consistent with the estimates of larval abundance from the larval survey, which helps validate the index.

The authors noted that they are confident that the survey currently covers most of the spatial extent of the larval population in the Balearic Sea because larvae are retained in specific areas which can be identified from surface dynamics analyses. Thus, when the environmental data suggests that there will be a large number of negative stations in a given area, the sampling strategy can be adapted to ensure that the surveys also cover areas with a high probability of larval presence, while remaining within the set survey region. A similar approach could be used in the central MED for determining areas of larval retention.

It was also noted that different methodologies were tested for generating mortality curves and that in the end using one mortality curve (not one per year) was the most robust. The distribution of sizes was different among surveys, but not so different as to preclude combining the data from the different years to generate a common mortality curve.

Estimation of offspring fitness in Atlantic bluefin tuna: a potential recruitment index (Dr. Patricia Reglero)

The authors presented their work on the creation of an index of survivors. They are using larvae to look forward and estimate recruitment rather than looking backward to estimate spawning stock biomass. Offspring fitness is the integrated survival probability from egg stage to post-flexion stage; assuming this is the stage of life where most of the mortality occurs. The objective of the current work is to understand how environmental variability acts on eggs and larvae and what factors need to be taken into account (i.e., temp, prey, light, predators). There is a strong relationship between temperature and larval spatial distribution – the water must be warm enough for eggs to hatch and larvae to grow – leading to the question of why spawning does not last longer since the water is still warm in August. Worldwide, BFT larvae are not found in water colder than 20ºC and in the lab it was found that maximum growth rates occurred from 23-28ºC. Field data indicates that larvae primarily occur in oligotrophic waters where there is less chlorophyll and little prey. Two primary prey sources are *Nauplia* and *Cladocera*. A bioenergetics model (including prey and temperature data) indicated that optimum growth for BFT larvae occurs earlier in the year due to food limitations later in the summer – after Julian date 200 (approximately mid-July) the temperature is too high and food (specifically *Nauplia*) is too low for larvae survival. The field data indicate that tuna larvae are growing at the highest growth rate possible (max growth based on lab study), which suggests that if there isn't enough food, they starve and die. The spatial spawning strategy does not seem to be related to food availability. Fast-growing larvae can survive in extremely oligotrophic environments unless the temperature is too high; higher temperatures can be beneficial if prey abundance is high, but critical if not. This finding could have important implications for the evolution of the stocks, since, under the expected scenarios of global warming caused by climate change, important shifts in larval survival could occur. Integrating these mechanisms in a recruitment index would constitute a major contribution to stock assessment.

It was noted that it would be interesting to have more prey data from other locations, such as the GOM. However, in general it appears that larvae are growing at the maximum rate everywhere.

One question that was raised was about the possibility of there being physiological differences between stocks/substocks. Some genes have been identified as being related to temperature tolerance in samples from the eastern MED. BFT from the GOM may be adapted to higher temperatures.

Another question was raised about the effect of rapid changes in temperature (or large swings within a year) on survival if these changes create a mismatch between larval presence and prey availability. In principle, higher temperatures are good for survival, but large/rapid changes may not be.

It was also noted that it is important to think about how to integrate this type of science into stock assessment and management advice. The following points were raised for consideration:

- Recruitment to post-flexion is highly correlated with the recruitment to year 2 so maybe this data could also be integrated into assessment models. Larval data could potentially be used to decide which recruitment model (high/low/medium) to use or as an indicator for environmental impacts and to examine how environmental factors drive changes in productivity.
- Annual mortality rates (or survival rates) for larvae could be incorporated into the assessment. Usually what goes into the assessment are indices of abundance.
- It is convenient to separate the objectives of the basic research on larval biology and ecology from those directed towards fisheries management, although in the end there is a need to integrate the new knowledge from this basic research into fisheries management, mainly through the new indices that can be developed.
- In the MSE, regime shifts are modelled, so process-oriented studies could be useful for identifying the mechanisms behind changes, to give an early indication of future trends (e.g., expected shifts due to climate change).

Ichthyoplankton surveys in Sicilian waters (Dr. Angela Cuttitta)

The author presented the work of the CNR (Consiglio Nazionale delle Ricerche) research team, composed of various researchers from the ISMed CNR and the IAS CNR. These teams have been studying fish eggs and larvae, including tuna, in the Sicilian Channel for many years using multidisciplinary approaches. Dr. Cuttitta introduced her colleagues, Drs. Dino Patti, Marco Torri, Stefania Russo and Marilena Di Natale

The BANSIC ichthyoplankton monitoring program: methods and ecological insights from Mediterranean studies (Dr. Marco Torri)

The author presented information on the BANSIC monitoring program that began in 1997 in the central MED around Sicily. This is a region with complex hydrodynamics, with an upwelling zone in the NW area abutting the coastal zone with colder saltier water, resulting in a retention area between Sicily and Malta. The survey focus is anchovy, so sampling is concentrated near the coast (distance between stations: 4 nautical miles), but data is also collected on ichthyoplankton, zooplankton, and environmental data from a broad area including offshore waters (distance between stations: 12 nautical miles). The surveys are undertaken in the summer on board different R/Vs (Urania, Minerva Uno, Dallaporta). A bongo 40 net with 200 µm mesh size is used at each station and tows are oblique. In addition, CalVet net (vertical haul, 150 µm mesh size), a Bongo 90 net (oblique tow, 1,7mm net mesh size and 0.8mm collector mesh size) and a Multi-Plankton Sampler net (MPS, mouth 35x35 cm, 5 net of 200 µm mesh size are used to investigate the vertical distribution of fish larvae) at some stations irregularly distributed within the study area. At each station, a rosette equipped with CTD probe, fluorimeter, oxygen sensor and Niskin bottles is used for the collection of the environmental parameters. A larval dispersion model was used to see how larvae move in the study area. The dispersal models were correlated with the age of larvae to estimate how old they will be at sampling. It is also possible to run the model back to see where larvae come from (i.e., where spawning is occurring).

Tuna larvae in the Strait of Sicily (Dr. Stefania Russo)

The results of the Italian survey (see above) specifically for tuna larvae were presented. The goal was to understand environmental drivers of distribution, to study larval biological and ecological traits, and to understand the distribution and abundance of the larvae of three tuna species. BFT abundance is higher in the SE zone (the edge of the retention zone), with larvae originating from different areas aggregating here. Temperature, chlorophyll data and the environmental conditions experienced by the larvae were estimated and it was found that larvae of the same length could be in different developmental stages, with earlier development occurring in warmer water. The flexion stage is reached earlier in larvae with greater body depth.

It was noted that *in situ* data and back calculated data are available for examining development. Age estimates were derived from length using otolith-length relationships obtained from a different study.

