REPORT OF THE ICCAT GBYP WORKSHOP ON ATLANTIC BLUEFIN TUNA REPRODUCTIVE BIOLOGY

Anonymous

SUMMARY

ICCAT GBYP Workshop on Atlantic Bluefin Tuna Reproductive Biology was held in Madrid, 26-28 November 2018. It was attended by numerous experts in the field who gave presentations and discussed on various topics including discrepancies in eastern/western reproductive parameters, reproductive physiology, reproduction in captivity, larval ecology, spawning habitat modelling, life history, effects of fisheries practices on sampling and implication on MSE and assessment.

RÉSUMÉ

L'atelier du GBYP de l'ICCAT sur la biologie reproductive du thon rouge de l'Atlantique s'est tenu à Madrid du 26 au 28 novembre 2018. Il a réuni de nombreux experts dans ce domaine qui ont fait des présentations et discuté de divers sujets, y compris les écarts dans les paramètres de reproduction de l'Est et de l'Ouest, la physiologie de la reproduction, la reproduction en captivité, l'écologie larvaire, la modélisation de l'habitat de frai, le cycle vital, les effets des pratiques de pêche sur l'échantillonnage et les implications sur la MSE et l'évaluation.
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RESUMEN

El Taller de ICCAT sobre biología de reproductiva del atún rojo del Atlántico se celebró en Madrid, del 26 al 28 de noviembre de 2018. Asistieron numerosos expertos en la materia que expusieron presentaciones y debatieron diversos temas, entre ellos las discrepancias en los parámetros reproductivos del este y el oeste, la fisiología de la reproducción, la reproducción en cautividad, la ecología de las larvas, la modelación del hábitat de desove, el ciclo vital, los efectos de las prácticas pesqueras en el muestreo y sus repercusiones en la MSE y en la evaluación.

KEYWORDS

Bluefin tuna, biological studies, sexual maturity, spawning fraction

1. Opening, adoption of the agenda and meeting arrangements

The ICCAT GBYP Workshop on Atlantic bluefin tuna reproductive biology was held from 26-28 November 2018, at ICCAT Headquarters in Madrid.

The Workshop was opened by Mr. Camille Jean Pierre Manel, ICCAT Executive Secretary, who welcomed all the participants (the list of participants is given in the **Appendix 1**) and wished them to have fruitful discussions with strong outcomes. The GBYP Coordinator welcomed the participants and presented the tentative agenda (**Appendix 2**), which was adopted with some changes in the presentations order.

2. Appointment of the Chairman and sessions rapporteurs

The participants nominated Ana Gordoa (E-BFT Rapporteur) to chair the meeting.

The following served as rapporteurs:

Section I, II (first part) – Enrique Rodríguez Marín, Dheeraj Busawon
Section II (second part), III, IV - Haritz Arrizabalaga, André Boustany
Section V, VI- Patricia Reglero, Ashley Pacicco
Section VII, VIII – Diego Álvarez Berastegui, Ashley Pacicco
General discussion – Ashley Pacicco

3. Presentations and discussions

3.1 Report of the independent reviewers on the discrepancies in eastern/western reproductive parameters

Jessica Farley and Seiji Oshimo: Review and Insights into the Differences in Reproductive Parameter Estimates between Eastern and Western Atlantic Bluefin Tunas Stocks

This presentation provided a review of reproductive studies for eastern and western stocks of Atlantic bluefin tuna (ABFT), focusing primarily on age-at-maturity/spawning estimates used in stock assessments. The maturity ogive used in the East assessment is based on a study of ovaries collected from multiple sampling locations within the Mediterranean Sea and used an appropriate histological classification scheme to differentiate mature from immature females. However, the low number of immature females sampled, and the sharp change in the maturity ogive from 0% immature to >50% mature at ~100 cm fork length suggests that the sampling may be biased towards mature females and that the spatial variation in the distribution of immature and mature fish both within the Mediterranean Sea and the Atlantic Ocean needs to be accounted for. Western studies of ABFT maturity apply three methods; analysis of ovary histological, endocrine (hormone) and catch data, using samples/data from the Gulf of Mexico (GOM) and the NW Atlantic. The spawning fraction ogive used in the West stock assessment is based on the analysis of sex-combined catch data from the GOM and assumes all fish caught are mature and that the GOM is the main spawning ground for the western stock. The ogive will be biased if fish younger than those present in the Gulf of Mexico spawn elsewhere. Speakers noted that the two ogives were measuring different parameters (proportion mature vs spawning fraction) and suggested that differences in maturity ogives for the stocks may not be as large as currently observe. They recommended, based on other tunas maturity studies, that comprehensive reproductive studies using consistent methods across ocean basins are undertaken through large-scale collaborative sampling programs, using precise criteria to differentiate mature and immature fish (e.g. maturity markers in ovary histology) and applying appropriate statistical analysis methods. The authors suggested that it may be possible to obtain an independent maturity ogive for each stock through a well-designed, length stratified, sampling program throughout areas where both immature and mature females are present.

In the following discussion it was argued that introducing additional samples of immature fish from the Mediterranean to the eastern maturity ogive would not change its shape. The speaker responded noting that the maturity ogive would change if large numbers of immature fish of bigger sizes were caught/sampled within or outside the Mediterranean. The young spawners sampled on the Mediterranean spawning grounds may represent only a small fraction of those sizes classes, with the rest being immature in feeding areas in the Mediterranean or in the Atlantic. The length of time that maturity markers (MM) remain visible in the ovary after spawning was also discussed. It was noted that MM are only visible in ABFT ovaries for a month. However, the speaker noted that MM have been identified in other tuna species, including southern bluefin tuna, for much longer periods of time

(>4 months) and research is needed to assess these markers in ABFT. Questions were also raised about how to study young potential spawners outside the spawning grounds. Farming facilities were suggested as an option for performing reproductive biology research (e.g. MM), although certain experiments may be biased because of culture conditions (e.g. earlier maturity). A participant asked which consistent methods should be applied to both stocks and whether east and west ogives could be similar. Speaker answered that blind reading of ovary histological based on broad sampling programs (length, months and locations) could be applied and that ogives could be different for ABFT stocks. A participant remarked that not all the available references were taken into account by the study.

