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REPORT OF THE ICCAT GBYP WORKSHOP ON BLUEFIN TUNA LARVAL STUDIES AND SURVEYS

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SUMMARY

The workshop focus on the exchange of research results pertaining to early life history of tunas, mostly Atlantic bluefin, including aspects of the ecology, growth and survival of pelagic fish larvae. Scientific studies covered not only work derived from field surveys but also laboratory experiments and modelling. All topics were discussed in the context of how this research can contribute to reducing uncertainty in stock assessment results for ICCAT managed species, particularly in the development of indices of abundance for Bluefin tuna based on larval surveys. The workshop also discussed the broad relevance of icthyoplankton research in support of ecosystem based fishery management.

RÉSUMÉ

L'atelier se concentre sur l'échange des résultats de la recherche concernant les premières étapes du cycle vital des thonidés, principalement le thon rouge de l'Atlantique, y compris des aspects de l'écologie, de la croissance et de la survie des larves de poissons pélagiques. Les études scientifiques ont couvert non seulement les travaux obtenus des enquêtes de terrain, mais aussi les expériences en laboratoire et la modélisation. Tous les sujets ont été discutés dans le contexte de la façon dont cette recherche peut contribuer à réduire l'incertitude dans les résultats des évaluations de stocks pour les espèces gérées par l'ICCAT, en particulier dans le développement d'indices d'abondance pour le thon rouge basés sur les prospections larvaires. L'atelier a également discuté de l'importance générale de la recherche sur l'icthyoplancton à l'appui de la gestion des pêcheries fondée sur l'écosystème.

RESUMEN

El taller se centró en el intercambio de resultados de investigación relacionados con el ciclo vital temprano, principalmente del atún rojo del Atlántico, incluidos aspectos relacionados con la ecología, el crecimiento y la supervivencia de las larvas de peces pelágicos. Los estudios científicos cubrían no solo el trabajo derivado de prospecciones de campo, sino también experimentos de laboratorio y modelación. Se debatieron todos los temas en el contexto de cómo podría contribuir esta investigación a reducir la incertidumbre en los resultados de las evaluaciones de stock de las especies gestionadas por ICCAT, principalmente en la elaboración de índices de abundancia para el atún rojo basados en prospecciones de larvas. En el taller se debatió también la importancia de la investigación sobre ictioplancton en apoyo de una ordenación pesquera basada en el ecosistema.

KEYWORDS

Bluefin tuna, sampling, larvae, habitat, modelling, index of abundance

1. Opening, adoption of the agenda, meeting arrangements

The meeting was held during Sept 12-14, 2016, at ICCAT headquarters in Madrid. Dr. Antonio Di Natale welcomed all the participants (**Appendix 2**) in the name of the GBYP and the ICCAT secretariat. Dr. David Die chaired the meeting which was organized as described in the approved agenda (**Appendix 1**). A number of papers and presentations were made at the meeting (**Appendix 3**).

2. Appointment of the sessions rapporteurs

The following participants served as Rapporteurs for various sections of the report:

Section	
1 & 2	David Die
3	Sarah Privoznik, Christine Hernandez, Kathryn Shulzitski,
4	Estrella Malca
5	Stasa Tensek, Alfonso Pagá
6	David Die

3. Bluefin tuna larval ecology

This section of the meeting covered the research done in both the Mediterranean and the western Atlantic. The following two sub-sections summarize the presentations of such research separately and a third subsection synthesizes the joint discussion pertaining to the two areas.

3a. Review of the available knowledge for the Gulf of Mexico

SCRS/P/2016/054 covered the following research areas: Trophic dynamics, Age and growth, Oceanographic observations, Larval distribution and Modelling. Past work by the research team at the Southeast Fisheries Science Center has included exploratory sampling for bluefin spawning habitat, building larval habitat models to improve the larval bluefin index, and developing climate models to study the impacts of climate change on bluefin habitat. Much of this work was carried out in collaboration with colleagues from IEO. Studies on pelagic food webs and trophic levels of larval tunas has been a recent area of emphasis closely engaged with scientists from the IEO in Malaga and Palma who are leading the project. Other strong collaborative projects include age and growth, bluefin habitat models, and larval index development. Future work includes a continuation of the climate work "Predicting the impact of climate change on physical and bio-geochemical processes in the Gulf of Mexico". In addition the authors are pursuing efforts to develop a "close kin analysis" and develop methods to relate to *bottomup* drivers of recruitment in the Gulf of Mexico.

The authors have been looking at mechanisms to reduce the CV of the GOM larval abundance index. Approaches have included defining the larval BFT habitat, in relation to the position of the loop current to better predicting larval occurrence. Improving survey coverage of the habitat has turned out to be a major challenge as habitat based on oceanographic features is essentially a moving target, difficult to accommodate for survey cruise plans. Habitat models indicate that the predicted reduction of spawning habitat in the GOM due to climate change may be accompanied by increases in habitat area somewhere else.

Participants discussed the implications of having GOM larval surveys, which are conducted in April through June, that for many years were restricted to US EEZ. Although recently these surveys have been expanded to include other areas in the GOM (Yucatan Peninsula, around Cuba and north of the Bahamas) they do not cover these areas regularly. Larval BFT were found in all areas in varying concentrations, suggesting some level of spawning in a number of areas outside the US GOM. The participants acknowledge that spawning locations for BFT are likely more widespread than just the GOM and the MED.

SCRS/P/2016/056 presents research on habitat models developed by the laboratory of Fisheries.