Study of large pelagic fish in the Central Mediterranean Sea and projection under future climate change scenarios: molecular approaches (Dr. Marilena Di Natale)

The presentation focused on the use of molecular approaches for species identification, studying population structure and determining the effects of environmental changes on larval development. These types of approaches are especially useful for young larvae or eggs which are hard to identify using conventional methods. Researchers used DNA barcoding, and forensically informative nucleotide sequencing of mitochondrial DNA (mtDNA) and nuclear DNA (first internal transcribed spacer (ITS1)) to generate phylogenetic trees where sequences of the same species are grouped into clades. Specific molecular biomarkers are being developed that are associated with a specific environmental stress (i.e., high temperature or better nutritional conditions) to look at molecular mechanisms that affect differences in development. These markers will be used to address the question of whether the conditions that have been associated with positive traits are really good from a molecular point of view or are they a response to stress. It will also be possible to assess nutritional status of larvae using RNA/DNA ratios (i.e., to assess condition and growth).

Tuna larval surveys and BFT larval index in the GOM and Western Caribbean (Dr. Glenn Zapfe)

Information from SEAMAP surveys was presented. This survey provides fishery independent data to NOAA, based on a systematic survey that has been carried out in the northern GOM since the seventies. More recently the survey has covered other areas in the southern GOM and Caribbean. A key concern is maintaining the standard used for historical data to maintain the integrity of the index and the long-term data set. There have been some

changes over time, for example to the track line, some of the gear used, and the number of passes. The survey collects zooplankton, environmental data (temperature, salinity, chlorophyll a, DO, fluorescence and transmissivity), larval fish abundance and specimens for a variety of studies.

There was some concern that with climate change the survey could be missing increased temperatures earlier in the spring, so they did some modeling of habitat availability (i.e., when it's too hot) and used that to inform the survey design. They are still capturing peak spawning time (based on water temperature), but the most important thing is to shift the survey to see if spawning is occurring earlier (so far that is not the case). If there was hard evidence that spawning was occurring earlier, they would consider changing the survey since it is very important to capture peak spawning. One option would be to extend the time window to start earlier.

Bluefin tuna: adaptive surveys (Dr. Estrella Malca)

The presentation focused on adaptive exploratory cruises (beginning in 2009) in the GOM, southern GOM, Caribbean Sea and Bahamas, outside of the spawning areas covered by the SEAMAP traditional survey. Researchers were interested in larval ecology and drivers of larval growth, the scale and timing of growth, and predator/prey dynamics. BFT larvae have been found in these areas but are few and far between. Applications of the survey data include studies on gear catchability, larval habitat maps, growth in the GOM, aging, trophic ecology, surveys outside main spawning grounds and times, close-kin genetics, vertical distribution, spatial variability and trophic dynamics, and habitat quality in the GOM, etc. Survey methods were adapted at sea as samples were analyzed. Aging protocols were developed for standardization. As part of the BFT larvae in oligotrophic ocean foodwebs (BLOOFINZ-GOM) project, when larvae were found, the ship stayed with that water mass and ran various experiments (i.e., plankton sampling, larval guts and otolith processing, growth curves, growth trajectories, abiotic data). It was found that there was not much difference in productivity between areas with BFT and those without but differences in otolith trajectories were observed between the two years of the study. Patches of abundant larvae showed no interannual somatic differences but there were differences in otolith trajectory patterns, depending on ontogeny and food availability. Temperature did not influence larval growth, but diet was a driver of growth with preferred larval prey enhancing growth of older larvae. A similar set of experiments will be undertaken with BLOOFINZ-IO (Indian Ocean).

It was noted that data from the adaptive cruise is not included in the abundance index.

Tuna larval distribution throughout the western Atlantic: implications of a broad distribution to regional indices for the Slope Sea and Northern Gulf of Mexico (Dr. David Richardson)

This presentation focused on larval data from the western Atlantic. In 2016, work was published showing spawning of BFT in the Slope Sea. Larval data from the western Atlantic was compiled from peer reviewed papers, reports, databases from various sources, and museum and archived samples. Identification of larvae was based on morphology with some targeted genetic ID´s to ensure reliability. Spawning takes place later in the year as you move north (April/May in GOM; June/July in Slope Sea). Larvae in the northern part of the Slope Sea are too small to have been produced in the GOM. These larvae are seen in a very broad area – too large for a single survey to adequately sample the full larval distribution. Several hypotheses for spawning ground selection in western Atlantic have been advanced: 1) size structured but single western Atlantic population; 2) environmentally driven but single population; 3) MED, Slope Sea and GOM are independent populations with natal homing; 4) Slope Sea and MED are one population and the GOM is a separate population; or 5) something more complex. They have laid out support for size structured spawning hypothesis. Based on reproductive sampling, fisheries catch data, and electronic tagging, it appears that bigger fish are spawning in the GOM and smaller fish are spawning in the Slope Sea. The relative magnitude of spawning is predicted to be reasonably stable in each area (30-50% in GOM). Preliminary analysis shows similar numbers of larvae in the two areas. These findings have implications for stock assessment, i.e., if there is size structured spawning the larval indices are likely valid, but indices could be biased if spawning ground selection is influenced by environmental factors. If there are independent populations, or the Slope Sea is part of the MED population, population structure in the models should be reconsidered but regional larval indices could be unbiased. Larval size at age (based on otolith increments) was used to estimate ages of all larvae collected in the 2016 survey to estimate spawning locations. Most larvae were collected in the Slope Sea (97%) and growth trajectories suggest that they remained in the Slope Sea for rearing (prior to ability to swim against currents like the gulf stream). Over the medium to long term, a survey here would give information on the variability and stability of spawning among areas and provide an index of larval abundance for the area.

There was some discussion of sampling opportunities. Currently, sampling is done opportunistically as part of the marine mammal survey in the area; they don't determine where the ship goes so stations are not ideal but there is an underlying sampling design. Plankton tows are done at dusk and noon and sometimes in the morning. Suggestions about how to change gear to increase the number of captures on non-standard tows would be welcomed.

A question was raised about availability of young of the year (YOY) data (from the fishery) to show if larvae stay in the Slope Sea. However, YOY in W. Atlantic are very rare. The few that have been collected were in the Straits of Florida and from the edge of the Slope Sea.

Tuna larval surveys in Tunisian waters (Dr. Rafik Zarrad)

The author presented information about the Tunisian ichthyoplankton surveys in the central Mediterranean, which have been undertaken for many years. The surveys (named ESPOIRS) are conducted in summer and in winter on board the R/V Hannibal. A bongo 60 net with 335 µ mesh size is used and tows are oblique. The distance between stations is 10 nm. Following the summer surveys, the presence of BFT larvae were observed in different areas around Tunisia (North, East and South). The relatively highest abundance was located along the east coast of Tunisia. Larvae occurred in patches that were clearly related to the Modified Atlantic Waters (MAW).

It was clarified that the survey also collects larvae in the northern area but that the main catch is in the eastern part. Also, larvae from the survey were not post-flexion. It was pointed out that other ichthyoplankton surveys specifically targeting BFT larvae were carried out in Tunisian waters some years ago, and that the data from these surveys should also be provided to the group.