A second presentation provided overview of known biological parameters and management measures for Pacific bluefin tuna (*Thunnus orientalis)*, as a comparison to ABFT. Pacific bluefin tuna has two main grounds like Atlantic bluefin tuna. One is the Nansei area and the other is the Sea of Japan. The spawning grounds of this species are limited to the western Pacific. A new spawning ground, Sanriku area, has been reported recently. Spawning grounds of Pacific bluefin tuna are segregated by age or size. The reproductive parameters of Pacific bluefin tuna are different by area as they are for Atlantic bluefin tuna. Studies in the three Pacific spawning areas have used ovary histology for estimating the proportion mature-at-length/age. Maturation ogives in eastern and western Atlantic were estimated through catch data analysis and ovary histology, respectively. As the analysis methods are quite different, maturity cannot be compared directly. Maturity ogive differences between Pacific areas are similar to those from the Atlantic. These differences of reproduction parameters might be caused by the differences of spawning strategy by size/age or migration (resident-migrant mixing).

Despite having found different spawning grounds, Pacific bluefin tuna is managed as a single stock. One participant commented that there is a continuous spawning gradient with decreasing size from southern to northern spawning grounds. It was also disputed if the fishing gear (sampler) can cause bias by catching different size compositions of fish, since purse seine catch smaller fish in the northern Pacific spawning ground, meanwhile long-line is catching bigger fish in the southern grounds.

3.2 Reproductive physiology (Endocrinology: Behaviour vs endocrine profile; Hormonal and social basis of skipped spawning)

Antonio Medina: Reproduction of Atlantic bluefin tuna. Are eastern and western stocks so markedly different?

This presentation reviewed key factors that drive the reproductive performance and behaviour of ABFT. Attention was largely focused on biotic factors that directly influence ABFT reproductive potential (maturity at age, spawning frequency, fecundity, sex-ratio at age), especially in the eastern stock, though mention was also made on exogenous factors that play a role in the species' reproductive life history (food abundance and availability, migration patterns, spawning habitat selection, and egg/larval distribution and dispersal). A number of endogenous variables (e.g., spawning omission, egg and offspring viability and survivorship, parental effect) remain poorly investigated due to the enormous challenge of implementing the required research surveys across the distribution range and over time. These aspects, however, might significantly affect the reproductive performance and productivity of the stocks; therefore, further attention should be paid to such topics. There is a major debate as to the extent of similarity/disparity in reproductive traits between eastern and western ABFT stocks. The two stocks are considered to display distinct reproductive strategies that are exemplified, among others, by earlier maturation in the eastern population. Large differences may thus result in higher spawning rates, and hence higher reproductive output, in the eastern stock. Yet, similarities have been found in major reproductive aspects suggesting that discrepancies between stocks may not be as broad as often assumed. Future research actions towards bridging current gaps in ABFT reproduction science were proposed.

It was argued that finding mature bluefin may not imply that those specimens were spawning. It was also noted that batch fecundity, spawning frequency and periodicity estimations were influenced by the fishing gears selectivity and fleet behaviour. Purse seiners capture bluefin tuna that is distributed close to the surface and are actively spawning, while longliners capture specimens over a broader area and show lower spawning activity. This implies that sampling by multiple gears sampling may introduce bias in parameter estimation. It was also observed that diving behavior obtained through electronic tags can be used to detect spawning. The Strait of Gibraltar seems to be an area with a resident population whose distribution is modified during the spawning season. This fact has also been observed in other areas of the Mediterranean. An acoustic curtain in the Strait of Gibraltar could provide insight into spawning migrations, but maritime circulation and military constrains makes deployment difficult.

Aldo Corriero: First sexual maturity in the Atlantic bluefin tuna, Thunnus thynnus

Efforts to determine the reproductive state of ABFT have been carried out for several decades using different methods: staging of gonads according to arbitrary macroscopic scales; evaluation of the seasonal changes in gonadal relative mass (Gonadosomatic Index); gonad histological analysis; and quantitative analysis of maturity-related hormones and vitellogenin. The available information converges to indicate that, in the Mediterranean Sea, ABFT reproduce for the first time at the age of 3 to 5 years. Tuna farming, as well as several domestication experiments carried out in Spain and Italy, provided further and indisputable evidences that 3-4 years old ABFT undergo vitellogenesis and spawn in captivity, either spontaneously or after hormonal stimulation. Recently, 9 males and 8 females 134-185 cm CFL (age ≥5 years) were found to have a pituitary gonadotropin content similar to that of Mediterranean spawners, thus conforming historical observations reporting mature gonads in age 4 and 5 individuals caught in the western Atlantic and providing further evidence that younger adults spawn outside the GOM.

A question was raised about the possibility of obtaining more samples to improve the Mediterranean maturity ogive, given that they may be spatial variation in the proportion mature. It was clarified that genetic studies do not show different populations within the Mediterranean, although there may be different contingents with different spawning behaviour.

Samar Saber: Are the tuna researchers using the same maturity scales?

The objective of this presentation was to point out some difficulties found in macroscopic maturity staging, and the importance of agreeing on the descriptions of maturity stages given in maturity tables. The assessment and management of fish populations relies on the correct estimation of the spawning stock biomass, which is based on maturity ogives of the population. Maturity ogives are usually estimated through macroscopic maturity data, i.e. the maturity stage of fish based on macroscopic observation (visual stating method) of the gonads. A more accurate evaluation of the reproductive gonad stages requires histological examination. It is important that the maturity scale used for each species is consistent across the laboratories and countries involved in stock assessment.

The discussion highlighted the importance of standardization of the descriptions of maturity stages (macro and microscopic). Different studies have been conducted in the past, but not all of them used the same classification, and the comparison of results becomes a non-trivial exercise. Using the same terminology is important, especially when comparing results and making joint analyses. The Group recommended that multiple reads of gonad histology samples are undertaken across labs. The group noted that the ICCAT manual also needs to be updated (maturity table and whole chapter, including standardization of terminology).