Oceanography for Recruitment Climate and Ecosystem Studies (FORCES) at the Southeast Fisheries Science Center (SEFSC). Historically, models were developed using decision trees informed with in situ physical oceanographic observations. Next generation of models were developed using remote sensed data and artificial neural networks (ANNs). These models have been useful to 1) predict spawning sites and 2) understand the effect of anthropogenic impacts of Atlantic bluefin tuna spawning sites. In our most recent work we have begun using generalized additive models (GAMs) and our results demonstrate that GAMs perform as well as ANNs. In addition to habitat models, GAMs are being used to develop models of annual average catch per unit effort of larvae by the SEAMAP survey. We show there is a strong relationship between the North Atlantic Oscillation (NAO) and annual average catch per unit effort. Combining an NAO model with a habitat model is shown to more accurately predict the presence of larvae than models without the NAO. Next generation habitat models are in development, which include spatial gradients. Additionally, collaborative models are in development with Diego Alvarez-Berastegui and Patricia Reglero (Instituto Espana Oceanografica de Palma) to learn if combining our datasets can allow us to better understand the larval habitat of Atlantic Bluefin tuna. Finally, we are beginning to use data assimilative oceanographic models to 1) backtrack larval collections to probable spawning sites and 2) use models in a heuristic fashion to better understand the ecology of bluefin tuna larvae.

The presentation noted the positive results to date and discussed how the models have been used to test remote sensing and to model spawning outside oil spill influence zone. The focus of more recent studies incorporated GAM/NAO with habitat models indicated that the inclusion of the NAO improved the accuracy of predicting larval presence (explained a significant portion variability). Caution was noted on the potential significance of the GAM relationships. The models also indicated that spawning was likely occurring near fronts which are temporal/spatially variable. Transferring the approach to the MED may not be straight forward as the MED is different from the GOM in terms of oceanography.

3b. Review of the available knowledge for the Mediterranean Sea

SCRS/P/2016/029 acknowledges the past research on bluefin research done during the seventies by C. Piccinetti and A. Dicenta which already recognized the importance of the Balearic Islands as bluefin spawning grounds. But not until the 2000 did ICCAT recommended the carrying out bluefin larval surveys to understand the early life ecology in relation to environmental changes. This recommendation led the IEO to carry out the TUNIBAL bluefin larval project aiming to understand the influence of environmental factors of bio-physical nature on larval growth variability, condition and the distribution of larval abundances. The presentation highlighted some of the major findings that were achieved in TUNIBAL particularly in providing the first insights for the determination of bluefin spawning habitat. The TUNIBAL project was also important in that it opened the collaboration between the IEO team and the FORCES lab from the Southeast Fisheries Center (NOAA). Some of the highlights of TUNIBAL were to:

- Develop a novel methodological approach to the sampling strategy, using systematic grid, concurrent sampling with mesoscale oceanographic conditions, and 90-cm 500 micron undulating bongo tow, from surface to 20 m
- Characterized BFT spawning habitat on the basis of abiotic, temporal, spatial, biological variables.

After TUNIBAL the collaboration between the IEO and NMFS groups continued to be strengthened within the framework of the several subsequent research projects on tuna larvae carried out within the framework of the SEFSC Larval bluefin tuna research program in the GOM, and those leaded by the IEO in the Balearic Sea, as BALEARES, ATAME and BLUEFIN, realized in collaboration with other Spanish research institutions, as University of Cadiz, AZTI, CSIC and large scientific facilities, as SOCIB. All that occurred under the umbrella of the CLIOTOP program, which helped in standardizing some techniques and sampling strategies and develop joint studies. Thus, within this collaborative framework, the comparative approach between the Gulf of Mexico (GOM) and Mediterranean (MED) has been mainly applied to the modelling of the spawning and larval habitat, focusing on the influence of mesoscale hydrographic features and seascape metrics on larval abundance and distribution and to the study of the early life ecology of the bluefin larvae from both ecosystems, as well as, the analysis of the different larval growth strategies.

Ahead lies a new project that it is just beginning, ECOLATUN, focused on bluefin larvae trophodynamics which integrates formally the research teams from NOAA, IEO but also ECOSUR that was described in a subsequent presentation.

SCRS/P/2016/057 reports that the occurrence of bluefin tuna larvae relates to water temperature, both at worldwide and regional spatial scales. The temperature range for this life stage is similar across spawning areas suggesting a minimum at 20ºC. A potential larval survival index for the Balearic Sea that combines a model parameterized by temperature-controlled experiments performed on Atlantic bluefin tuna eggs and larvae and field data is also presented. The index, covering years 2000-2015 identified years with good larval survival (as 2003) and poor larval survival (as 2013). The index developed is spatially-explicit and estimations for two different years were shown, however, it still needs to be discussed how such a survival index may be assimilated by the stock assessment process. It is also important to consider how the index may allow us to predict the effect of sea surface temperature increases on the survival of BFT larvae and therefore on the relevance of such impacts on the larval ecology and population dynamics of bluefin tuna. Other results presented suggest that there might be spatial and ecological interactions between BFT larvae and other species, particularly as there is field evidence of larval piscivory.

Participants noted that the egg survival index correlates with the larval index presented in SCRS/P/2016/051. Unfortunately, stock assessments teams have not yet determined how to incorporate such larval survival indices into the stock assessment process. Incorporating such index is a similar challenge to the incorporation of environmental data.

Given that the model was developed for the Balearic region of the western Mediterranean, the participants were wary of applying it to the rest of the Mediterranean, where larval survival may be affected by other processes not considered in the current model. This is in spite of the fact that some of the model equations come from experiments in the lab which in theory are not restricted to represent a particular area of the basin. The reason is that the index depends also on in situ data obtained during surveys and such data is not available for the Eastern Mediterranean.

The global objectives of the project presented in SCRS/P/2016/030 are to characterize the Atlantic Bluefin Tuna larval trophodynamics and assess its influence on larval growth and predator-prey interactions with other top predator larvae (*Katsuwonus pelamis*, *Thunnus atlanticus*, *Auxis rochei*, *Euthynnus alletteratus*, *Coryphaena hippurus* and *Sphyraena sphyraena*), from a comparative ecosystem approach of their main spawning regions, the GOM and MED, which show contrasting environmental conditions. The project is funded by Spanish government, led by IEO, NOAA, ECOSUR with collaborations from COMA, COG, WHOI, CUNY, COB and SOCIB. The project seeks to standardize field sampling methodologies through Bongo-20, Langrangian buoys, S-10 net and Bongo-90. The project rationale is that feeding and growth information will give insights on likelihood of larval survival.