Tuna larval surveys in the Eastern Mediterranean (Dr. Sinan Mavruk & Dr. Banu Yalim)

The presentation focused on the larval surveys undertaken in the eastern Mediterranean. It has been hypothesized that there may be a resident population that does not migrate to the Atlantic and spawns in the eastern Mediterranean (note that this is not yet supported by field data). Tuna spawn in deep oligotrophic waters and use temperature and photoperiod to time spawning. The area between Cyprus and Türkiye was identified as a spawning area in 2004. The goals of the project were as follows: undertake 3 surveys over the last 5 years to provide quantitative information on larval abundance using standard methods; develop a larval index for the eastern Mediterranean as a baseline for stock management; investigate larval habitat characteristics; and improve capacity for a regular monitoring program. Lower larval abundance was observed in the eastern Mediterranean than in the Central and Western Mediterranean, although observations were in the range of previous studies from different spawning areas. This area may have a lower SSB, or the observations may be a result of differences in the spatial scales of surveys. Distribution pattern changes among years may be related to circulation patterns. Larval BFT select warmer thermal habitats in the eastern Mediterranean compared with central and west possibly due to day of year preference or a local adaptation. It is important to develop new models for the area to take into account different optimum larval habitats. A regular monitoring program is being proposed with annual surveys in Antalya Bay. They would also like to initiate a research project looking at environmental drivers of larval population dynamics (collaborations would be welcomed), otolith microstructure, larval growth, larval condition based on RNA/DNA ratio, and larval trophodynamics based on stomach contents and stable isotope analysis.

It was noted that the starting and ending position of the sample grid was determined by where the commercial fleet was fishing (as an indicator of greatest fish density).

There was some discussion about how adults tolerate higher temperatures. Because the GOM also has warmer temperatures than the western Mediterranean, it would be nice to look at otolith microchemistry and compare growth of increments between GOM and eastern Mediterranean. GOM temperatures are sometimes as high as 28ºC, although BFT are mostly caught at 25 or 26ºC.

It was also noted that spawning may be associated with photoperiod such that fish preferentially spawn on the longest day of the year and therefore temperature preference may change between areas in order for the tuna to spawn on the selected day. This could explain differences between sites in the Mediterranean. Fish might prefer warmer waters in the eastern Mediterranean because that corresponds to the summer solstice, but that day may be colder in the western Mediterranean so that is why they appear to prefer cooler temps there. It is important to test the hypothesis of whether spawning is occurring at a fixed time or if fish are responding to a temperature cue. If it is fixed, then problems may arise due to changing temperatures and prey mismatches.

Interest was expressed in further investigating the possibility of a local resident population in the eastern Mediterranean, since BFT are seen all year in Türkiye waters.

There was some discussion about the importance of standardizing methods across regions because changes in methodology can change results. To compare values between areas, it may be desirable to work with different models. For example, there is little data at the 2mm size range and variation is high so it might be better to interpolate age data to a larger size so the error is less and remains within the data distribution The effect of this change on the index is unclear, but it should reduce the uncertainty. Other questions and comments raised on this issue include the following:

- Is it possible to use the mean distribution of larval size for the back calculation? There are environmental factors that affect differently the study areas, so need annual/region survival trends to account for this.
- Would it make sense to use an abundance index of larvae that have been fully recruited? Why extrapolate to larva that were not selected by the gear? Why not use older larvae? Need to make assumptions about efficiency of the gear.
- Distribution of early larvae is patchy, and some years early larvae are sampled but other years they are not. Thus, some years the complete distribution is not sampled. This is not a problem of the sampling but rather of the distribution – with 100 tows there may be 3-4 stations with high amount of yolk sac larvae but with fewer tows those stations are missed but this doesn't mean the larvae aren't there.
- One goal is to minimize uncertainty in both retro calculations and forward projections.

Tuna larvae trophic ecology: implications for survival (Dr. Raul Laiz)

The presentation addressed applications of stable isotope analysis for understanding tuna larval ecology. There is an implicit hypothesis that food availability and starvation/growth rate and predation vulnerability influence larval survival. For most species, the larval stages hold the greatest potential for regulating year-class size since the highest mortality occurs during this period. Stable isotope analysis can be used to trace assimilated food but needs a baseline analysis (typically from primary consumers) to determine the trophic position of the consumer. For tuna larvae, only isotopic signatures of the postflexion stages can be used to infer trophic information, while the isotopic signature of preflexion (or eggs) stages will provide maternal trophic information and has implications for growth variability. Through the ECOLATUN project, which compares samples from the MED and GOM bluefin tuna spawning ground, the following were observed: higher trophic enrichment in the MED; more zooplankton biomasses in GOM; GOM larvae with higher growth in length and weight and higher otolith increments related to a higher trophic position; comparable isotopic niches in the GOM and the MED, meaning habitats had the level of prey necessary for survival; and a broader isotopic niche in GOM ABT larvae, suggesting a more diverse diet than in the MED. Of the 4 cohabiting scombrid species studied, larvae looked more similar in the GOM than in the MED, with isotopic segregation between bullet and BFT larvae, even in those found at the same station. Differences were also seen in the amount of maternal influence observed in BFT from the MED and GOM in relation to the other cohabiting species. Models predicting the maternal signature, along with preflexion larval size at age, found that there was an overlapping isotopic niche, suggesting that larvae from different spawning areas have a similar signature, so parents may have been consuming food with common isotopic signatures. This finding supports the common feeding ground hypothesis. Through the BLOOFINZ-GOM project, nutrient sources and food-web structure of BFT larvae demonstrated a preference for habitat at edges of anticyclonic loop eddies. Advective transport from the shelf region is needed to resolve the nitrogen budget. Differences in availability of preferred prey can alter larval growth rate trajectories during development. The principal growth drivers were related to food limitation/availability, whereas ingestion of preferred prey better explained growth variability than total ingestion. Otolith growth variability was correlated with the food limitation index. Spawning areas along the shelf break optimized the tradeoffs when associated with transport of productivity from the shelf. The research group is now involved in similar projects in the Indian Ocean to characterize Southern Bluefin Tuna larval trophodynamics and to assess the influence of these dynamics on larval growth and predator-prey interactions with other top predator larvae like yellowfin tuna, bigeye tuna, albacore tuna, skipjack tuna in the Indian Ocean common spawning region (INDITUN).

It was noted that, with stable isotopes, differences between caged fish and wild caught can be determined but it can't be determined if the signature is because parents were caged or not. Stable isotopes can provide information about the centroid of the different communities, while inside the community the species are distributed to niches.