Hanna Rosenfeld: Timing of puberty in Atlantic bluefin tuna - the endocrine approach

A recent European study that closely followed captive-reared ABFT undergoing first sexual maturity, has successfully established a novel endocrine criteria for differentiating sexual-immature from sexual-mature specimens. However, no robust conclusion could be drawn addressing whether the observed puberty in 3-year (3Y) ABFT males is a general feature also occurring in wild populations, or a phenomenon induced by the culture conditions. Measurements of the two gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are the pivotal regulators of gonadal development and gamete maturation, revealed a clear discrepancy in the pituitary FSH to LH ratios in immature vs. mature ABFT. In summary, the useful FSH/LH criteria for screening sexual maturity among ABFT populations exemplifies the potential of the endocrine approach to identify clinical biomarkers for assessing a vast array of reproductive traits. Following the same rational, future studies combining new advances in genetics and endocrinology may further our understanding of the interplay between energetic balance, growth and maturation in ABFT. As a result, benchmarks could be determined enabling discrimination between ABFT populations that successfully spawn and those who fail to complete their reproductive cycle. Such criteria will, ultimately, help resolve the controversy of "skipped spawning phenomena" among western ABFT stocks.

The group discussed on vertical behaviour before spawning, noting that in the wild, apparently they cross the thermocline just before spawning. Knowledge on this issue from Pacific bluefin tuna kept in captivity as well as farmed and wild Atlantic bluefin tuna in the Mediterranean was shared among the group. It was discussed that the range of spawning behaviors observed in captive fish may not capture the full range of behaviors in wild fish, due to limited space in the cages. It was mentioned that in cages it has been observed that, while eggs are being produced, storms can suddenly stop reproduction of ABFT.

Gilad Heinisch: *Sexual maturation in western Atlantic Bluefin tuna and other perciforms*

Sexual maturity is characterized by energy allocation from growth to reproduction and is regulated by key hormones. Among the morphological-physiological changes that are linked to sexual maturation are growth inhibition and changes in pituitary gonadotropins profiles. The study performed histological and endocrine analyses to investigate sexual maturation in 93 mature and presumably immature ABFT (134–292 cm curved fork length, CFL) sampled on NW Atlantic foraging grounds, and in seventeen young of the year (YOY) sampled off Virginia. The results indicated that spawning during the previous or the next reproduction season was possible for all fish > 134 cm CFL, confirming their status as mature. These results do not support the postponed maturity schedules assumed for western ABFT stock, and suggest that alternative spawning scenarios, such as early maturation and additional spawning grounds, are likely.

The group noted that maturity (what is studied in this piece of research) means that the fish are capable of spawning, and the results cannot confirm whether they did actually spawn or not. The study was not based on samples taken during the spawning season, but just after (June to October), and the authors assumed that the fish they detected to be mature could spawn several months before or after the analysis was conducted. The authors confirmed that they did not perform genetic assignments of the individuals, so the possibility that they belonged to the Mediterranean population exists. However, the authors noted that the area where the samples were taken (Gulf of Maine) was extensively studied for fat contents, movements, energetics etc, which allowed to have additional supplementary information around the maturity study conducted.

Ashley Pacicco: A histological assessment of Atlantic bluefin tuna gonads sampled in the Northwest Atlantic from 2007-2017

An updated histological assessment of Atlantic bluefin tuna gonads in the northwest Atlantic was performed in order to address the uncertainties in the most recent bluefin tuna stock assessment. Reproductive analyses were conducted using histological examination of male (48%) and female (52%) gonads. Ages were determined using sectioned otoliths. Ovaries were observed in the spawning capable phase (tertiary vitellogenesis) during March-July in the Gulf of Mexico (n=69), south ATL (n=6), mid-ATL(n=4), and the Gulf of Maine (n=2) and the actively spawning sub-phase (post ovulatory follicles (POFs) and/or final oocyte maturation) in the GOM (n=106), south-ATL (n=2), and the mid ATL (n=2) from April-July. May and June had the highest estimated spawning fraction (the total number of ovaries with POFs in each month/all females in each month) among all months of 64% and 33%, respectively. Ovaries classified in the spawning capable and/or actively spawning sub-phase ranged in size from 185-307 +/- 2.96 SD cm curved fork length (CFL), with assigned ages from 8-33 years. Length at 50% (L50) and 95% (L95) sexual maturity, defined as females which are capable of being reproductively active (beginning at the onset of vitellogenesis in the ovary), was estimated at 172 cm +/-31 SD and 216 cm +/-40 SD CFL, respectively. The inflection point where whole gonad weight increased relative to CFL occurred around 216 cm, comparable to L95. Testes were observed in the spawning capable phase from February-September in all regions except Nova Scotia. Males in the spawning capable phase ranged in length from 105-328 +/- 55 SD cm CFL and had assigned ages between 2-21 years. Increased sampling coverage of Atlantic bluefin tuna gonads is warranted throughout the NW Atlantic for a comprehensive analyses of spawning seasonality and sexual maturity.

This study provided new information on gonad histology of individuals from the GOM and the NW Atlantic. The group noted that samples collected from bluefin tuna captured outside the GOM (throughout the U.S Atlantic) were limited, and agreed that improved spatial and temporal coverage were vital in correctly interpreting future data. Specifically, the group felt that additional samples from pelagic longliners operating in the Atlantic would be quite useful. The group also suggested to use alternative maturity thresholds(e.g. Vitellogenesis 3 instead of Vitellogenesis 1) and see their impact on the maturity ogive. It was noted that Vitellogenesis 3 was used as the indicator of maturity in SBT and other tuna species.

Ivan Katavić: New findings on the onset of gonadal maturation of Eastern Atlantic Bluefin tuna in the Adriatic Sea

The gonads of 91 females and 95 males of eastern ABFT captured in the central Adriatic from May 26 to June 08 in 2017 and 2018 were analyzed histologically. The age analysis based on the count of dorsal spine readings showed that the vitellogenic oocytes were present in 16% of 2 years old specimens at average body size 83.2 cm fork length (FL) and 12.5 kg round weight (RWT). Three years old females at mean body size 97.1 cm FL and 18.1 kg RWT were all in the process of maturation, whereas 75% in the advanced maturational stage, some with hydrated oocytes present. The beginning of spermatogenesis of the 2 years males was recorded in 33% of bluefin tuna (mean FL 80.6 cm, RWT 10.6 kg), and 89% in 3 years bluefin tuna specimens (mean FL 96.7 cm, RWT 16.9

kg), respectively. The authors conclude that the eastern Atlantic Bluefin tuna is physiologically ready to start gametogenesis in just two to three years, indicating the capacity to produce ripe gametes in the early juvenile stage which process might reach its end with spawning under adequate environmental conditions.