To achieve this it has a series of specific objectives:

1. Larval population genetics to look at genetic links from each ecosystem
2. Larval trophodynamics of GOM vs. MED, using bulk stable isotope analysis, compound specific isotope analysis, and stomach contents
3. Larval growth using otolith microstructures and increment widths to characterize growth variability and daily growth patterns
4. Oceanographic features characterization, relating hydrobiological processes in 2 ecosystems
5. Larval fish community abundance, distribution, assemblages, diversity, and the link to presence/absence of BFT in both systems
6. Relational ecology synthesis – pool all objectives and methods together to determine relationship between two systems (GOM and MED)

During the project, BFT larval collections from surveys in GOM, Cuba, Bahamas, Caribbean, Balearic Islands and Levantine Coast will be analysed. ECOLATUN kick-off meeting took place 8-9 September 2016, where we presented all projects, started brainstorming, first collaborative get-together.

Participants commented that the GBYP reports in the ICCAT webpage the biological data which has been collected by the program on young-of-the-year and juvenile tuna. Some of the data includes identification of stock of origin. Such data may be useful to the people studying larvae and are trying to relate their results to the two stocks of BFT. The evidence of separation between eastern and western stocks is strong from genetic analysis of all life history stages, but less clear with isotope analysis. It was also mentioned that there is on-going work on trophic relationships of various tuna species, including BFT. There is a paper in review considering the 4 species, pre- and post-flexion, in the Mediterranean. Such work should be expanded to other species and regions SCRS/P/2016/058 reports that a critical part of next generation stock assessments models is improved consideration of environmental variables. However, one of the key factors limiting the performance of environmental covariates in assessment practice is an incomplete understanding of the mechanistic relationship between the environmental covariate and resulting biological effects. This lack of understanding can be addressed by examining the impact of mesoscale oceanographic features and sea surface temperature on larval growth of Atlantic bluefin tuna (ABFT, *Thunnus thynnus*) in both the Gulf of Mexico and Mediterranean Sea, the two major spawning grounds for this species. First, you need to quantify the mesoscale oceanography of the GOM and the MED using published algorithms and assign individual ABFT larvae to mesoscale feature location. Second, you must standardize methods for otolith microstructure analysis and calibrate measurements of larval growth across geographic regions by calibrating across readers and explicitly evaluating inter-reader precision using a test set of otoliths that represent samples obtained across regions (i.e., GOM and MED) and larval sizes. Third, you have to quantify recent (i.e., last two full days of growth) and daily growth rates of larvae across mesoscale features for three years of collections from both the GOM and the MED and will compare larval growth among mesoscale features, years, and regions. Fourth, you need to use empirically derived growth rates to develop a predictive model of growth as a function of mesoscale feature and sea surface temperature. Such a model could then be used for predicting growth in space and time to obtain an index of growth potential, ABFT_G_Index, which could be used as an environmental covariate on recruitment deviations in an integrated assessment model. Finally, you have to assess the impact of mesoscale feature, sea surface temperature, and year on variability in larval size at age and quantify the sample sizes required to characterize this variability. This information could be used in future work to develop a 'correction' for the larval index currently used in the stock assessment for western ABFT that assumes constant larval growth in space and time.

Participants noted that, ideally this project would use three years of data. Also that daily rings had not been validated with aquaculture experiments for ABFT, but that daily growth/rings have been validated for other Scombrids. There was agreement that the relationship between growth rates and environmental factors may differ between regions. In general the temperatures used in the models are SST, it is hypothesized that the vertical distribution of temperature would not have as much of an effect in the distribution of tuna larvae in tropical waters. It was agreed, however, that for BFT, SST only reflects the surface conditions and therefore that the temperature profile may affect the larval distribution; The method proposed by the authors, however, uses temperature and salinity integrated over the entire mixed layer.

SCRS/P/2016/055 included estimations of Atlantic bluefin tuna (*Thunnus thynnus*) age-length relationships using otolith increment analysis and relate recent somatic growth as revealed by recent otolith growth to environmental parameters. Otoliths (sagittae) from larvae collected during spring spawning seasons (2000-2012) in the GOM (259 larvae, 2.1–10.9 mm body length, 0–15 daily increments) were aged. Larval growth for the GOM was compared to historic larval collections in the Straits of Florida (southeastern United States) and in the Balearic Sea (western Mediterranean Sea).

Larval growth for GOM larvae was significantly faster than reported for the Straits of Florida or Balearic Sea larvae, indicating differences in larval ecology between the two main spawning grounds. Multivariate statistical models suggested that different environmental variables (temperature, fluorescence, and salinity either at the surface and/or at 100m, location, among others) influenced larvae at different stages of development. Results from this study are being tested in the development of the annual Atlantic bluefin tuna larval index which currently uses an age-length relationship based on larvae collected solely off the Straits of Florida collected more than 30 years ago. Improved knowledge of larval BFT ecology will also contribute to ongoing ecosystem and modelling studies of this species. In addition, future collaborations with IEO's ECOLATUN project at the Malaga and Palma IEO Lab, and its links with ongoing BLUEFIN project, carried out in collaboration between IEO and SOCIB, were discussed and will be incorporated in temporal and spatial comparisons. Standardizing ageing methods is crucial for ecosystem comparisons and to incorporate into modelling approaches for the GOM and the MED.

Participants noted that it is not known when ABFT larvae absorb their yolksac, but that Pacific BFT does it at between 2-3 days, or about 80 hours, depending on temperature. Also noted that, even in the smallest preflexion larvae, the yolk is fully absorbed. A large part of the discussion centred around shrinkage concerns. Shrinkage is different depending on preserving solution and time. Over time, shrinkage progresses and gets worse; it can be as high as 10-20%; it may not happen, however, for a few months. Much of the shrinkage happens when larva dies. Larval death, however, depends on the gear used for collection, and in some gears, certain samples are in the water shorter than others and may die well before larvae are preserved. The need to correct the larval index for net selectivity was also discussed. It was proposed that, for comparison, it would be best to always calculate abundance to refer to the age when the first ring was deposited in the otolith.