Making the most of your larvae: proven larval processing method to meet close kin genetics needs with flexibility to retain otolith and gut samples (Dr. Kristen Walter)

This presentation focused on methods of sample handling and preservation for tuna larvae to be used in genetic studies. The current Close-kin Mark Recapture (CKMR) project developed in the Western stock is a collaboration between groups that may use different collection and processing methods, so the issue of larval preservation is very important. Genetics requires strict handling and storage protocols to prevent contamination and keep quality

high over time. CKMR is used in a similar way to traditional tagging methods to generate estimates of stock size. However, parent-offspring pairs are used with the offspring (larval) tissue as the mark and parents as the recapture. It is necessary to collect 10*sqrtN samples, where N is the spawning size estimate, to ensure finding sufficient pairs for estimating population size. There are additional considerations such as the proportion of different stocks in the catches and relatedness between larvae. Larvae are preferred over juveniles for CKMR as they are easier to collect and sample. The processing method has been optimized to increase the quantity of tissue used for genetic work while also maintaining other tissues (i.e., otoliths, guts) for different analyses. The steps involved are as follows: 1. remove eyes, 2. separate trunk/tail, and 3. dissect the head just anterior to otoliths. With this method it is possible to prep otolith, gut and genetic samples all at the same time and it can be used for even the smallest larvae. Anti-contamination procedures (clean/sterilize between fish) and preservation methods (use buffered ethanol) have also been improved.

There was some discussion about the large sample sizes needed for CKMR. For example, in the 2018 SEAMAP survey approximately 3000 larvae were collected. Adult samples were obtained from collaborators and the fishery, with approximately 2000 received from the US recreational and commercial fishery and the Canadian commercial fishery. The goal for CKMR was about 2500 for the western spawning stock so the number of larvae was adequate. It was noted that the eastern stock is larger so more pairs will be needed (an estimated 30 000 larval fish). Both parents and offspring (YOY or larvae, but larvae are cheaper and easier to sample) are needed for CKMR, but if the fish is large (i.e., for the parents), the whole fish does not need to be collected.

Different objectives for sample collection were also discussed. For example, development of standardized abundance indices is one objective and for this design sampling should be standardized. This is a different goal from that of collecting the maximum number of larvae. Researchers should think about including the latter in campaigns. Good larval habitat models are needed in order to know where to go to get more larvae. However, it may be undesirable to change larval surveys because they need to follow systematic sampling to maintain the integrity of the larval index. Opportunistically, you could spend a little more time in one area to get these other samples for CKMR if you found a large patch of larvae. Some suggested that it would be best to maintain design-based surveys over time rather than switch to opportunistic sampling. Large enough numbers are needed for the initial phases of CKMR to identify stock structure, which the larval surveys are valuable for since they can't move and thus represent stock structure of area. The value of larvae for CKMR is that when you find a pair the adult can be assigned to the spawning area directly; older juveniles could have moved from rearing area so don't create the same real time monitoring of spawners. Optimal sampling will also be discussed at a CKMR workshop in March 2023. However, it was noted that some sample handling and preservation methods on the boat might need to be changed if larvae are being collected for genetic analyses; this needs to be known in advance.

It was further noted that an important consideration for the beginning of the CKMR process is that there can be high sib-ships in larval samples so a greater number of larval samples is needed. Targeted sampling can end up with higher numbers but sibship may be higher in a concentrated area. Siblings mark the same parents and so are redundant. The spawning population in the Mediterranean is large so the probability of sampling siblings is reduced. If a survey already gets a high number (i.e., like in the Balearics), then that is likely sufficient, but some combination of methods (standard survey design and opportunistic sampling) is valuable in any case to ensure a sufficient number of samples for the different analyses. Also, surveys that won't yield an index of use for the first few years can still provide data that can be used immediately in a pilot CKMR study.

3. Workshop discussion

The discussion addressed the topics laid out in the agenda included as **Appendix 1.**

3.1 Proposed formation of a working group on Early Life-History.

An Early Life-History Group was proposed in 2016 and the SCRS supported the creation of this group, but in the end the proposal was too broad, and the group was never formed. There is already a technical sub-group approved for bluefin larval surveys, but it is unclear that any tasks beyond organizing this current workshop are approved under the SCRS/ICCAT work plan. The group recommended that a new proposal be brought to the BFT Working Group (WG) to see if there is enough interest in forming a subgroup. The BFT WG needs to see a proposal from the group prior to petitioning for official status as sub-group. It was noted that the BFT working group currently has a defined management procedure based on larval indices, and hence there is a need to maintain these larval indices of abundance, but in the future these mandates may be revisited.

Due to time constraints at the September 2023 BFT species group meeting, the presentation of a proposal must be very focused. The following recommendations were made for the proposal:

- Need to have clear terms of reference (TORs), objective and deliverables to define what the subgroup would do to further the goals of the working group and the SCRS; the SCRS has a specific role in advising the Commission so the new group would need to stay within the auspices of the SCRS.
- Areas of focus could include ecotrophic relationships, quality of genes, impact of environmental factors, stock structure, input for CKMR, and indices standardization.
- Describe how surveys/processing will continue in the future.
- Main objective: standardize and improve methodology to have comparable larval abundance indices across the different areas.
    - These indices are provided to the assessment process via the CPCs, produced/funded via IEO (EU, BFT-E) and NOAA (USA, BFT-W). The group is not proposing new funding for this work, simply to serve as a coordinating body for standardization and improvement.
- Smaller objectives/work areas: provide samples to CKMR, development of potential recruitment indices.
- Deliverables must focus on management tools to be of use to BFT working group.

The following points were also raised for consideration:

- It would be useful to provide a summary from this meeting to present at the CKMR workshop in March 2023, so that the value of larval sampling can be discussed. It would be helpful for the CKMR group to know the extent of surveys for each area and number of potential larval samples that could be made available. These numbers should be put together by coordinators.
- There was additional discussion around the utility of forming an early life stages group at the ICCAT level (or under Ecosystems), not just focused on BFT, but it was determined that in the short term it is more practical to focus on indices for BFT because there is a clear need.
- The group needs to think about how CPCs should contribute to the effort since any requests for funding must be well justified. Currently CPCs fund the surveys in their relevant areas; this would continue. CPCs may not necessarily provide the index but provide data to other entities who are then calculating the index. It was further proposed that the final index would be provided by the sub-group rather than the CPCs presenting their indices. In the future, a standardized index could be provided by the group but based on data from CPCs.

It was decided the group would move forward informally until the proposal could be made to the BFT WG. Drs. Walter Ingram and Diego Alvarez were nominated as the initial coordinators and tasked with laying out an agenda and work plan, Then, coordinators can rotate if the group moves forward. A representative will also need to be sent to communicate with the BFT WG. The coordinators will draft the TORs which can be presented to the WG in September 2023.

3.2 Standardization of sampling methods

The group discussed the utility and constraints around standardizing sampling methods for BFT larvae. It was noted that in practice the strategies will need to vary between regions depending on the oceanography, area surveyed, continuity with historic efforts, funding constraints, and processing capacity, etc. It was agreed that groups would share their protocols, with the intent of establishing some ideals within the group, but that protocols could then be modified as needed by different groups. GBYP will set up a folder for shared documents. Specific considerations were discussed as described below.

