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

SUMMARY

The ICCAT GBYP Workshop on Atlantic Bluefin Tuna Reproductive Biology concentrated on reviewing the current knowledge on bluefin tuna reproductive biology and identification of research priorities, aiming at providing advice to the GBYP Steering Committee in regards to the feasible activities to be carried out within the GBYP biological studies and preparing the agenda and the list of invited speakers for the Workshop on this topic requested by the SCRS BFT Species Group that is going to be held within GBYP Phase 8.

RÉSUMÉ

RESUMEN

KEYWORDS

bluefin tuna, biological studies, sexual maturity, spawning fraction

1. Opening and meeting arrangements

The ICCAT GBYP Workshop on Atlantic bluefin tuna reproductive biology was held during 14 and 15 February 2018, at ICCAT Headquarters in Madrid. Miguel Neves dos Santos, Assistant Executive Secretary, welcomed all the participants (the list of participants is given in the **Appendix 1**) on behalf of ICCAT Secretariat. The participants nominated David Die (SCRS Chair) to chair the meeting.

2. Meeting objectives and agenda

The chairman, along with the GBYP Coordinator and former GBYP Coordinator, presented the agenda to the participants and commented on the objectives of the meeting. They explained that, even though this workshop was conceived initially as a sort of informal brainstorming, it should be considered a formal GBYP meeting because the results will have direct or indirect influence on GBYP Phase 8 activities. It was reiterated that one of the objectives of the meeting was identifying the feasible priorities of biological studies which could be carried out within the GBYP, especially in Phase 8, while the other one was preparing the more extended workshop on ABFT reproductive biology in Phase 8 as requested by the SCRS BFT Species Group, including drafting the agenda and the identification of experts to participate as invited speakers.

3. Review of the current knowledge and identification of the research priorities

In order to facilitate the discussions, the former GBYP Coordinator, along with current GBYP Coordinator, had prepared a factsheet (**Appendix 2**), which was circulated among the participants, identifying some current assumptions and open questions related to the bluefin tuna reproductive biology. The group mostly agreed with the assumptions. It identified several gaps and shortcomings in the current knowledge on bluefin tuna reproductive biology, concentrating especially on the huge discrepancies about the presumed age of sexual maturity between the eastern and western stock and the unknown amount of spawning fraction by age within each stock. It was pointed out that further sources of uncertainity arise from the existence of additional spawning grounds in the Atlantic Ocean (i.e.: the Slope Sea) other than the two well-known spawning areas in the Mediterranean and Gulf of Mexico, the spatial inter-annual dimensional variability of spawning areas and the limited temporal inter-annual variability of spawning season. It was also noted that the current conclusions on bluefin reproduction might be partly biased because most of the bluefin tuna adults were sampled only in the spawning areas of Gulf of Mexico and Mediterranean and/or during the spawning season. The group concluded that ideally bluefin adults would be sampled throughout the year cycle and also out of the spawning areas, but also agreed that it might not be feasible to carry out such a sampling due to the lack of fishing activities in some of these areas and/or periods.

After brief discussions, aiming in some cases at identifying possible methodologies that might be used for providing new insights, the group decided to concentrate only on the activities that might be carried out within the GBYP Phase 8, considering the short time framework, budget constraints, the practical feasibility and the priority for the current MSE uses.

The group identified seven research activities to be possibly done within the framework of the GBYP in Phase 8:

1) Sampling blood /pituitary glands/gonads for hormonal levels.

Recent studies have revealed that the bluefin tuna maturity might also be confirmed by analysing the pituitary concentrations of LH (Luteinizing hormone) and FSH (Follicle stimulating hormone). In particular, the analysis of pituitary gonadotropin content showed that the FSH/LH ratio is significantly different between juveniles and adults and then it can be used as a reliable indicator of the maturity state. The conventional histological methods don't confirm whether the individual has actually spawned in the previous spawning period, unless sampling is conducted before the reabsorption of post-ovulatory follicles, usually within 1-2 days after spawning. On the contrary, the analysis of pituitary gonadotropin concentration may provide information on the maturity state of fish sampled outside the reproductive season. It was recommended to perform in Phase 8 a limited sampling to evaluate the feasibility and costs of carrying out an extended sampling in Phase 9 and the subsequent analytical tasks to determine the hormonal levels. It was also mentioned that this sampling should not be done in farms, because the hormonal levels might be different under captivity conditions due to stress. Traps in Cadiz and Sardinia were identified as potential sampling sites.