SCRS/P/2016/029, presents results of an artificial rearing experiment on bluefin larvae to examine the ontogenetic evolution of stable isotopes. The research revealed stable isotope transmission from parent to offspring. During pre-flexion stages, bluefin larvae captured in the field have maternally transmitted stable isotopes of nitrogen which may provide clues on the maternal trophic condition. A recent paper; Uriarte *et al.* (in press) provides a formula to estimate the nitrogen stable isotope of maternal origin using pre-flexion larvae. Such a finding can open research questions, such as, the effect of maternal trophic condition on potential growth of larvae.

The participants noted that the isotopic signature of the eggs is exactly the same as the pre-flexion larvae. Unfortunately it is not clear how best to relate these signatures with that of adults because of the differences in the signatures between liver, hear, and muscle. Some tissues concentrate $\delta^{15}N$ more than others. Nonetheless, Uriarte et al. (in press) proposes a formula to estimate the maternal $\delta^{15}N$, which may represent a potential tool to analyse the maternal trophic condition with growth potential at its early stages. It was also mentioned that it would be interesting to look at historical samples preserved in formalin. This may be difficult to know whether samples preserved in formalin over so many years (1982-2010) could be used to make such comparisons. It was also reported that the differences in isotopic signatures between the GOM and the MED may be related to the differences in the prey field. Appendicularians, for example, that are a common prey of BFT larvae in the GOM, are a reservoir of recycled nitrogen. Furthermore, the GOM ecosystem is prone to have blooms of diazotrophic organisms, such as *Trichodesmium* spp. which conform a trophic chain on its own by being capable of assimilating atmospheric nitrogen introducing it to the ecosystem. As a result, this nitrogen is incorporated in the food chain, thereby reducing the ratio of $\delta^{14}N$: $\delta^{15}N$. Some participants noted that ABFT larvae are being used in Close kin analysis to attempt to estimate effective spawning size. Two challenging areas of such attempts are problems in the quality of DNA in the larvae and the requirement to have large sample sizes for analysis.

3c. Review of studies in other Oceans or growth in captivity

Presentation SCRS/P/2016/059 discussed the early life stages of fish paradigm that larger and/or faster growing individuals are more likely to survive than smaller and/or slower-growing conspecifics. This "growth–survival" paradigm has been given much attention in studies on recruitment dynamics of fish over the past quarter century. However, there have been contradictory results in both field and laboratory studies and among different ecosystems, taxonomic groups, and study groups. There is a need to understand, reconcile, and synthesize such contradictory results. The talk highlighted the perspectives from a symposium/workshop on "*Growth–survival paradigm in early life stages of fish: controversy, synthesis, and multidisciplinary approach*" held in Yokohama, Japan, in November 2015. The symposium participants aimed at extracting controversial issues on the paradigm (controversy), proposing ideas for reconciling and synthesizing contradictory results based on different perspectives from different study groups (synthesis), and promoting a collaborative framework for field, laboratory, and modelling studies (multidisciplinary approach).

After the Symposium a workshop proposed a conceptual framework for reconciling and synthesizing contradictory results related to the paradigm. Furthermore, the workshop listed up issues which are considered necessary for future breakthroughs in studies on the growth–survival dynamics during early life stages of fish. A total of 19 issues were summarized under 5 topic categories: (1) "predation mortality", (2) "endpoint", (3) "spatial and temporal scales", (4) "intrinsic factors", and (5) "miscellaneous". The list could also be a good reference for future studies on early life biology of bluefin tuna. The overall message of the symposium and workshop is that understanding growth–survival dynamics during early life stages is key to understanding recruitment processes and facilitating the prediction of recruitment dynamics. A wrong assumption of growth–survival relationship could lead to serious failure in prediction in IBMs.

The participants agreed that this studies require a clear definition of the predator field. For instance other pelagic fish species grow faster, in their early life, than BFT and in captivity have been seen to prey upon them (e.g., horse mackerel, *Auxis* spp., and other *Thunnus* spp.). There are also complex interactions between time of birth and growth rate. For example a possible scenario may be:

Hatch earlier □ cooler waters □ slower growth □ cannibalism on larvae that hatch later (early piscivory) □ greater survival.

It was agreed that it is essential to study daily growth rates of juveniles to determine which individuals survive, the fast growers or slow growers. This has been done for other fish like small pelagics. It may be possible to collaborate with the GBYP funded ASTI team that collects juvenile otoliths to do such study. If this study is done one must be careful in choosing samples for use in growth survival study; samples must cover size complete size distribution and distribution of sampling area. It also needs to be remember that many otoliths are cored for otolith microchemistry analysis, and therefore can't be used to measure growth from daily rings.

SCRS/P/2016/052 described how IEO started to research in bluefin tuna aquaculture twelve years ago with a couple of European Projects, REPRODOTT and SELFDOTT funded by V and VII FP. In the last five years, IEO has developed a technology to obtain eggs and to culture larvae and fingerlings of ABFT, and it has been able to produce close to 30.000 fingerlings. This achievements were part of the framework of RDI Spanish projects but also supported by some private companies, Caladeros del Mediterráneo, Fortuna Mare and Piscifactorias Albaladejo, which have funded several programs and manage the broodstock and the ABFT fingerlings when they arrive into the cages. Several other research projects have been carried out to obtain knowledge about culture technology, nutrition, biochemical composition, biology and ecophysiology. In 2016 the team was able to, the first time, close the life cycle of ABFT, collecting eggs spontaneously spawned from tunas born in IEO in 2011-2012 and kept in cages in the sea. The facilities allow the team to keep larvae, fingerlings and broodstock and to run several trials with larvae, which can be a tool to validate ecological models and hypothesis and to get relevant information about biology, physiology or behaviour of ABFT larvae. Hopefully the team will be able to obtain ABFT fertilized eggs coming from tunas kept in tanks in ICRA (infrastructure to control the reproduction of ABFT) in 2018-2019.

Participants discussed how it may be possible to improve the survival of larvae and juveniles. Some of the things that have been tried are changes in the technology of handling the fish, including lights in tanks to reduce collisions, use nets in cages with different colours and to enhance diets to improve the fitness of fingerlings.