Gear and sampling

While a diversity of gear has been used by different groups, the following parameters were suggested for optimizing efficiency for BFT larval sampling:

- Use a Bongo-90 net with 505 m
- Tow for 10 minutes at 2kn;
- Maintain a well calibrated, cleaned flowmeter;
    - Recommended use of an SBE39 (https://www.seabird.com/moored/sbe-39plus-temperature-depth-recorder/family?productCategoryId=54627473774), but it was noted that these must be wired to the ship;

    - SEAMAP uses a tracking sheet to make sure the left and right flowmeters are similar for each tow, and they recommended this practice to others;
- Preserve sample from one side of the net in formalin and a replicate from the other side in 95% buffered ethanol (see additional discussion on sample preservation below);
- Include a targeted, quantitative tow for microzooplankton. It was suggested that a 200mm net be placed above the Bongo-90 for this collection;
- Collect data on environmental variables using a CTD plus fluorometer, oximeter and possibly collecting water samples to analyze nutrient content. It was further noted that standard stations for CTD sampling is 300m but that some deeper sampling might be warranted depending on the region;
- Perform a quick sort/identification of the first sample that comes out of the net (while the net is being rinsed) so that it can be determined if adaptive/additional sampling would be worthwhile to increase numbers (this extra tow does not have to be quantitative). These sorted larvae should be kept cold, maybe frozen or stored for other analyses (Stable Isotopes Analyses, for example).

Some regional considerations were noted:

- The mixed layer depth is ~15 m in Turkish waters so sampling might not need to be as deep as 30 m.
- In Italian waters, oblique tows go to 60 m. These tows are for targeting anchovy larvae, but a Bongo-90 tow could be added to target BFT larvae in the offshore region.

Distance between sampling stations

It was determined that the distance between stations will depend on the area where sampling is taking place and the size of the mesoscale structures. For example, a grid of approximately 10nm is adequate in the Mediterranean (currently, 10nm are used for the Balearic sampling, 12nm for the Italian survey and 11nm around Türkiye) in order to match the size of the larval patches and properly characterize mesoscale hydrographic features. In contrast, the grid is 30nm in the GOM because of the total area that needs to be covered. In the GOM, it might be possible to add some targeted shallow sampling between stations to address issues of patchiness and look at smaller-scale variability. GOM sampling is also limited by ship time, which can be affected by oceanographic conditions/features such as the Loop Current. For this survey, the priority is to complete the grid so as to maintain consistency with historic data series, but it might be possible to add some stations if areas of interest were identified. In the Slope Sea, approximately 120 stations can be sampled with the given ship time and processing capacity, and this will determine the resolution that can be achieved. It was noted that in the Slope Sea, it is not necessary to use the same spacing east to west as is used north to south, because the Gulf Stream affects things very quickly in the north-south direction. Therefore, north-south transects can have closer spacing than east-west. This could be a good compromise that is oceanographically-driven.

Survey area

There was some discussion about the best ways to determine the survey grid and it was suggested that exploratory surveys to delimit the spawning areas could be useful. The MONGOOS model (mongoos.eurogoos.eu) is one such model that could be applied. In addition, it was suggested that teams could do some Lagrangian modeling to assess retention areas.

Temporal coverage

Issues pertaining to the timing of surveys were discussed as it is important to make sure that the surveys cover the peak of the spawning period as best as possible. It was suggested that a plot of gonadosomatic index and larval catch for each area could be useful for determining as precisely as possible the seasonal timing of spawning. Indices could then be corrected/standardized based on the match between the survey dates and the temporal distribution of spawning. For example, a spawning seasonality function is fit for the larval/egg indices for Atlantic mackerel in the northeast US. The index is then corrected for the timing of the survey relative to this spawning seasonality. Sensitivity analyses are done to evaluate how much a shift in spawning seasonality would affect the index values.

Sample processing

Sample processing and preservation were discussed in relationship to the different analyses performed. It was recommended that 100% of the sample be checked/sorted for BFT larvae. Currently some samples are being preserved in formalin and others are being preserved in 95% ethanol. It was noted that high quality samples well

preserved in ethanol are needed for any genetic analyses, especially for CKMR. In the GOM, it was found that large plankton volumes require a change of ethanol after 24hrs from the initial collection. At this time samples could be changed to buffered ethanol in keeping with the protocol optimized by Kristen Walter's group (see presentation summary in section 2; protocol provided as **Appendix 3**). Length should be measured from the formalin-preserved larvae (or if not formalin, then be very clear about which preservation method is used). Ideally, a calibrated image analysis software with a precision of 0.01 mm (2 decimal places) should be used and larvae should be measured from the end of the upper jaw to the end of the notochord (pre-flexion) or as a straight line from the end of the upper jaw to an orthogonal line from the notochord tip (post-flexion). This is the standard length for post-flexion larvae. If some larvae are very damaged or cannot be measured reliably, they should be counted but not measured. If the number of larvae at a station is very high, then measurements should be taken until the length distribution stabilizes (i.e., a unimodal distribution for a cohort becomes normally distributed) and then the mean length can be applied to the rest. If there are also a few large larvae (i.e., bimodal distribution), then those must all be measured and not contribute to the estimate of the mean length for the cohort. It was noted that, currently, several labs fix samples for measuring in ethanol rather than formalin, which can affect the length data. A database should be used to keep track of counts, actual measured lengths (i.e., not transformed data), numbers measured, how many are assigned the mean, developmental stage, body depths, etc. The group agreed to share measurement protocols and come to a future agreement on a common protocol.

3.3 Larval identification issues

Best practices for larval identification were discussed. Identifying BFT larvae requires knowledge of all the scombrids present in the region. In some places identification is very clear, but as a general recommendation, use of morphology is adequate but should be validated using genetics. To this end, each region should develop some expertise in identification and regions should collaborate to ensure robust morphological identification. Genetic validation for morphological identification should be used in each region while expertise is being built. The group identified the creation of a common larval identification guide for all Atlantic scombrids that includes all stages and pigmentation variability across regions as a useful tool. It was noted that Kristin Walter has photos of 3,000 BFT larvae that have been genetically verified. Some labs send samples to Poland for sorting and identification. It would be helpful if they could be provided with a new guide. It was also noted that all of the ichthyoplankton samples sent to this lab should be double-checked for scombrids, especially yolk-sac larvae. The question was raised of potentially hosting a future workshop on larval identification.

3.4 Data analysis for larval index calculations

Discussion focused on the best methods for data analysis. Length frequencies are used for calculating the loss rate. The minimum and maximum lengths are needed to generate a length frequency histogram (with suggested bin width of 0.1mm) and a curve is then fitted to this histogram using whatever will be the best model (i.e., exponential decay, spline, etc.). It was agreed that a standard method should be used to calculate the decay curve so that catch per unit area (CPUA) is comparable between regions. Walter Ingram has developed a method that is currently used for the GOM index, and this method could be used in other areas. It was agreed that each region should share length distributions (histograms) so that a common method can be agreed upon. In addition, Drs. Sinan Mavruk and Walter Ingram are going to use some population simulations to generate larval length frequency pseudodata for sensitivity analysis of loss rate. They will also look at different options for back-calculating larvae at a given size.