2) Aerial surveys outside Mediterranean for finding potential spawning areas.

Since these surveys need to be extensive in order to capture potential spawning activities, the group noted that probably it would not be realistic to include this activity in Phase 8.

3) Modelling of spawning habitat based on larval studies, aerial surveys for adult bluefin tuna and electronic tags.

It has to be considered that, due both to the inter-annual variation of the exact limits of spawning areas and of the optimal zones for spawning within these preferential areas, and given that the GBYP aerial survey is carried out on fixed polygons, the index obtained by aerial survey might be biased by the oceanographic conditions in each area and year. The group considered that the possibility of using a model of spawning habitat for standardising abundance index obtained from aerial surveys should be explored, improving the efforts done in GBYP Phase 1 to 3, taking advantage of the models already developed in relation to the larval indices.

4) Use of bluefin tuna operating model to test hypothesis about BFT reproduction.

It was noted that the OM developed within ABFT MSE might be used for testing different hypothesis and the outcome produced by the model might be used also for refining the possible sampling areas.

5) Review of available methods and techniques that might inform us about reproductive state of bluefin tuna and reproductive history.

Given that lately there has been lot of progress in the field of development of new methodologies for studying bluefin tuna reproductive status, the group agreed that it would be very useful to have a complete list of available methodologies, along with the details on possible strengths and shortcomings of each one.

6) Expanding larval surveys to integrate pilot acoustic studies of adults.

The group proposed to carry out an experimental acoustic survey, using high resolution multibeam sonar in one spawning area in order to determine bluefin tuna spawners aggregations size structure and biomass, with the ultimate goal of defining the spawning fraction. The group proposed to explore the possibility for carrying out the acoustic survey along with the larval studies already in place off Balearic Islands, in order to minimize the costs of the survey.

7) Independent panel to review the available information on east/west differences.

The group agreed that it would be useful that independent reviewers, preferably having a broad knowledge on bluefin reproductive biology and fisheries, elaborate a report reviewing the available information on east/west reproductive differences in order to provide insights about the causes of the current discrepancies in the assumption about the age at maturity (first maturity, 50% and 100%) and hopefully facilitate a global agreement on this important issue.

The group coincided that only some of these priorities are feasible and recommended only activities 1, 5 and 7 to be reconsidered by the GBYP Steering Committee as potential activities for Phase 8, if the budget availability will allow them.

4. Preparation of GBYP Workshop on bluefin tuna reproductive biology in Phase 8

With reference to the extensive workshop to be held in Phase 8, the group recommended that it should be conceived in such a way that it should answer or provide inputs on the following topics: 1) planning of the activities to be carried out within the GBYP biological studies in Phase 9, 2) affirming or rejecting the hypothesis of substantial differences in bluefin tuna reproductive biology between the eastern and western stocks and 3) identifying methods and related feasibility for estimation of the percentage of individuals contributing to spawning by age and area.

In order to address the controversy on the reproductive parameters currently used for the assessment of the two bluefin tuna stocks, the group recommended that the aforementioned report elaborated by independent experts within the framework of the GBYP, reviewing the available information and drawing conclusions on this issue be drafted prior to the GBYP workshop and the final report should be finalised before the BFT Species group meeting

in September. The group recommended that the reviewers be independent, don't have any conflict of interest and preferably have experience on other tuna species. As potential candidates, the group initially identified the following experts: Kurt M. Schaefer, Jessica H. Farley, Tom Polacheck and other reproductive biologist from Japan (to be identified taking into account the recommendation by Ziro Suzuki). It was decided that GBYP initiate preliminary contacts to explore their availability and contract two of these experts for the task. The group recommended that the report be based on the review of all the relevant documents on reproduction, not only the peer review papers, and should take into account SCRS reports on meetings in relation to bluefin tuna (Stock assessment session, Data preparatory meeting, Species group meeting, previous Workshops on BFT biology). The reviewers should get enough information to understand which is the methodology that was used for defining the maturity of the eastern stock and which is the one used for the western stock. They should also understand in which way these assumptions are currently used for the bluefin stock assessment and devote special attention to the assumption that the percentage of each age group that contributes to spawning doesn't necessarily correspond to the proportion of mature fish at age (if some mature fish hypothetically skip spawning). The final report provided by the independent reviewers should provide the recommendations on which is the appropriate way for 1) defining size at maturity and 2) defining spawning fraction for the assessment purposes. It was envisaged to discuss the report in detail at the GBYP Workshop on BFT reproductive biology and to present in at the BFT Species group meeting in September 2018.