4. Bluefin tuna larval sampling methods and larvae identification

SCRS/P/2016/050 discusses two basic requirements for development of larval indices: right taxonomic identification and representative sampling. In order to set the basis for future coordinated and standardized quantitative surveys oriented to the estimation of ABFT larval indices, potential sources of error or bias affecting basic data generation, have been revised. Firstly, evidence of misidentifications of bluefin tuna larva have been presented, its implications discussed and some ways solve them proposed. Secondly, potential sources of bias affecting tuna larvae samples representativeness, as spatial-temporal variability of spawning area; larvae catchability issues (escapement, extrusion, avoidance) and larval spatial distribution (patchiness, position in water column), illustrated with some examples from Balearic Sea tuna larvae campaigns have been explained and discussed. Finally, proposals to minimize these sources of bias, based on experiences from IEO tuna larval surveys, dealing both with sampling strategy and sampling methodology, have been presented.

Participants discussed whether early identification problems remain a problem or not, or if by now they were easily resolved. There was no full agreement on whether the within species variability in the pigmentation remain an issue for the identification of larvae based on the distribution of pigments. There was also extensive discussion on whether the use of oblique tows ensured the full habitat were larvae may be found was sampled appropriately. There was an agreement that surface net tows (Nueston 1 mm) faster tow ~3-4 knts can be used to collect larger sized larvae; however, in the GOM, the abundance of such larvae seems low and therefore questions the efficacy of such sampling.

Comments were also made in regards to the paucity of taxonomic expertise. One of the reasons for it may be that this topic is does not reward provide high rewards for those who publish in it because of the low impact factors that journals which specialize in taxonomy have. This is in spite of the importance of the topic of biodiversity. Able taxonomic illustrators are also in declining in numbers even though their work is essential in taxonomy. The GBYP has developed a process to reduce taxonomical mistakes in their treatment of biological samples through and integrated quality control system. Clearly the ideal procedure is to validate species identifications with genetics.

Invasive species, common now in the MED are increasing the challenges of taxonomic work and its application to the estimation of abundance and distribution of fish larvae. In fact, larval communities are responding to climate change, as new species from adjacent regions come into areas they were previously absent. Unfortunately very few larval assemblage baseline studies have been conducted for pelagic fish.

It was requested that the SCRS should decide on a standard publication reference for the identification of tuna larvae as it is done for juvenile and adult fish.

5. Planning Bluefin larval surveys (for the Western Atlantic and the Mediterranean Sea) aiming at the development of abundance indices: needs and constraints

SCRS/P/2016/051 presents an overview of the spawning ecology of bluefin tuna in the western Mediterranean, which is highly linked to mesoscale oceanography in the area. Various studies using different methodologies show the relevance of environmental variables such as sea surface temperature, salinity and geostrophic sea surface high, always forced by the temporal window of the spawning season. Recent studies developed in the framework of the BLUEFIN tuna project by IEO and SOCIB show that seascape ecology metrics, providing information about oceanographic processes improves our ability to link the location of the spawning areas and the environmental information. In basis of these relations, larval habitat, defined as the presence/absence of larval at any length, has been modelled to improve quality of the Bluefin Tuna larval indices, by standardizing abundances at station level. Same information should be applied to standardize inter-annual variability of larval indices at basin level. Also, location of spawning grounds, identified from abundances of Bluefin tuna larvae under 4.5 mm, have been modelled from remote sensing data, allowing near real time forecast of the spawning habitat. Forecasting spawning habitats allow today spatial management of closure areas and appropriate design of field work campaigns.

Discussion concentrated on the methodology of the study. It was explained that the standardized density is defined as the number of larvae per square meter. Some consideration was expressed that the total abundance might not be correctly estimated because only the upper part of water column is sampled.

Catch methodology was also discussed, especially considering the maximum depth on which the larval net is towed, in correlation with the vertical distribution of larvae. Nets used in these studies include a standard tow up to 20 meters depth. The teams conducting studies in Balearic Sea have never found any Bluefin tuna larvae below 25 meters depth and they hypothesize that larvae can be found only above the thermocline. During early studies in Mediterranean, other than Balearic Sea, larvae were found below the thermocline and even below 50 meters depth. There was not complete agreement among participants on the relevance of such historical observations in relation to more recent observations about depth distribution of larvae.

Larval schooling in earlier stages was also discussed. This type of behaviour is common in larvae 20-30 days old, but is not confirmed for the smaller ones. The fact that smaller larvae stick together might be due to the schooling behaviour as well, but not necessarily. Dispersal of larvae out from the spawning site is mainly due to the passive dispersal (drift) and in minor part due to the active swimming, but latter is limited to predator avoidance.

SCRS/P/2016/053, gives an overview of the development of larval BFT indices. One of the most valuable applications of larval growth studies is the development fishery-independent yearly time series index of spawning stock biomass (SSB). This technique has been executed using the abundance of bluefin tuna larvae collected in the Gulf of Mexico (Ingram et al. 2008; 2010) and the western Mediterranean (Ingram et al. 2013; 2015). Collection, analysis and dissemination of such information are a paramount function of the National Oceanic and Atmospheric Administration, National Marine Fisheries Service (NOAA, NMFS) and the Spanish Institute of Oceanography (IEO).

Methodologies concerning general ichthyoplankton surveys conducted by NMFS in the Gulf of Mexico have been extensively reviewed (Richards and Potthoff 1980; McGowan and Richards, 1986) and recently updated and summarized by Ingram *et al.* (2010). Ichthyoplankton surveys were conducted from numerous NOAA vessels during mid to late April through May from 1977 through 2014 in the offshore waters of the U.S. Gulf of Mexico with double oblique plankton tows located on a 30-nautical-mile grid. Resulting index values were highest in the early years of the survey and much lower in recent years. Therefore, there is an obvious decrease in catch rates of Atlantic bluefin tuna larvae, which likely indicate a decline in the spawning stock biomass in the U.S. Gulf of Mexico. Such declines have been a concern during several of the Atlantic Bluefin Tuna Stock Assessment conducted by the ICCAT (Anonymous 2014).