This study suggested that the Adriatic might be an opportunistic spawning ground for BFT, based on macroscopic evaluation of the gonad maturity stage, that showed that during late May and early June some ABFT caught in the Adriatic Sea were reproductively active (hydrated oocytes, milt in seminal ductus). The group agreed that wider sampling of fish from the Adriatic, paired with histological sampling would be beneficial.

3.3 Tuna aquaculture/reproduction (Factors contributing to spawning success -age at maturity, egg fitness; Hormonal profile of spawners; Spawning frequency; Fecundity; Parental contribution)

Fernando de la Gándara: Recruits from farmed ABFT in Murcia?

It has been demonstrated, at least in the Murcia area, that the captive ABFT for fattening activities reproduce actively in the farming cages in the natural spawning season (early June – middle July). Tens of millions of fertilized eggs from these cages had been collected over the last few years and some have been cultured in the facilities of the Spanish Institute of Oceanography (IEO) in Mazarrón (Murcia, SE Spain) and grown up to juveniles, demonstrating their viability. Taking into account that an ABFT female could spawn roughly hundreds of thousands of eggs per kg during the spawning season and thousands of tons of ABFT adults have been farmed in the Murcia coast during the last years, the total fertilized eggs could reach hundreds of billions every year. Obviously the conditions in the Murcia coast are different to the natural spawning areas, such as in regard to food availability but also the massive presence of many egg and larvae predators present in the wild. It was suggested that prospecting surveys could be undertaken with the aim of shedding light on this possible effect of farmed ABFT on recruitment.

While the group found this study to be informative towards understanding spawning dynamics in ABT, there were questions about the representativeness of these studies to wild fish given the captive fish were treated with hormones. It was noted that spawning was demonstrated in purse seine caught fish in the Balearics without hormone treatment.

Aurelio Ortega: Reproduction of ABFT in captivity. The role of land based facilities improving knowledge

Over the last ten years, and in the frame of different projects, captive spawning of ABFT in cages in Murcia (SE Spain) has been achieved by IEO. Some hundreds of millions of fertilized eggs from different broodstock and also from recently captured tunas from Balearic Island and owned by Tuna Graso, have been obtained. However, knowledge of the reproductive biology of these tunas is limited. This presentation showed some of the results obtained from studies, particularly with a way to solve the observed gaps in order to get a deeper understanding about the reproductive biology of ABFT by using the new land based facilities that IEO owns in Cartagena (SE Spain). This facility called ICRA (Infrastructure for the Control of Reproduction of ABFT) currently holds 70 1+ juveniles (mean weight 15 kg) and 200 0+ juveniles (1,5 kg) kept in two different tanks and it is expected that the eldest tuna will begin to reproduce in 2020 season.

Members of the group noted that cessation of spawning due to sudden changes in environmental temperature is also believed to occur in wild fish, based on studies of the presence of larvae correlated with environmental conditions. There was some discussion on the diel patterns of spawning in wild fish, with the observed patterns that are highly influenced by estimation methodology. Direct observation of spawning from plane and ship based surveys suggested that spawning occurs during the day, but direct observations of spawning in cages, histological sampling of spawning fish, and behavior data recorded from electronic tags suggest most spawning takes place at night. It was noted that the optimal temperature for spawning (~23 C) based on fertilization rates, hatching rates, and presence of developmental issues in the larvae, is cooler than those temperatures over which much spawning in wild fish is observed to occur.

3.4 Larval ecology (Contribution to and validation of spawning habitat models)

Patricia Reglero: Presentation of the Advances on the Research Conducted to Identify Spawning and Larval Habitats in the Western Mediterranean

This presentation provided analysis on how offspring fitness constrains spawning phenology in Atlantic bluefin tuna. The evidence was combined from long-term field data, temperature-controlled rearing experiments on eggs and larvae and a model of egg fitness, and the results showed that Atlantic bluefin tuna does not spawn to optimize egg and larval temperature exposure. The timing of spawning leads to temperature exposure considerably lower than optimal at all spawning grounds across the Atlantic Ocean. The potential alternative drivers to temperature that set time constraints for reproduction and prevent later spawning from the offspring and the adults' point of view were described. Highlighting those processes that are more likely to drive bluefin tuna spawning phenology can help us to identify the areas that need further research to understand the spawning dynamics in this species.

In addition to the hypotheses raised in the presentation on drivers of the timing of spawning relative to the "optimal" environmental conditions (prey abundance, predator avoidance, thermal tolerance of adults, trying to maximize growth before winter) it was suggested that peak spawning may occur earlier than the thermal optimal window as a way to minimize cannibalism. Under such a scenario, there would be an evolutionary pressure to spawn as early as possible so that the larvae could reach post-flexion before the larvae of other species. It was also noted that, during warm years in the Mediterranean Sea, spawning occurs both earlier and later than usual. In response to this, it was commented that while this may be true, there is likely to be little spawning and/or larval survival below 19º C.

Rafik Zarrad: Evidence of Bluefin Tuna Spawning in the Central Mediterranean

The presentation showed the results of survey carried out from 23rd June to 9th July2008 off the eastern coast of Tunisia. The ABFT larvae were abundant in the offshore stations where they were concentrated on the oceanic side of an eddy, at the limits of the shelf break, 50 to 90 miles from the Tunisian coasts and 30 to 40 miles from Pelagie Islands (Lampione and Lampedusa). The stations of occurrence of *T. thynnus* larvae formed a continuous area. *T. thynnus* exhibited a high positive correlation with depth, SST and zooplankton biomass

After the presentation, the presenter informed the group that spawning was also seen around farms in Tunisia, but no attempt was made to collect eggs. The author further clarified that the presentation summarized data from a single survey, but there are data from additional surveys available.
The group noted that although eggs cannot be visually analyzed, they can be genetically analyzed, and can also be used, in addition to larvae, to study the spawning habitat.