Some concerns were raised about whether the use of log-transforming and back-transforming can distort the data/index, which could be a barrier for making comparisons among regions/areas. On the other hand, the log-transformation allows the use of a linear model for multiplicative effects. The question was raised of whether spatio-temporal models could better deal with non-random spatial sampling of the observations (i.e., Gaussian Latent models, spatial-mesh, etc.). Changes to the models can affect the historical time series of the index, which poses a challenge for integration into assessment/management tools. It's important for the group to evaluate the robustness of the model parameters to changes in environmental variables, etc. It is very challenging to figure out how to update the model without changing the previous years. It is important to provide a high-quality and stable index to the management process, but also to scientifically improve understanding of larval abundance, comparison among areas, move towards aggregated indices for BFT-E and BFT-W that incorporate the various spawning areas, etc.

3.5 How to improve the existing indices

There was a discussion about how to improve the existing indices and how they can be of use for other assessment or management tools. The next BFT assessment is scheduled for 2026/2027 so there is a margin of time to work together and improve the indices. While the nature of the stock assessment considered for 2026/27 timeframe has yet to be determined, it will follow the traditional process of evaluating the suitability of the available indices for

inclusion into the assessment. As outlined in Rec 22-09, the Commission and the SCRS shall complete a review of the Management Procedure (MP) by 2028. The aim of the review is to ensure the MP is performing as expected and to determine whether there are conditions that justify its continuation, or that warrant reconditioning the MSE operating models; retuning the existing MP; including new indices into a new MP; and/or considering alternate candidate management procedures or development of a new MSE framework.

Currently, the larval indices are relative indices. There was a question as to whether it would be possible to combine the larval index with fecundity and other biological/environmental data to move towards being able to generate an absolute estimate of spawning output or SSB. It was suggested that this is possible mathematically, but there are a lot of caveats. Interest was expressed in exploring this further for WBFT using data from both the GOM and Slope Sea, and then comparing this analysis with the assessment estimate of SSB. A good correlation has been observed between SSB and larval abundance in the Balearics so there was a question as to whether this relationship could be used to improve the estimates of the model parameters, although it was pointed out that there may not be enough data to trust that these correlations are consistent through time. Even without a time series, having these comparisons between larvae and SSB could be useful for stock assessment (but currently this is not a priority from the stock assessment perspective). So, for areas where you don't have consistent sampling (for example, the Slope Sea), targeted sampling for a couple of years before an assessment would be useful and could then be used to inform the assessment process. It was also noted that there may be a mismatch between the areas covered by a given index/sampling and the full area that contributes to the estimate for SSB.

Some work has been done by Dr. Dave Richardson on egg production methods using data from Atlantic mackerel. There are some egg data available from the Balearic samples, but the amount of data is sparse because eggs have a short duration, are very patchy, and are dispersed across the offshore region.

3.6 Potential development of recruitment indices

There was a discussion about developing other indices and specifically a recruitment index. This would not be a measure of expected/absolute recruitment values, but rather an indication of deviations/anomalies in early life-history survival (a relative value). Based on environmental conditions, the index would produce a prediction of higher-than-usual or lower-than-usual recruitment and would examine variability about the recruitment curve. A better term for this might be a larval survival index rather than recruitment index. A similar method has been developed in the Baleares and this work, including software, could be shared with other regions interested in running simulations. Data input needs include: satellite temperature data; a histogram of larval lengths; consideration of variability in food availability between years, or the assumption that there is sufficient food. It was determined that MONGOOS should be contacted about providing environmental data. Laboratory work on understanding temperature-dependent metabolic costs would help improve the model. Another consideration is the possibility of local adaptation to warmer temperatures in the Eastern MED. An Individual Based Model (IBM) that incorporates larval drift, environmental conditions, and a larval survival model would also be an improvement. Comparing this index to deviations from the stock-recruit relationships from the assessment would give an idea of performance and increase the ability to detect the stock-recruit relationship. This kind of indicator could also provide information about regime shifts and could contribute to ecosystem report cards.

3.7 Larval sampling and processing for next-generation genetics, stock structure, and close-kin mark recapture (CKMR)

The group discussed the types of genetic analysis that could use larval samples and the best way to provide those samples. The point arose that for some analyses, in particular for CKMR, more samples might be needed than could be collected on the regular surveys and/or that it might be helpful to have samples from more locations than are currently samples. It was determined that it was important to maintain the current systematic sampling for the larval index, but that it might be desirable to add adaptive sampling strategies for other scientific needs. Adaptive sampling strategies could be incorporated in high density areas by doing some extra tows to accumulate additional larvae. The question arose of whether this would result in higher capture rate of siblings, which are less useful for CKMR. It was suggested that because spawning aggregations are big enough, the chance of sampling sibs is low; however, it is better to add stations than to increase effort at one station. McDowell *et al.* (2002) concludes that, although sibship increases, it's worthwhile to add extra sampling in high-density places. In addition, sibship may vary between regions because of size-dependent fecundity (e.g., GOM might have higher sibship than other regions because the biggest fish spawn there). NOAA has evaluated the issue of sibship levels in targeted vs dispersed surveys. An increase in sibship was observed in dense areas but not enough to negate the benefit of targeted surveys. It was determined that it would be worth validating this observation in the MED. Increased effort will be most important in areas with lower density of spawning, but would not be necessary in the Balearics, for example,

where regular sampling may provide enough larvae for CKMR. For the initial phase of CKMR, the priority is to sample large numbers of larvae. Collections from multiple spawning grounds could also be used to validate stock structure, which would then inform study design for recaptures.

There was some continued discussion about sample processing specifically for use in genetic analyses. It was recommended that the sample from one side of the bongos be preserved in ethanol for use in genetic studies. This increases the laboratory burden for processing the larvae. On board sorting is recommended, although some noted that sometimes sorting on board is not feasible due to the amount of plankton in the sample, the vessel schedule, etc. Potential costs will be discussed at the CKMR workshop, including the additional costs to the larval surveys for collecting/preserving/processing additional samples. One suggestion for increasing survey design efficiency was to perform the plankton tow first, then process/sort/identify samples while the ship is holding the station for the CTD vertical cast. During opportunistic sampling, sites with higher density and older/larger larvae should be prioritized. Older larvae have more tissue and are less likely to be siblings. It was noted that SEAMAP uses habitat modeling to add sampling stations in between those of the standard grid; this is a way to get more samples even if it is not possible to sort and identify them in real time.

3.8 Support for other surveys (additional to the GOM and Balearic surveys)

The group discussed various ways to support larval surveys apart from the well-established surveys in the GOM and around the Balearic Islands. It was felt that the group could play a helpful role in clearly delineating the benefits of larval surveys so that when funders are approached this information is clear. If the BFT WG recognizes the subgroup, this can be taken to NOAA and to relevant CPCs for funding considerations.