The resulting index values had relatively high variance in index values. This could be a result of the lack of explanatory variables and not just the large proportion of zero catch rates. Environmental information at individual sampling stations, such as water temperature, salinity, dissolved oxygen concentration, etc. could explain much of the variability in catch rates. Also, research is currently being conducted concerning the characterization of larval bluefin tuna habitat based on the analysis of satellite imagery, current patterns, and sea surface height anomalies, along with variables used to track climate change such as the North Atlantic Oscillation and the mean north Atlantic sea surface temperature down to 100 m. Findings of these studies will be incorporated into current modeling approaches, in order to both reduce the variance in annual abundance indices and to ensure an accurate index of SSB.

In 2001, the IEO started a series of standardized ichthyoplankton surveys around the Balearic Islands, recognized as one of the main spawning areas of Atlantic bluefin tuna within the Mediterranean (Garcia et al. 2004; Alemany et al. 2010), which provided quantitative data on BFT larvae from 2001 to 2005 adequate for Larval Index estimation. Later projects, as ATAME for 2012 and BLUEFIN from 2011 to 2015, has provided the data for continuing this time series of larval indices. Methodologies concerning general ichthyoplankton surveys conducted by the IEO have been summarized (Garcia et al. 2004; Alemany et al. 2010). More recently, several papers dealing with the elaboration of spatial models defining or predicting the potential spawning and larval habitats off Balearic islands, have been published (Reglero et al., 2012; Alvarez et al., 2014, 2016. Based on these findings, three larval indices were computed to assess the effect of improving the habitat information on index development. The first model, denoted as "basic larval index" (BLI), included no environmental variables in its formation. The second model, denoted as "standard larval index" (SLI), included salinity and sea surface temperature to evaluate if there were any linear effects of these environmental variables, following previous versions of the larval index in the Balearic Sea (Ingram et al. 2013). The third model, denoted as "habitat corrected larval index" (HLI) included a potential habitat variable obtained from a general additive model (GAM), predicting the presence/absence of larvae. This model was constructed from a model selection process where sea surface temperature, sea surface salinity and geostrophic velocities were combined with day of the year, spatial location and gear type. This habitat covariable used in the HLI accounted for nonlinear effects of sea surface temperature, sea surface salinity on the characterization of the spawning habitat. In order to have an independent reference for evaluating the adequacy of the larval index as indicator of the SSB trends and which of the three larval index models could be a better estimator, results from the BLI, SLI and HLI models were compared against estimates of spawning stock biomass (SSB) obtained from the VPA of the ICCAT stock assessment in 2013 (ICCAT 2013). The three larval indices follow the same general trend of the SSB, with higher values of larvae abundances in the period 2012-2013 than in the 2001-2005 years. Correlation coefficients indicated that the three models are well correlated with SSB trends. The HLI had the highest coefficients. When we compared data from years 2001-2005, the two correlations coefficients for the BLI and SLI were low while the correlation between larvae abundances from the HLI model and the SSB from VPA attains the higher value.

The results from the ichthyoplankton surveys conducted in the Balearic Sea and the Gulf of Mexico have shown that spatial location of larval habitats of Atlantic bluefin tuna are strongly influenced by mesoscale oceanographic processes. Therefore, larval index values may be influenced by the type of habitat sampled among years. In addition, ongoing climate change could impact the oceanography and ecosystems, causing changes in the larval habitats of Atlantic bluefin tuna. Improving the knowledge of how habitat information can increase the performance of larvae index models is of paramount importance to the advancement of fishery-independent stock evaluation methodologies.

During the discussion it was noted that the range of larvae lengths that are properly sampled should be established before introducing it into the model because some large samples add noise. Model now assumes that selectivity and mortality don't change over the years, which is not in line with the current knowledge. Annual age length key should in theory be developed for this purpose, but it is difficult to collect enough samples for creating the proper ALK. An alternative might be to use growth rates dependent on the temperature, but only if a model is sensitive enough.

According to the current sampling results on length distribution, the catches from bongo 60 and bongo 90 are different in a way that with bongo 90 there is a bigger number of smaller larvae (yolk sac). It should be noted that these two nets were not used in the same years. Selectivity of both gears should be studied in detail and taken into account. If selectivity of nets proves to be similar, it means that the noted difference is probable due to the different mortality associated with each net or due to inter-annual difference of survival. Different vertical distribution of larvae might also be the key. If not all water column where they are is sampled, it means that a bias is introduced because larvae are not distributed in the water column uniformly, but rather according to their particular ontogenic development (stratification). Tows should cover the entire vertical column where larvae are distributed and sampling should be performed both during day and night because of different activity of larvae in dependence of the light. For instance, during the night, larvae tend to be closer to the surface. All sampling which is not done in a proper way should be removed, not to introduce bias. Proper sampling includes sampling up to depth where larvae are distributed or just a bit below it. If sampling is done in too deep where there are no larvae, there is a risk not to calculate correctly their density. Using oblique tows is recommended, as well as testing current vertical distribution before each sampling operation.

Participants reiterated the need to use a common methodology and common gear between different groups is essential for being able to compare the results. Standardization in the habitat models is a priority as well, beginning with selecting the variables.

SCRS/2016/176 is a short overview of the current and past knowledge about the main spawning areas for the Atlantic Bluefin tuna of both stocks and the development of the knowledge over the centuries and especially in the last decades. The overview of the limited knowledge about additional spawning areas outside the Gulf of Mexico and the Mediterranean is also provided, including past hypotheses and recent data from larval studies or satellite tags or presence of small YOY. These basic knowledge provides the necessary background for better defining the scientific needs for extended larval surveys. Future larval surveys should cover the entire Gulf of Mexico and the Mediterranean Sea, including all known areas, in order to have a comprehensive coverage of all known main spawning areas, having all different oceanographic conditions. The replicates over the years should be able to provide a larval index and many elements for refining the environmental models; research should be as inclusive as possible in terms of countries. In addition to these surveys, exploratory larval surveys are needed for confirming the presence and/or importance of additional spawning areas. Various scientist confirmed occasional findings of larvae out of known spawning areas (Gulf of Guinea, off Guinea Bissau) and in time of the year different from the known spawning season (March).