3.5 Spawning habitat modelling (Definition of potential spawning areas from knowledge on larval ecology and data on spawners distribution during reproductive period)

David E. Richardson: An update on research on Atlantic bluefin spawning and early life history in the Slope Sea

Atlantic bluefin tuna are generally depicted as having two spawning grounds, one in the Gulf of Mexico and the other in the Mediterranean Sea. However, in 2013 early-stage bluefin tuna larvae were collected during opportunistic sampling in the Slope Sea off the northeast United States, greater than 2000 km from the Gulf of Mexico. Larval abundances during the 2013 Slope Sea sampling exceeded average levels in the Gulf of Mexico. These larval collections, when combined with electronic tagging data and reproductive studies, were consistent with size-structured spawning across a wide area in the western Atlantic, a life history model first proposed in the late 1950s. Here the authors present the results of further sampling in the Slope Sea in 2016. Larval bluefin tuna abundances during the 2016 sampling were similar to the 2013 sampling, and again indicated that the Slope Sea is an important western Atlantic spawning ground. The results were presented of an examination of archived 1976 Slope Sea samples housed at the Harvard Museum of Comparative Zoology, which contained bluefin tuna early juveniles (< 40 mm), and an examination of larval bluefin tuna growth rates in the Slope Sea. Overall, these updated results highlight the importance of continuing research into bluefin tuna spawning in the Slope Sea, and the need incorporate this additional spawning ground in the development of modelling and population structure studies.

This study provided a summary of Atlantic bluefin tuna larvae in the Slope Sea (published in Richardson et al. 2016), further evidence of bluefin tuna larvae being caught in the same area after 2013, review of historical reports of reproductive fish from commercial fisheries in the past in the area, evidence of post-flexion larvae or juveniles of bluefin tuna from analyses from museum-samples, similar growth rates between the GOM and Slope Sea larvae from comparative otolith analyses. It was suggested a significant amount of spawning occurs outside the Gulf of Mexico. It was emphasized the need of a cruise targeting bluefin tuna larvae in the Slope Sea. The group questioned where the nursery habitats (age 0-1 BFT) are located in the area. It was suggested the nursery areas are usually limited to the coastal areas, but more work is needed to locate nurseries in this area. The overall consensus was positive and the group was pleased with the new data since the original paper. It was applauded in the efforts of the collaborators in taking advantage of cruises when possible. A long term, consistent sampling program for the larvae is desired.

Diego Alvarez Berastegui: *Linking bluefin tuna spawning and larval habitats with mesoscale oceanography in the Western Mediterranean*

The Balearic Sea is one major spawning ground of bluefin tuna in the Mediterranean. The historical research on early life stages in the area, and the modern oceanographic observation systems are now joined to advance the understanding of how the environmental variability affects tuna spawning and its early life ecology. The studies linking the spatial distribution of bluefin tuna larval with the oceanographic scenarios, and show a relevant relationship between the ocean dynamics and the spawning and larval habitats. Very recent advances on high resolution hydrodynamic modelling has shown that the Balearic Sea present unique hydrographic characteristics in the Western Mediterranean Sea during the spawning season, which could provide new insight on the ecological drivers that explain the restricted distribution of bluefin tuna spawning grounds. The results are discussed and compared to other results obtained in the Gulf of Mexico.

This study showed a summary of results of research conducted after collaboration between oceanographers and ecologists. The study reviewed spawning strategies for different tuna species, analyses of having oceanographic data at different spatial scale, review of operational model for larval habitats, the incorporation of habitat to estimate the larval index as a proxy for spawning stock biomass, retention studies and potential larval habitats in the Atlantic Ocean based on relationships between oceanography and larval occurrence in the Gulf of Mexico and Mediterranean.

Consensus of the group was that there is no good recruitment data. There was discussion on how to incorporate habitat data and recruitment in the assessment models and how to test the effect in the assessments. The group agreed on the complexity of the Mediterranean and the need to improve knowledge of the use of the different spawning grounds within the Mediterranean. It was raised as a concern that using the larval index only from the GOM is biased if the Slope Sea is a major spawning area, as well as other potential spawning areas in the Atlantic Ocean. The question on what makes an adult BFT to go somewhere to spawn was raised. It was suggested that if we can understand variability in the spawning grounds then we can standardize better abundance and the variability.

Samar Saber: A possible new spawning area for bluefin tuna (Thunnus thynnus) in southern Gulf of Mexico

This study showed results from gonad analyses in Atlantic bluefin tuna adults captured by the Mexican longline fleet targeting yellowfin tuna between January-April 2015 at temperatures between 22-27ºC. Histological analysis indicates that the spawning activity in Mexican waters occurs in March and April, when the sea surface temperature was 25.5°C ± 0.687 in March and 27°C ± 0.685 in April. The results confirmed spawning in the southern Gulf of Mexico, although additional studies are needed to improve the understanding of migratory patterns.

Conducting larval surveys in the area was suggested but it would only be possible if funding was available. It was reiterated that hand drawn figures from the pasts have already suggested spawning in that area, as well as in Africa and Brazil, which are probably areas that will be “re-discovered” in the future. The Slope Sea, Canada, and north of Bermuda have been suggested as spawning grounds already in the past. Tagging data align with Saber’s results.

3.6 Life history (implications on reproduction)

Molly Lutcavage: Atlantic bluefin tuna life history: Linking Energetics, Reproduction and Migration

The presentation provided the review of ABFT life history and old documentation of other potential spawning grounds. There are multiple spawning areas and times for ABFT in the western Atlantic. Based on energetic allocations from life history models it was suggested that smaller fish will spawn closer to their foraging grounds and the decision to skip spawning is suggested to be limited to fish in earliest years of sexual maturity/puberty. Skipped spawning was suggested to be unlikely and it was not reflected in the growth curves (same growth curves for all). Tracks (even with more than 300 days at liberty) show fish that did not visit assumed spawning locations such as the GOM and Med. The assumption of fidelity to natal origin may be a question, as the assumption that there are only few spawning areas located inside the Mediterranean and the GOM. The need of biological assumptions used in ABFT assessment to be consistent with life history and physiology was emphasized.

It was suggested that SCRS needs to consider the non-entrance of adult ABFT into the GOM and the Med. It was said that it is unknown which stock these fish belong to. It was discussed if it is energetically possible to spawn more than once, and it was concluded that it would be difficult to build gonads to a spawn state more than once during a year, but that needs to be confirmed. Aquaculture and more research may be able to give insight on whether it is possible for the same adult spawn more than once in a year. It was discussed that there is some evidence of females being able to stop spawning and spawning again two months later, but further information was not available. Similar evidence was available for a small school of tunas in the central Mediterranean in 2011.