Some region-specific issues were discussed as detailed below.

Slope Sea

In the past the Slope Sea survey has mostly been run opportunistically in conjunction with the Atlantic Marine Assessment Program for Protected Species (AMAPPS) marine mammal surveys (approx. every 3 years), which also collect plankton data relevant for studies of planktivorous whales. Larval sampling is constrained by short night hours and the need to get back to the correct starting point in the morning to continue visual surveys. The plankton tows are performed with standard Bongo 60 nets fitted with 333□m mesh, with oblique tows to 200m depth. It is hoped that additional shallow tows can be incorporated in future survey efforts. In terms of expanding to more systematic annual and spatial sampling, there is not much additional ship time available through NOAA, so other platforms must be considered.

Levantine/Turkish area

No cruises are currently planned in this region, but funding for a regular monitoring survey will be proposed at a meeting next month. This would not be using competitive funding but would be a Turkish Ministry of Agriculture and Forestry funded program. There should be an answer regarding this funding in June 2023. The proposal includes annual smaller-scale surveys (e.g., near Antalya), with a large-scale survey every few years. Some opportunistic sampling with a professional fishing vessel may also be possible. Other funding opportunities (i.e., competitive funds) are also being sought. It was stated that establishing a regular monitoring program in the Eastern Mediterranean can provide critical information for determining the biomass of spawning contingent/stock in this area. This knowledge could be very helpful for species management.

It was noted that political boundaries restrict the possible sampling area, but the fishery tends not to go south so it's unknown if fish spawn there. There is some historical operational data from the area south of Cyprus that suggests this is a potential spawning area.

It was emphasized that the methods used by the Antalya and Orkinos cruises in 2018-2021 matched the standardized/accepted methods from the Balearic survey, and also results in a calculated CPUA, so this has potential to be integrated into ICCAT processes. Preliminary results from the surveys indicate that the same trends are observed in the Eastern and Western MED; however, it is not yet clear if there is a mixed stock or two stocks tracking the same environmental processes. It is possible that CKMR can help resolve this question; if there are half-siblings in the larvae from the different spawning areas, this is strong evidence of stock mixing. Identification of parent-offspring pairs (POPs) from CKMR will also help provide information about adults, abundance, etc., and provides a different way to think about tagging.

Malta/Tunisia/Italy/Central Mediterranean

No larval index surveys have been carried out yet by the local governments in the central Mediterranean. It was suggested that perhaps Tunisian or Italian vessels could cover the area, but it might be a good idea for Maltese scientist(s) to be involved in that sampling, maybe through some multi-lateral agreements. There are some European funds that are available for projects that involve collaboration with neighbor countries, for example, bilateral agreements Italy-Tunisia, Italy-Malta, and possible opportunities with Türkiye as well. It was noted that the Italian survey cannot expand the dates for the whole Central MED area, but that maybe a larger standardized survey could be achieved with two vessels. It was further noted that there is a Tunisian plan for ichthyoplankton sampling through 2023, but that the vessel has had some technical problems.

Italy

The Italian survey was originally undertaken to target/monitor small pelagics, but it has now expanded to also target large pelagics. There have been some problems with vessels. For example, there is one vessel that must be shared by all CNR scientists, which usually spends the summer in the Adriatic Sea. The next scheduled survey is in September, which is not ideal timing for BFT larvae or the small pelagics that are the primary focus of the monitoring survey. In addition, there is a spatial mismatch with greater BFT catches in the Ionian Sea and the eastern part of the current anchovy survey grid. There is currently a proposal to add stations to the eastern part of the standard sampling area. There was a suggestion that this survey might apply for funding through the EU, following the example of the TUNIBAL project. A national representative/point of contact needs to be identified.

It was noted that the MEDIAS acoustic survey is coastal but it reaches the shelf break and so it might be possible to take advantage of this regular monitoring survey to do nighttime sampling offshore during the cruise. There were some vessel constraints for nighttime work, so there would need to be further discussion with the MEDIAS team to assess the feasibility of this suggestion.

Other regions

A question was raised about Mexico's capacity for sampling and their interest in collaborations. It was determined that Dr. Estrella Malca would reach out to scientists there.

There is a UN initiative to increase oceanic studies (OCEANS-30), particularly in the high seas that might be of interest for various sampling programs.

There was also a discussion about the ICCAT focus on climate change and the need for resilience and forward-thinking for data collection: https://www.iccat.int/Documents/Recs/compendiopdf-e/2022-13-e.pdf. For example, larval surveys are helpful for other species besides BFT, and for understanding the ecosystem as a whole. It is important to leverage these climate change priorities from ICCAT to make it clear that the larval surveys can contribute to knowledge pertinent to this goal.

The group determined that a map should be produced showing where sampling is currently possible, the numbers of larvae currently being caught, and sizes. This would also be really useful for the CKMR workshop for planning purposes and would help motivate the surveys.

3.9 Integrating outputs into Management Strategy Evaluation (MSE)

There was some discussion about how to integrate larval indices into a Management Strategies Evaluation approach. The existing Atlantic Bluefin tuna MSE implicitly allows for environmental regime shifts but does not have explicit, mechanistic links to environmental process. Reglero *et al.* 2019 provides mechanistic, process-oriented approaches to linking environmental changes to larval survival within either assessment models (Sampedro *et al.* 2022 - EBFT assessment), similar to the Shutlzitski *et al.* 2018 larval survival index. This may be valuable for informing potential environmentally induced changes in productivity. Further, this may inform any future operating model reconditioning to include more explicit environmental linkages to specific biological process, which might allow for incorporation of climate change scenarios as well as the potential development of climate-informed management procedures, responding to ICCAT Rec 22-13.

3.10 Repository for relevant papers

It was suggested that GBYP create a repository for useful background papers as well as for papers presented during the workshop.

3.11 Other comments

It was noted that some experts in the field are quite skeptical of larval indices, so the group needs to think about how to address those criticisms going forward. Especially when coming to present a proposal to the assessment group. The following example was provided from the recent assessment:

> *1.1.2 Use of larval survey as an index of spawning biomass as noted, the rapid changes and recent increases in the estimated larval survey seems contrary to other indices of similar sized fish. That is, it seems very unlikely that the adult population could increase to the extent observed. The simple-minded analysis showed that the process error assumption would differ substantially from the other indices should the index be reliable. All of the models fit these data poorly with a serious pattern of negative results for the first several years. This is because the increase suggested by the Larval survey is inconsistent with most all other information on incoming recruitment (that would have contributed to the spawning biomass which this index is intended to apply). Using this index in any assessment as a proxy for adult spawning biomass. This is basically applying these data as if there were reliable egg-production sampling that has been done in other parts of the world for pelagic and other species. The track record for these data being useful within stock assessment settings is poor (e.g., see Armstrong et al., 2001). [from Iannelli 2023]*

It was also noted that it could be useful to look at sources of uncertainty, like a sensitivity analysis, and focus on which sources have higher impacts on the larval index.