During the discussion about an expansion of the larval index for the Mediterranean, it was mentioned that the larval index developed by IEO for the Balearic Islands will be used in the operating model developed by Tom Carruthers, for the purposes of management strategy evaluation, as agreed during the BFT data preparatory meeting in 2016. The BFT working group will consider whether the index can be included in the next assessment of BFT during the data preparatory meeting in 2017. Concerns were expressed by some participants about whether such index, developed for one area, can be extrapolated to the whole eastern stock. The issue of how you may weight such index in relation to other indices used in the assessment was also discussed. The relative number of spawners in each spawning area is not known and there might be a considerable inter-annual variability in such relative number. Therefore assigning a real weight to each area is challenging. It was pointed out that this is a general problem for most indices of abundance used in the assessment. In general, larval experts seldom participate in ICCAT stock assessments in spite of the fact that larval indices, like climate variables and indicators of habitat quality may be useful for population assessment models. Presently larval indices are consider as indices of the spawning biomass and not necessarily reflect recruitment. It was noted that the larval index for the Mediterranean is being considered as an alternative of the index of spawning developed by the GBYP through aerial surveys of spawning BFT.

In order to present recommendations for a possible Mediterranean-wide larval survey to the GBYP, the participants discussed the criteria which may be useful to use in the selection of sampling areas to be covered by the survey. A preliminary list of criteria was developed by the participants and it was combined with the list of major spawning areas described by the GBYP Aerial surveys (**Table 1**). The table was populated with estimates of the various criteria based on the data available at the meeting. It was also pointed out that such a survey should not be seen as only be relevant to BFT. Its value to support assessments of other tuna species, and ecosystem research should be highlighted.

It is important that any future survey design can accommodate the possible changes in survey areas due to the inter-annual variability of oceanographic factors or that the areas be big enough. Also, an extensive survey of entire Mediterranean which would be done from time to time might be a good solution to review the definition of study areas. The possibility to examine the oceanography and then adapt timing or space of survey was mentioned, however, everyone's in the room agreed that it's harder to move the time of the survey rather than the area surveyed.

The current Balearic index seems to be a good initial proxy and might be a base for building a more developed one, specific for each spawning area. An international project would be needed, which is a great challenge from a logistic point of view and especially considering finding available funding. For instance, funding which at the moment is provided to the IEO Balearic team is only for Spain and it is not probable to extend the study to other countries. The Balearic larval index will be continued in 2018 with investment from IEO but it will need long term investment to be continued. An international larval survey would only be possible with a strong SCRS support. As a first step, a group should develop a clear set of objectives for the survey. These objectives should not just include objectives related to BFT but also to other species and to broad ecosystem issues. The set of objectives could then be passed on to all ICCAT WG that would be interested in the outputs of the surveys (e.g. BFT, ALB, SWO, SMT and Ecosystems).

It was explained that funding would be challenging. To obtain similar funds as those obtained for the GBYP or AOTTP any proposal would have to demonstrate benefits to the broad public, not only as a scientific achievement. Also, even if it were possible to obtain EU funds, according to the experience, several years would be needed to strongly promote this idea before finally getting the financing. Inclusion developing countries can help obtaining EU developing funds. It was agreed that it was imperative to keep looking other sources of funds outside those associated with ICCAT.

6. Other Matters

The workshop participants noted the usefulness of the meeting and the exchange of ideas that took place in it. Furthermore the participants suggested that it would be beneficial to have a structure to facilitate this kind of exchanges and to advance the research that is important to ICCAT in relation to early life history dynamics. The participants decided to propose to the SCRS the formation of an ad-hoc ICCAT working group on early life history dynamics. After discussion, the participants developed a draft set of Terms of reference (**Appendix 4**) for a possible ad-hoc SCRS working group on early life history dynamics, identifying the goals and an initial work plan. The participants requested that this draft should be presented to SCRS, first to the meetings of the species working groups and then to the SCRS plenary for consideration.

Table 1. Criteria to inform the design of an extended larval survey.

Criteria	Feasibility/Importance (High/Medium/Low)			
	Balearic	Southern Tyrrhenian	Central southern Mediterranean	Levantine
Existing larval survey for BFT	Yes	Some (not ongoing)	Some (not ongoing)	Some (not ongoing)
Current CV (LS)		n.a.	n.a.	n.a.
Area covered area of the AS box sqKm	61,933	53,868	93,614	56,211
Cost proportional to area Euro	350,000[1]	305,000	530,000	320,000
Representative of the index to the total population (mean relative abundance, % of abundance, and CV from AS)	5,025 t = 12.14% (43% CV)	5,353 t = 12.96% (76% CV)	26,028 t = 62.88% (84% CV)	4,978 t = 12.03% (124% CV)
Logistical feasibility	Y	Y	Y but with some logistic constraints	Y with some logistic constraints
Likelihood of being sustainable	High, Depending on funding	Medium, Depending on funding	unknown, Depending on funding; political constraints	unknown, Depending on funding; political constraints
Technical capacity to conduct the survey	Y	Y	Y need for technical support	Y need for technical support
Representativeness of the Commission membership	SP	IT	IT, ML, TN, LI	GR, CY, TU, SY
Opportunity for funding for CPC	Y	Not at the moment	Limited, but not at the moment	Not at the moment
Validated hydrodynamic model	Y	Y	Possibly	?
Availability of national vessels – consistency of platform	Y	Y	Y	partly
Usefulness for other species/ecosystem	High	High	High	High
Funding and capacity for IDs of larval sorting	Y	Y	Y need for technical support	Y need for technical support
Challenge of monitoring vs research	Y	Y	Y	Y
Existing surveys for other pelagic species	Offshore planktonic, Acoustic (shelf)	Acoustic (shelf)	Acoustic (shelf)	Acoustic (shelf)
Existing surveys for other species	Bottom trawl	Bottom trawl	Bottom trawl	Bottom trawl (partly)

[1] The cost for the Balearic survey was used as a reference in the calculations.