Barbara Block: Electronic Tagging & Genetics reveal life history and spawning information on Bluefin tunas

The Stanford University Tag-A-Giant (TAG) research program has electronically tagged ABT since 1996. To date, 1,400 electronic tags (internal archivals, pop-up archival satellite (PAT), and acoustic tags) have been deployed on adolescent and mature fish primarily in the western Atlantic Ocean along the eastern seaboard of North America. Seventy-five electronic tags have been put out in the Mediterranean Sea and in the eastern Atlantic along the coasts of Morocco and Ireland. This electronic tagging effort has created a detailed time series for ABT from 4 to 25 years of age, with over 40,000 tracking days from western Atlantic releases and 50,000 acoustic detections. Tracks from 1 to 5.5 years have been obtained and the TAG team can obtain annual tracks with PAT satellite tags attached to giant bluefin tuna as they move between foraging and spawning areas. Archival tags require recovery- and to date 23% of the deployed archival tags (n=737) have been recovered. These valuable data sets, once processed, have been made available to other modelling teams through the GBYP data recovery program. Maintaining the data stream requires a continuous effort that begins the year of tagging, and ends when data is recovered upon tag recovery, processed and then archived. It is often a multi-year process for a single data set from deployment date to the final archive date. Population assignments of a proportion of the ABT from the western Atlantic with track data have been possible using genetics of fin clips taken during tagging events. Assignments to a population are also possible (although not 100% sure) when mature electronically tagged fish visit a known spawning site. Models incorporating these types of data have improved the capacity to predict spatial habitat use by population, maturity, natural and fisheries mortality, and to impact our capacity to project biomass trajectories. Information on the time of first maturity is also garnered if tags are on sub-adult fish prior to making a spawning migration. For ABT, critical aspects of the current stock assessment models require inputs of natural and fisheries mortality that are vital to modelling predictions of recruitment and biomass. Electronic tagging data has provided new tools for assessing and modelling natural and fisheries mortality in the context of northern bluefin tunas as well as other large pelagics. New models with tag data indicate that empirical estimates of natural mortality from tagging data are possible, and it is possible to assign-cohort specific values of M for large ABT tagged in Canadian waters. Electronic tagging can also inform scientists about the energetics and foraging success of bluefin tuna, and increase our understanding of different physiological stresses (e.g. reproduction energetic costs for females) which may lead to earlier senescence for females and impact mortality rates and distributions as fish age. The archival and satellite tags have also been used to improve the capacity for spatial modelling with the Mast and M3 mixing models.

There was a discussion about the ABFT in the Eastern Mediterranean being resident and adapted to higher temperatures so that they may be genetically different and, if so, new genetic technology, besides new tagging data from the Eastern Med could help in confirming this hypothesis. It was also noted that available information from previous genetic analyses carried out within GBYP do not support this hypothesis. Before using archival tags, however, the potential recapture rate, the size of the year class among other factors should be considered. The time and location of tagging along the northwestern Atlantic coast was discussed. It was noted that, according to the spawning behavior from the tracks, there are some fish from the GOM which migrate to Canada without apparent

spawning in the Slope Sea, without disagreeing that other fish may spawn in the Slope Sea. It was noted that the key to conservation of BFT is to protect biodiversity if they are to survive a changing climate. In this sense, it was suggested that new populations could be recognized in the future and that using genetics to identify them was a good approach.

Haritz Arrizabalaga: BFT reproductive biology: an Atlantic perspective

This presentation showed the first results of multiple research lines developed in the Atlantic. The results from 7 archival tags deployed on juveniles in the Bay of Biscay (including those published in Arregi et al. 2018) were presented, to inform on when the BFT entered the Mediterranean for the first time. The results show Atlantic residency until at least age 5 and migrations into the Mediterranean between age 6 and 8-11. On the other hand, gonad analyses in BFT caught in the Bay of Biscay (n=49) suggest a delayed maturity schedule compared to the one assumed for the Mediterranean. The study also suggested ways to use hard parts to inform about potential reproductive migrations and/or events. Acknowledging that current sample sizes are low, insights were provided for future research required to solve the reproductive biology issue on BFT.

It was suggested that fish tagged early and late in the season could potentially show differences with respect to entries into the Mediterranean, but the authors clarified that all fish tracked were tagged in a similar period, so this should not be an issue. It was also asked if juveniles tagged could have arrived from other areas than the Mediterranean. The authors clarified that both otolith chemistry and genetic analyses suggest that juveniles in the Bay of Biscay are mostly from the Mediterranean, although there are no tools yet available to identify potential Slope Sea natal origin.

3.7 ABFT fisheries (Feasibility of sampling, temporal changes in the exploitation, factors that can influence our ability to understand spawning structure/dynamics)

Antonio Di Natale: ABFT Fisheries: Temporal Changes in The Exploitation Patterns, Feasibility of Sampling, Factors That Can Influence Our Ability to Understand Spawning Structure/Dynamics

The presentation summarized the information about changes in the bluefin tuna fishing patterns which has occurred in the ICCAT Convention area over the years. The author presented the situation by main management area, gear and CPC. The complex overview was provided along with the correlated opportunities, problems and limits for carrying out the sampling, and how specific situation were or can affect or bias our ability to understand the bluefin tuna spawning structure and dynamics.

The group noted that ichthyoplankton surveys exist in areas identified as having a lack of data. The author suggested tagging and possibly sampling in the Southern Atlantic, as there are limited data available. It was proposed to improve sampling efforts through EU Data Collection Framework and to get the biological data already collected by this important programme. It would be useful to draft a summary table where all the information is sorted according to the question it could provide answer to, including assessing its levels of importance. The possibility of using observers for collecting ABFT samples was discussed as well. It was explained that currently the ICCAT ROP observers are present on purse seiners, farms and traps, while the longline fishery is under the responsibility of the CPC through national observer programs. GBYP has agreements with Regional Observers Programs to get tissue samples, but it is not always possible. It was also noted that the primary purpose of observer programs is for fishery compliance purposes and not for biological studies and biological sample collection. It was suggested that SCRS propose to the Commission to redefine the status of observers and enable them to collect increased numbers of samples.