The group recommended that the Workshop be held during three days, where the first two days would be reserved for the presentations and discussions on programmed topics, while the last day would be dedicated to the general discussion and drafting of the final report. It was also recommended that each invited speaker hold the presentations not only on the programmed topic, but also to provide the comments on the reviewer's report, from his/her own area of expertise. The invited speaker should also inform on state of art methodologies and studies in relation with the topic. It was recommended that, apart from invited speakers, whose travel expenses and per diems will be covered by GBYP, the invitation be extended to other scientist who are willing to participate and cover their own expenses. Nevertheless, the allotted time for presentations of these scientists would be a total of 5 minutes per topic, while it was recommended to limit the time for presentations of key-note invited speakers to 30 minutes per topic.

The group envisaged that the Workshop shall focus only on previously identified topics, which would be ensured by the presentation(s) of invited speaker(s), which could be possibly complemented by other presentation(s) and subsequent discussions. The group identified numerous scientist that might participate in the workshop and contribute with their expertise, but it was indicated that, due to the budget constraints, the list of attendant experts should be limited. It has to be noted that the final list of invited speakers was not completely based on the expertise and accomplishments of the individual scientists, but was also influenced by the repercussion to the GBYP budget the invitations might have due to the travel expenses, prioritising in some cases the most economic option. The tentative agenda, with the proposed key-note speakers on each topic is given in the **Appendix 3**.

5. Other issues

Within the scope of discussions on new methodologies for determining the bluefin tuna maturity, Tim Lam presented a scheme of available methodologies and techniques for confirming the spawning event in correspondence to the time distance from the event (**Figure 1.**). According to the scheme, while the exact moment of spawning event can be confirmed only by a direct observation, histological analyses of oocytes 24 hours before and up to 48 after the event can also provide the reliable evidence of spawning. It was explained that the reliability of the evidence decreases with the time distance from spawning, in both directions. Therefore, a conclusion that a spawning event took place which is based only on a presence of adult fish over a spawning ground, should be considered less reliable, as it is only a very indirect inference. Consequently, using exclusively catch length frequencies of the fish in a spawning ground for determining maturity could not be considered as a reliable method to this end.

Gary Melvin presented to the group the recordings of new state-of-the-art sonar he had the opportunity to test in the Bay of Biscay. It was explained that the sonar allows geo-referencing each fish and tracking it afterwards and that there are software packages already available on the market that permit automatic counting and even measurement of each individual. It was stated that the precision and the sensitivity of the sonar would be enough

to track even smaller particles like larvae and eggs. The group agreed that this methodology has a great potential and that its possible use for developing fisheries independent index of abundance should be further explored.

Antonio Medina showed a underwater video, from the documentary Ultimatuna (https://www.youtube.com/watch?v=K-lJ-pOcgxM), that recorded the moment of spawning of a group of bluefin tuna. It was explained that the fish were inside a cage and were not treated with any hormone or any chemical ingredient. In addition, before spawning, the cages were left in the open sea enough time for the fish to get used to the natural environment and to follow its natural physiological processes without spawning being artificially induced in any way. It was visible from the video that various individuals within the group were spawning, although it was not possible to track any individual fish. It was explained that, according to observations spawning of a group takes place during several consecutive days, during which all the individuals will spawn, at least during one day, but not necessarily all individuals from the same group spawn in the same day. Additionally, one archival tag was implanted in an individual from the same bluefin tuna group, in order to get more insights on a spawning behaviour i.e. to find the pattern in vertical movements, characteristic for the spawning event.

Appendix 1. List of participants

Invited experts

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Appendix 2. Fact sheet on current knowledge on bluefin tuna reproductive biology

(drafted by Antonio Di Natale and Francisco Alemany)

WHAT WE KNOW:

1) There are two major spawning grounds for the Atlantic Bluefin Tuna (ABFT): the Mediterranean Sea and the Gulf of Mexico.
2) Within these two large areas there are various zones where usually the ABFT preferably concentrate for spawning.
3) Within the Mediterranean Sea, the major demonstrated ABFT spawning concentration zones are the Balearic Sea, the southern Tyrrhenian Sea, the central-southern Mediterranean Sea and the Levantine Sea around Cyprus. The existence of these preferential spawning grounds has been confirmed by the presence of ABFT larvae. Most of the remaining Mediterranean areas, except the northernmost areas of the different sub-basins, maybe are opportunistically used for spawning when the conditions are adequate, as suggested by observations of spawning behavior of adults in such zones.
4) Within the Gulf of Mexico, the spawning grounds cover probably almost the full area, but the northern part is the most studied, while the southern and SW areas are less monitored. ABFT larvae have been found both in the Gulf of Mexico and in the Caribbean Sea.
5) There are other spawning areas in the Atlantic outside the two major ones. The large area between E Florida, Bahamas, the Slope Sea and Cape Cod is an area where larvae and early juveniles have been reported since the early '80s and then confirmed again for the Slope Sea in very recent years. The large area between the Canary Islands and the Atlantic Morocco was documented as a potential spawning area since the early beginning of the past century up to nowadays due to the presence of small YOY. However, it must be pointed out that ichthyoplankton surveys carried out around Canary archipelago have never detected the presence of ABFT larvae. Similarly, the coastal areas in the western part of the Strait of Gibraltar, both along the southern Spanish coasts and the NW Moroccan coasts, have been also reported in the past century and up to the most recent years as potential spawning areas for ABFT due to the presence of very small YOY. However, ABFT larvae have never been found in these areas, in spite several summer ichthyoplankton surveys have been carried out there. The westernmost record of ABFT larvae within the Mediterranean is a couple of larvae found off Cabo de Gata. ABFT larvae were found in the Western part of the Gulf of Guinea in the first half of the '60s (confirmed by Richards). Other proposed potential spawning areas are the one around the Azores, in spite recent larval surveys carried out there during ABFY spawning season did not found any ABFT larvae) and the area north of the archipelago of Madeira.
6) The abundance of spawners among the main four spawning areas within the Mediterranean Sea may vary depending on the year. According to the observations of the aerial survey, shifting of SSB abundance among areas in a given year seems correlated with the oceanography and e climate events.
7) The usual spawning period in the Gulf of Mexico is between late April and June, but it might be annually moderately variable according to the climate and the oceanography.
8) The usual spawning period in Mediterranean Sea is between May and July (in the Balearic Sea larvae have been reported from June to September), but it might be annually moderately variable according to the climate and the oceanography.
9) *Thunnus thynnus* larvae have been found in the Slope Sea from June to August.
10) The ethology of the ABFT plays an essential role before and during the spawning season. Several studies have been carried out on the behavioral aspects of the ABFT during spawning, particularly in the Mediterranean Sea (mostly in the southern Tyrrhenian Sea).
11) Several studies are available about the environmental and oceanographic factors influencing spawning spatial patterns and ABFT larval survival. Larval Habitat models have been developed, and tested, for both Western and Central Mediterranean, and Gulf of Mexico, from larval distributions in several years and in situ and satellite data about oceanographic conditions.