4. Summary of proposed Early Life-History Group objectives and outputs

The group identified several objectives and proposed outputs for the Early Life-History Group, should it be developed further.

4.1 Group objectives

1. Provide more robust larval indices of abundance that are comparable between spawning grounds;
2. Serve as connection with the CKMR group to maximize sampling strategies;
3. Provide mechanistic based indices of larval survival [i.e., for the ICCAT ecosystem report card];
4. Facilitate a network for access to external funding.

4.2 Proposed outputs

1. Standardized protocol and guidelines for larval identification, sampling, sample processing and data analysis;
2. Standardized larval abundance in Western, Central and Eastern MED, the GOM, and the Slope Sea;
3. Preserve high quality larval samples for the CKMR group using standardized laboratory protocols;
4. Generate a larval survival time series for all spawning grounds;
5. Create a time series of ecological meaningful environmental variability indicators on spawning grounds for assessing regime shifts and anomalous years.

5. Action Items

The following action items were identified by the group:

1. Early life-history (ELH) working group coordinators will put together information on the extent of surveys for each area and the number of potential larval samples that could be made available for CKMR.
2. ELH coordinators will draft TORs that can be presented to the BFT working group; the next working group meeting is in September.
3. Everyone will share their protocols that can then be modified as needed by different groups. GBYP will set up a folder for shared documents.
4. Glenn Zapfe will work with Walter Ingram to determine which areas would be of most interest for adding sampling stations within the GOM for finer resolution.
5. Each team will identify a model for their region and run some Lagrangian tests to assess retention areas. Suggested 10 years of simulations.

6. Groups from each area can make a plot of gonadosomatic index and larval catch to identify as precisely as possible the seasonal timing of spawning.
7. Groups will share measurement protocols and agree on a common protocol.
8. A common larval identification guide for all Atlantic scombrids will be put together, with all stages represented and including pigmentation variability across regions. It was noted that Kristin Walter has photos of 3,000 BFT larvae that were genetically verified to species.
9. Each region should share length distributions (histograms) so that we can agree on the backcalculation method.
10. Sinan Mavruk and Walter Ingram will use some population simulations to generate larval length frequency pseudodata for sensitivity analysis of loss rate.
11. A larval survival method has been developed for the Baleares survey and software will be made available so simulations can be run in other regions as well.
12. MONGOOS will be contacted about providing environmental data
13. Estrella Malca will reach out to scientists in Mexico to determine their interest in collaboration and their sampling capacity.
14. A map will be made of where sampling is currently possible, the numbers of larvae currently being caught, and larval sizes to present to BFT working group.
15. GBYP will create a repository for relevant papers: those presented here, plus others that contain useful background information.

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Appendix 1

Workshop on Atlantic bluefin tuna larval indices
(*Palermo, Italy, 7-9 February 2023*)

Agenda

Opening Session:

9:00 – 10:00 Arrival & Registration

Greetings and introduction | 10:00
- Salvatore Capasso (CNR ISMed)
- Prof. Gabriele Carapezza Figlia (LUMSA University)

I part (day 7, 10:00 to 13:00 and 14:30 to 18:00)

Presentations on current status of larval surveys (survey strategies, sampling methodologies, biological samples and data analyses)

- Need for collaboration to improve the larval indices used for Management Strategy Evaluation (MSE) (Dr. Francisco Alemany)
- Using larval fish abundance to index spawning stock biomass (Dr. Walter Ingram, NOAA)
- Presentations by each team:
  - History and methods of the larval survey for Pacific Bluefin tuna (Dr. Atsushi Tawa and Dr. Yohei Tsukahara)
  - Bluefin tuna larval abundance indices in the Balearic Sea: History, methods, results, impacts (Dr. Diego Alvarez)
  - Estimation of offspring fitness in Atlantic bluefin tuna: a potential recruitment index (Dr. Patricia Reglero)
  - Ichthyoplankton surveys in Sicilian waters (Dr. Angela Cuttitta)
  - The BANSIC ichthyoplankton monitoring program: methods and ecological insights from Mediterranean studies (Dr. Marco Torri)
  - Tuna larvae in the Strait of Sicily (Dr. Stefania Russo)
  - Study of large pelagic fish in the Central Mediterranean Sea and projection under future climate change scenarios: molecular approaches (Dr. Marilena Di Natale)
  - Tuna larval surveys and BFT larval index in the GOM and Western Caribbean (Dr. Glenn Zapfe)
  - Bluefin tuna: adaptive surveys (Dr. Estrella Malca)
  - Tuna larval distribution throughout the western Atlantic: implications of a broad distribution to regional indices for the Slope Sea and Northern Gulf of Mexico (Dr. David Richardson)
  - Tuna larval surveys in Tunisian waters (Dr. Rafik Zarrad)
  - Tuna larval surveys in the Eastern Mediterranean (Dr. Sinan Mavruk & Dr. Banu Yalim)
  - Tuna larvae trophic ecology: implications for survival (Dr. Raul Laiz)
  - Making the most of your larvae: proven larval processing method to meet close kin genetics needs with flexibility to retain otolith and gut samples (Dr. Kristen Walter)

II part (days 8 and 9, from 10:00 to 13:00 and 14:30 to 18:00)

Standardizing methodologies and exploring the possibilities for implementing new BFT larval index surveys.

- New "larval" subgroup within BFT SCRS group
- Optimal survey strategies and tuna larvae sampling and processing methodologies
- Larval identification issues
- Data analysis for larval index calculations
- Improvement of existing indices
- Other potential larval derived indices: feasibility of recruitment indices
- Larval sampling and processing for next-generation genetics, stock structure and close-kin mark recapture
- Support for additional surveys
- Integrating outputs into Management Strategy Evaluation
- Miscellaneous

Appendix 2

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Appendix 3

Ethanol Buffering
Standard Operating Procedure
NOAA Miami, Florida

Equipment Needed:

- 200 proof/100% ethyl alcohol
- 5-gallon Nalgene carboy
- Tris buffer
- Graduated cylinder

Notes on buffering:

1. Plankton samples should be changed into buffered ethanol when displacement volume/biomass is measured
2. Prepare a well-marked carboy with buffered ethanol, to be used to fill squirt bottles and jars

Buffering Procedure:

1. Check that carboy is fully empty, to ensure accurate measurements
2. Fill carboy with fresh ethanol to the 5 gallon mark
3. Measure 125ml Tris buffer into graduated cylinder
4. Carefully pour buffer into carboy
5. Screw cap tightly on carboy, and carefully invert 2-3 times to mix
6. Allow to settle for 24 hours
7. When performing displacement volume/biomass procedure, use well-marked squirt bottles and carboys for rinsing and filling of the sample. Discard used unbuffered ethanol.
8. Label jar/squirt bottle/carboy as “buff EtOH” (internal label also)