David Macias: Approaching the Eastern Atlantic Bluefin Tuna Spawning Fraction Ogive Based on Frequency Data Recorded in the Spanish Traps during the Spawning Migration

The presentation explained that the differences in the estimated parameters related to the reproductive biology of eastern and western stocks of Atlantic bluefin tuna are perceived as inconsistent with those assumed for other traits of the species life history. One of the possible causes could be the dissimilar approaches used in estimating those parameters. The study was made for estimating of the eastern stock bluefin tuna spawning fraction ogive by using a method equivalent to the one implemented by Porch and Hanke (2017), based on size frequency data collected in the Spanish traps during the bluefin tuna spawning migration from the Atlantic feeding grounds to the Mediterranean Sea spawning areas. It was noted that the obtained spawning fraction ogive is very similar to that estimated for the western stock of Atlantic bluefin tuna.

The Group argued that the data set may be incomplete, because, due to the quota regime, the fishery is managed in a way to obtain the preferred fish sizes and therefore the samples obtained represent only a fraction of the spawning stock. Particularly, ages 4-8 in the traps may be under-represented. The group discussed possible alternative interpretation of the results. Age structure and spawning fraction estimated from the fishes captured by Spanish traps when fishes are entering into the Mediterranean differ from that estimated from other fleets operating in the Mediterranean. An important question was asked about how best to combine all gears into one analysis. It was explained that the method is a way of dealing with biased sampling that occurs when only individuals from the Mediterranean are used to estimate to the proportion of matures of the whole population. It was pointed out that an integrated analysis was needed to obtain more complete information on ABFT life history. It was also noted that studies carried out in the past (when the fishing season was fully open and fish were harvested at landing) showed no major maturation discrepancies among the various areas.

Jose Luis Cort: Temporal changes in fisheries and manipulation adversely affecting sampling for reproductive studies of Atlantic bluefin tuna

The presentation gave insights on how changes in fishing systems and handling of fish caught have affected reproduction studies of ABFT over the years, based on audio-visual and photographic material. Fishing operations have changed over time causing biological sampling to be more complicated. The historical video material was provided to the ICCAT cloud. Whereas up until the 1980s-1990s tuna processing in the canning factories was paused so that sampling and other kinds of studies, including those of reproduction, could be carried out on incoming fishes, in the last 30 years, however, practices have changed radically. In the past, the gonads remained inside the fish for a long time before being left to dry in the sun. In recent years, however, the immediate decapitation and gutting of fishes and the speed with which the fish are manipulated in general made it very difficult to carry out sampling for reproduction studies. The problem has now been further exacerbated by the growth in fishing for fattening farms, which has rendered sampling for studies into reproduction highly difficult in practice.

3.8 Implications on MSE/assessment (Alternative hypothesis considered in current assessment OMs)

Ai Kimoto (on behalf of Matt Lauretta): Maturity assumption effects on the stock assessment of West Atlantic bluefin tuna

The presentation offered the results of testing different hypotheses of age-at-maturity in the 2017 stock assessment models of West Atlantic bluefin tuna. The hypotheses tested were knife-edged maturity (proportion spawning contribution) across the range of ages-at-50%-maturity equal to 4, 6, 8, and 10, which covered from younger to older maturity assumptions considered in the 2017 stock assessment. Maturity assumption had no noticeable effect on the estimated abundances-at-age from VPA and SS, including estimated recruitments. Therefore, the assumption did not influence the current harvest advice estimates based on target fishing limits equal to F0.1. However, the maturity assumption has some effect on estimates of current stock status, but in general, spawning biomass benchmarks scale with the estimated biomasses. In the long term, the maturity and fecundity assumptions are expected to be important assumptions and data in the close-kin mark-recapture approaches.

The Group discussed that, based on the results, changes on the maturity ogives could affect predictions of stock status, or MSE not based on specific F objectives, but on MSY objectives. It was also pointed that analyses on F=0.40 should be tested. The parameters MSY and SSB were discussed and it was recommended that in the next BFT assessment SCRS revise biomass estimates, at least for the eastern component for BFT.

As for the results on close-kin analyses that were presented, the Group commented that a potential bias may result from not including the Slope Sea (and other additional potential spawning areas) and that the high sample requirements for conducting this type of analyses might be difficult. The Group agreed that more information on the basic concepts behind this technique would be useful to understand its implications.

André Boustany: New Information on Methods to Incorporate Age of Spawning Estimates into Stock Assessment Models

The presentation addressed differences in Pacific bluefin tuna reproductive dynamics between spawning regions. It discussed that the same techniques could be applied in the Atlantic to resolve the calculation of the proportions of mature fish, biased from not having data of the whole population or having data from different spawning grounds. Pacific bluefin tuna (*Thunnus orientalis*, Scombridae) range throughout the Pacific Ocean but are known to spawn in two geographically isolated regions, one off the coast of Taiwan and the Ryukyu Islands and the other

in the Sea of Japan. Fish on these two spawning regions have different age structures, with the former being composed only of older fish (>8), and the latter region exhibiting spawning by younger (ages 3-6) fish. Currently, the spawning schedule used in the stock assessment is based only on the Sea of Japan spawning ground, which may not be representative of the spawning dynamics for the entire population of Pacific bluefin tuna. Authors use age distribution and spawning ogive data from both spawning grounds and relative spawning output from each of these spawning areas to estimate the proportion of Pacific bluefin spawning in each age class for the entire population. This spawning ogive is driven largely by the relatively small contribution of the Sea of Japan to the overall spawning output. To explore the effects of this estimated spawning schedule, an age-structured population model was created, and projections were run under several fishing mortality rates. Under a 90% reduction in fishing mortality the spawning stock biomass is projected to decline for an additional 10 years using the updated spawning schedule, compared to 4 years under the schedule currently used in the stock assessment. A similar analysis could be conducted for East and West Atlantic bluefin tuna populations, which may also exhibit different reproductive dynamics among spawning regions.

The Group expressed some concerns regarding the methodology used, namely regarding selectivity of the fisheries or annual change in the age structure. The Group also discussed the effect of assuming that 100% of all fish are mature, which was debated to be true. This is linked to the need of considering all of the spawning grounds.