12) In the Mediterranean Sea, the minimum recorded temperature associated to ABFT spawning events is 19ºC. Usually the ABFT spawners prefer a SST of minimum 20.5°C and up to a maximum of 26/28°C, with a best preference between 22 and 24°C; a well-established thermocline, with an upper mix layer having a minimum depth of 8/10 m and a max depth of 22/24 m, having a strong gradient of temperature (min 3°C) in very few meters, is also preferred. Other factors might drive the ABFT spawning activity but they are less defined; however, the oceanographic dynamics plays anyway an important role. ABFT larval spatial distribution, mostly reflecting spawning events, has demonstrated to be, both in Balearic Sea and Central Mediterranean, influenced by the dynamics of inflowing recent Atlantic surface waters. Both in Gulf of Mexico and Mediterranean it has been observed the mesoscale oceanographic scenario shape the larval distribution. Spawning was noticed at temperatures as low as 17°C in traps and cages; the stress factors are considered to drive these events. Rearing experiments demonstrated that hatching success is null below 19°C.
13) In the Gulf of Mexico, the SST during the spawning season are between 22/24°C and 27.5°C; the upper layer has a constant temperature down to 30 m, and the thermocline is found hundreds of meters deep. However, both in Gulf of Mexico and in the Mediterranean, ABFT larvae are usually restricted to the first 20 m of the water column.
14) In the Mediterranean, the very first spawners are reported at the end of age 2; 50% of the spawners in the spawning area are reported at age 3, while 100% are mature at age 4; the data are coming both from histological and a large amount of direct observation analyses. Currently, the VPA for the EBFT set the 100% of maturity at age 5.
15) In the Gulf of Mexico, the studies on BFT maturity are much limited; most of the direct observations were carried out along the eastern US coast in the ‘40s and ‘50s; according to these data, the first signs of maturity start at age 3, while more fish mature at age 4 and it seems that many fish are mature at age 5; almost all examined fish at age 6 were post-spawners. For the northern area of the Gulf of Mexico the studies are mostly derived from the age classes frequencies coming from the US fishery, which catch larger fish. Currently, the VPA for the WBFT set the maturity at age 8, but for many years it was at age 15.
16) The ABFT shows an asynchronous oocyte development and therefore a sequential multispawner. The few available studies report that it can spawn for more than one month. There are some e-tags evidences which suggest that the same ABFT can spawn in more than one area.
17) The gonad-somatic index is available for the Eastern ABFT stock and, even if less documented, for the Western ABFT stock. The weight of the gonads is proportional to the total weight of each fish. Therefore, the fecundity increases in both areas with the size of the fish.
18) Even the most larger ABFT which have been fished in the spawning grounds were active spawners; therefore, there is no reason for supposing that the older fish attain an age at which they are not able to spawn anymore.
19) The age structure of the ABFT catches during the spawning season in the two main spawning grounds is well documented. It can be assumed that the age structure of the spawners within these catches was representative of that of the spawners in the whole population for the years before the enforcement of the quota system in the Mediterranean Sea, while it should be better examined for the Gulf of Mexico, due to the longer enforcement of the quota regime in this area and the existence of alternative spawning grounds outside the Gulf of Mexico.

WHAT WE DON’T KNOW:

a) If the “additional” spawning areas in the Atlantic Ocean (both documented or potential) receive ABFT spawners every year or only according to specific environmental situations. At the same time, it is not known the proportion of SSB going to these areas.
b) It remains unknown neither the proportion of spawners among different spawning areas within the Mediterranean nor its interannual variability (synoptic aerial and larval surveys could provide some cues…). Moreover, it remains unclear if a given individual can spawn in different preferential spawning areas within the same year; since few e-tags data seem confirm these events.

c) It is unknown if the observed interannual variations in the extent and location of spawning areas results in significant changes in the number of spawning events.
d) In the years when the spawning events are documented to happen in an extended period of time, it is unknown if the survival of both fertilized eggs and larvae is very different during the marginal periods or not. Rearing experiment suggest that cold temperatures are more limiting for hatching success and larval survival than higher ones
e) It is unknown the real age-at-maturity in the full spawning ground of the Gulf of Mexico.
f) It is essential to better document if there is a different age-at-maturity between the spawning ground in the Gulf of Mexico and in the spawning areas along the eastern US coast and if these spawners represents different age groups of the same population (having different preferential spawning areas) or if there are other substantial differences.
g) It is unknown if the ABFT larvae collected in the western area of the Gulf of Guinea were a very occasional event or if this area might be an additional or opportunistic spawning area for the ABFT.
h) It is unknown if the very small YOY found in the Atlantic near the Strait of Gibraltar and in the area between the Atlantic Moroccan coast and the Canary Islands come from opportunistic spawning in these areas. Such possibility should be confirmed through the presence of larvae.
i) It is unknown if all potential adult ABFTs spawn effectively every year and what could be the percentage of non-spawners; it is unknown if this percentage may change according to the age. According to the few studies available so far, the percentage of ABFT that may skip spawning might be very low.

Appendix 3. The tentative agenda of the ICCAT GBYP Workshop on Bluefin Tuna Reproductive Biology (Phase 8)

ICCAT GBYP Workshop on Atlantic Bluefin Tuna Reproductive Biology (Phase 8)

The tentative agenda

Presentations (two days)

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

2. Reproductive physiology (Endocrinology: Behaviour vs endocrine profile; Hormonal and social basis of skipped spawning)
   Key note speakers: Antonio Medina, Aldo Corriero, Hanna Rosenfeld (or Gilad Heinisch or Constatinos Mylonas or Johnny Zohar)

3. Larval ecology (Contribution to and validation of spawning habitat models)
   Key note speaker: Patricia Reglero (or Barbara Muhling)