Appendix 1

Final Agenda

Workshop Organizer: Antonio Di Natale, ICCAT GBYP coordinator

Workshop Chair: David Die, ICCAT SCRS Chair

1. Opening, adoption of the agenda and meeting arrangements

2. Appointment of the sessions rapporteurs

3. Session #1 – Bluefin tuna larval ecology

   a) Review of the available knowledge for the Gulf of Mexico
      i. Presentations (Key-note speaker: John Lamkin)
   b) Review of the available knowledge for the Mediterranean Sea
      i. Presentations (Key-note speakers: Alberto Garcia)
   c) Review of studies in other Oceans or growth in captivity
      i. Presentations
   d) Discussion and conclusions

4. Session #2 - Bluefin tuna larval sampling methods and larvae identification

   a) Presentations (Key-note speaker: Francisco Alemany)

   b) Discussion and conclusions

5. Session #3 - Planning Bluefin larval surveys (for the Western Atlantic and the Mediterranean Sea) aiming the development of abundance indices: needs and constraints

   a) Presentations

   b) Discussion and conclusions

6. Other matters

Appendix 2

List of participants

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Appendix 3

List of presentations and background documents

Presentations (presenter is underlined)

Session #1 – Bluefin tuna larval ecology

Garcia, Alberto and Alemany F. Bluefin larval research highlights and milestones: results from the TUNIBAL years and its consequent collaborative projects.

Laiz-Carrión Raul, Uriarte A., Quintanilla J.M. and García, A. Using bluefin tuna eggs and pre-flexion larvae as an estimate of maternal stable isotopes.

Laiz-Carrión, Raul and Gerard T. Comparative trophic ecology of larvae of Atlantic bluefin tuna from Mediterranean and Gulf of Mexico (east v. west) spawning areas: the ECOLATUN project.

Lamkin, John. Larval bluefin tuna research in the western Atlantic, Gulf of Mexico and Caribbean. SCRS/P/2016/054.

Malca Estrella, B. Muhling, T. Gerard, J. Tilley, J. Franks, J. Lamkin, A. Garcia, J.M. Quintanilla and W. Ingram. Comparative growth dynamic of Bluefin tuna larvae from the Gulf of Mexico and the Mediterranean.

Ortega Aurelio, de la Gandara García F. ABFT larval rearing and juvenile production in captivity.

Rasmusson Leif. Individual-based modelling of larval bluefin in the Gulf of Mexico. SCRS/P/2016/056.

Reglero, Patricia, Balbin R., Ortega A., Mourre B., Alvarez-Berastegui D., Abascal F., Blanco E., Medina A., de la Gándara F., Juzá M., Kernec M., Tintoré J. and Alemany F. The effect of temperature and dispersal on bluefin tuna larval survival: applications in the Mediterranean Sea.

Shulzitski Kathryn. Examining the relationship between meso-scale oceanographic features and larval growth for Atlantic bluefin tuna in the Gulf of Mexico and the Mediterranean.

Takasuka A., Robert D., Shoji J. Sirois P., Fortier L., Oozeki Y., Garcia A. Summary of the symposium/workshop on growth-survival paradigm in early life stages of fish: controversy, synthesis, and multidisciplinary approach.

Session #2 - Bluefin tuna larval sampling methods and larvae identification

Alemany Francisco. Two pillars for Larval index application: right taxonomic identification and representative sampling. Problems and potential solutions.

Session #3 - Planning Bluefin larval surveys (for the Western Atlantic and the Mediterranean Sea) aiming the development of abundance indices: needs and constraints

Alvarez-Berastegui Diego. Bluefin tuna spawning and larval habitat, environmental dependencies, modelling and application to assessment.

Di Natale Antonio. Scientific needs for a better understanding of bluefin tuna (*Thunnus thynnus*) spawning areas using larval surveys.

Ingram Walter. Development of Larval Atlantic Bluefin Tuna Indices.

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Appendix 4

Draft Terms of Reference for ad-hoc working group on early life history dynamics

Background

Studies carried out during past years on early life stages of top predators species, as tunas, have been shown to be useful in understanding the population dynamics of harvested stocks. This reflects the hypothesis that early life dynamics is one of the main drivers influencing population fluctuations. This information has been incorporated in stock assessment, particularly as relative abundance indices of the spawning stock. There is the potential for scientists to contribute additional indices and data streams that could contribute to stock assessments, such as larval survival index; spawning and larval habitat quality predictions; population genetic structure, abundance estimates and stock mixing (through kinship analysis).

Under the context of EBFM, early life history studies can provide understanding of the structure and trophodynamics of plankton assemblages and sources of environmental variability that can feed into ecosystem models. They can also provide indicators of the plankton assemblages that are relevant for EBFM and the effects of climate change.

Goal and objectives

The goal of the working group is to improve the practical transfer of knowledge from early life ecology to stock assessment and ecosystem modelling. General objectives in service of this goal include:

1) Facilitate interdisciplinary and intersystem networking for SCRS scientists.
2) Identifying assessment questions that early life ecology may resolve
3) Identifying gaps in understanding of ecosystem functioning that early life history may resolve
4) Designing studies on early life history that can provide answers to questions identified in objectives 2 and 3.
5) Developing methodologies for the integration of ecology and assessment **Identify links of these objectives to the SCRS strategic research plan.

Initial work plan/priorities:

1) Standardize sampling design, sampling techniques, and methods for inclusion of habitat data in the development of larval indices for stocks of Atlantic Bluefin tuna.
2) Standardization of methodology of age and growth determination of larval bluefin tuna
3) Investigation of variability associated with plankton gear selectivity for bluefin tuna larvae
4) Further development of the BFT larval survival index through the integration of field and lab experiments
5) Advance the use of climate models for projections of spawning habitat of tunas
6) Analyse the trophic ecology of four tuna species (BFT, BLT, SKJ, blackfin tuna) in the Gulf of Mexico
7) Increase participation in the working group of scientists from additional ICCAT CPCs