4. Conclusions and recommendations for future studies

General knowledge state of the art

With respect to the current knowledge on Atlantic bluefin tuna reproductive biology, the Group concluded that available information is the following:

- The size class structure in the Gulf of Mexico fisheries
- Estimates of the proportion by size of the total population in the Gulf of Mexico (except age 0)
- The proportion of fish of each size class present in the Gulf of Mexico which is spawning
- The proportion of fish of each age class present in the Mediterranean Sea which is spawning
- Endocrine profiles of pituitary gonadotropins
- Gonad histology
- Gonad macroscopic observations

General knowledge gaps

The group identified following gaps in current knowledge:

- What proportion of Eastern and Western fish of each age class are in the non-Gulf/Med
- What proportion of Eastern and Western fish of each age class are spawning outside the GOM/Med
- Gonad histology in areas outside the Mediterranean and GOM
- Pituitary gonadotropins in areas out of the Mediterranean and Gulf of Mexico
- Larval spatial-temporal distribution in Slope sea and other potential spawning areas
- Interannual variations in the spatial-temporal patterns of spawning areas and their relative contribution to total spawning

Issues

Following knowledge gaps are identified as priorities for resolving:

- What proportion of Eastern fish of each age class are in the Mediterranean Sea and what proportion of each of those age classes spawn?
- What proportion of Western fish of each age class are in the Gulf of Mexico and what proportion of each of those age classes spawn?
- What proportion of Eastern and Western fish of each age class are in the non-Gulf/Med Atlantic and what proportion of each of those age classes spawn?
- Which is the distribution of WATL BFT age 0 fish?
- What is the spawning behavior and sexual maturation process (maturity cycle, spawning window, migration from foraging to spawning grounds) in the Atlantic?

Prerequisites

The Group identified that the prerequisite for carrying out any required analysis on reproductive biology is to have a solid knowledge on population structure and therefore it is necessary to have a reliable output from stock assessment.

Implications on the stock assessment

With respect to the current advice SCRS provides to the Commission, the Group concluded that it is based on assumptions which might not be correct and identified the following shortcomings:

- The maturity ogive for the Eastern population currently used in stock assessment, which is based on histological analysis of fish caught in the Mediterranean only, does not represent the fraction of spawners by age in the population
- The maturity/spawning fraction ogive for Western population currently used in stock assessment which is based on the age structure fish caught in the GOM, does not represent the fraction spawning in the population
- The Slope Sea is a spawning area. The origin of spawners is unknown. Fish present in the Slope Sea are of mixed size classes, also including small sizes. The contribution of this spawning group to BFT in the North Atlantic is unknown, but could be significant.

The Group concluded that both vectors of spawning fraction might be biased, while the magnitude of the bias is unknown.

The Group highlighted the need for developing specific research activities which will permit better estimates of the spawning fraction of Atlantic bluefin tuna populations to be obtained. For investigation of Atlantic bluefin tuna reproduction status, the Group promoted using of established physiological tools (histology and endocrinology) which are available.

Recommendations and guidelines for future studies

- Catch curve analyses and tagging data may shed light on the size distribution of fish entering the Gulf of Mexico and the Mediterranean, although information is needed on the proportion of fish that remains in the Mediterranean year-round.
- Integrated tagging and sampling across the distribution range of BFT, including areas were samples have not been collected (such as the Southern Atlantic), seasons and size classes for genetic, otolith chemistry, histological, endocrine and other molecular analysis is required to determine sexual maturation/spawning fraction and stock of origin.
- Revisiting the available larval habitat models and develop new mechanistic models in order to produce probability maps of potential spawning and larval habitats
- Overlay tracking and CPUE data with the outputs from the spawning/larval habitat models data to look for regions of overlap
- Develop quantitative assays specific for leptine and VTG to better understand reproductive physiology of wild fish.
- Set a working frame within GBYP to coordinate the integrated studies, including the potential sampling capacity of national and ROP observers.
- Use of aquaculture facilities to perform extensive range of studies on reproductive biology to set references to the wild populations.

Appendix 1

List of participants

Key Speakers

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Appendix 2

Tentative agenda

November 26

09:00-09:30	Opening, adoption of the agenda and meeting arrangements. Appointment of the Chairman and sessions rapporteurs.
09:30-11:00	Report of the independent reviewers on the discrepancies in eastern/western reproductive parameters. Open discussion. *Key note speakers: Jessica Farley, Seiji Oshimo*
11:00-11:30	Coffee break
11:30-13:20	Reproductive physiology (Endocrinology: Behaviour vs endocrine profile; Hormonal and social basis of skipped spawning). Open discussion. *Key note speakers: Antonio Medina, Aldo Corriero, Hanna Rosenfeld, Gilad Heinisch* Additional talks: Ashley Pacicco, Ivan Katavic, Samar Saber (2)
13:20-14:45	Lunch break
14.45-16:00	(Cont.) Reproductive physiology (Endocrinology: Behaviour vs endocrine profile; Hormonal and social basis of skipped spawning). Open discussion. *Key note speakers: Antonio Medina, Aldo Corriero, Hanna Rosenfeld, Gilad Heinisch* Additional talks: Ashley Pacicco, Ivan Katavic, Samar Saber (2)
16:00-17:00	Larval ecology (Contribution to and validation of spawning habitat models). Open discussion. *Key note speaker: Patricia Reglero* Additional talks: Rafik Zarrad
17:00-17:20	Coffee break
17:20-18:30	Tuna aquaculture/reproduction (Factors contributing to spawning success -age at maturity, egg fitness; Hormonal profile of spawners; Spawning frequency; Fecundity; Parental contribution). Open discussion. *Key note speaker: Aurelio Ortega, Fernando de la Gándara*

November 27

09:00-10:45	Spawning habitat modelling (Definition of potential spawning areas from knowledge on larval ecology and data on spawners distribution during reproductive period). Open discussion. *Key note speakers: Dave Richardson, Diego Alvarez Berastegui* Additional talks: Samar Saber (1)
10:45-11:15	Coffee break
11:15 -13:00	Life history (implications on reproduction). Open discussion. *Key note speakers: Molly Lutcavage, Barbara Block* Additional talks: Haritz Harrizabalaga
13:00-14:30	Lunch break
14:30-16:15	ABFT fisheries (Feasibility of sampling, temporal changes in the exploitation, factors that can influence our ability to understand spawning structure/dynamics). Open discussion. *Key note speakers: Antonio Di Natale, David Macias, Jose Luis Cort*
16:15 -16:45	Coffee break
16:45-18:00	Implications on MSE/assessment (Alternative hypothesis considered in current assessment OMs). *Key note speaker: Ai Kimoto (on behalf Matt Lauretta)* Additional talks: André Boustany

November 28

Morning session: General discussion (including coffee break).

Evening session: Report drafting (including coffee break). Close of meeting.