4. Tuna aquaculture/reproduction (Factors contributing to spawning success -age at maturity, egg fitness; Hormonal profile of spawners; Spawning frequency; Fecundity; Parental contribution)
   Key note speaker: Aurelio Ortega (or Fernando de la Gandara or Daniel Benetti)

5. Spawning habitat modelling Definition of potential spawning areas from knowledge on larval ecology and data on spawners distribution during reproductive period))
   Key note speakers: Dave Richardson, Diego Alvarez Berastegui

6. Electronic tagging (Contribution to spawning habitat model)
   Key note speakers: Molly Lutcavage, Barbara Block

7. ABFT fisheries (Feasibility of sampling, Temporal changes in the exploitation factor that can influence our ability to understand spawning structure/dynamics)
   Key note speakers: Clay Porch, David Macías, Antonio Di Natale, Jose Luis Cort

8. Implications on MSE/assessment (Alternative hypothesis considered in current assessment OMs)
   Key note speaker: Matt Lauretta (or Tom Carruthers or John Walter)

General discussion (half a day)

Drafting a report (half a day)

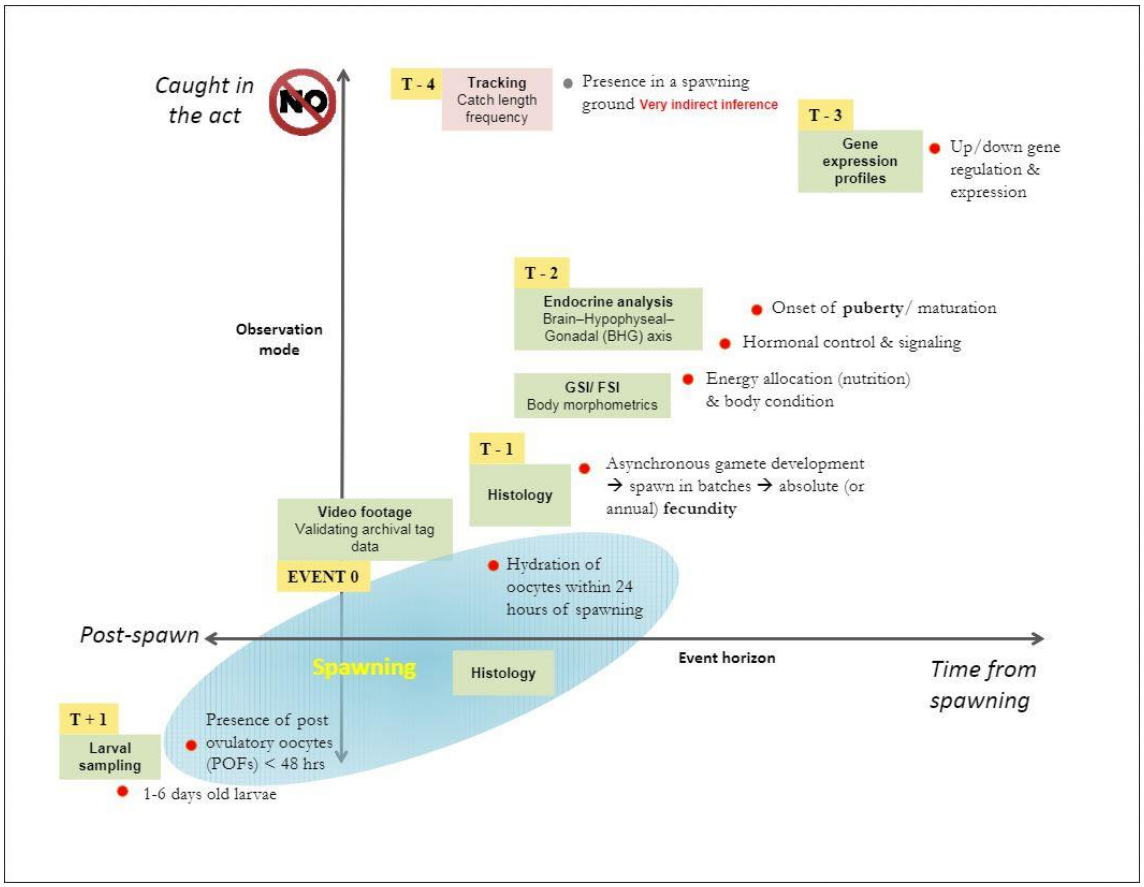

Figure 1. Scheme of available methodologies and techniques for confirming the bluefin tuna spawning event in correspondence to the time distance from the event