

SCIENTIFIC NEEDS FOR A BETTER UNDERSTANDING OF THE ATLANTIC BLUEFIN TUNA (*THUNNUS THYNNUS*) SPAWNING AREAS USING LARVAL SURVEYS

Antonio Di Natale¹

SUMMARY

This paper provides a short overview of the current and past knowledge about the main spawning areas for BFT 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. This provides the necessary background for better defining the scientific needs for extended larval surveys. Future larval surveys should cover the entire GOM and MED, including all known areas, in order to have a comprehensive coverage of all known main spawning areas, all having different oceanographic and hydrodynamic 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, exploratory larval surveys are needed for assessing a possible variability and for confirming the presence and/or importance of additional spawning areas.

RÉSUMÉ

Le présent document fournit un bref aperçu des connaissances actuelles et passées sur les principales zones de frai du thon rouge originaire des deux stocks et sur l'évolution des connaissances au cours des siècles et surtout au cours des dernières décennies. Un aperçu est également donné des connaissances limitées sur les zones de frai supplémentaires situées en dehors du Golfe du Mexique et de la Méditerranée, y compris les hypothèses passées et les données récentes provenant d'études larvaires ou de marques reliées par satellite ou de la présence de petits jeunes de l'année. Cela fournit le contexte nécessaire pour mieux définir les besoins scientifiques des vastes prospections larvaires. Les futures prospections larvaires devraient couvrir l'intégralité du Golfe du Mexique et de la Méditerranée, y compris toutes les zones connues, afin d'avoir une couverture exhaustive de toutes les principales zones de frai connues, toutes ayant des conditions océanographiques et hydrodynamiques différentes. Les répétitions au cours des années devraient permettre de fournir un indice larvaire et de nombreux éléments pour affiner les modèles environnementaux ; la recherche devrait être aussi inclusive que possible en termes de pays. En outre, des prospections larvaires exploratoires sont nécessaires pour évaluer une variabilité possible et pour confirmer la présence et/ou l'importance des zones de frai supplémentaires.

RESUMEN

Este documento presenta una breve visión global de los conocimientos actuales y pasados acerca de las principales zonas de desove del atún rojo de ambos stocks y del desarrollo de los conocimientos a lo largo de los siglos y especialmente en las últimas décadas. Se proporciona también una visión general de los limitados conocimientos sobre zonas de desove adicionales fuera del golfo de México y el Mediterráneo, lo que incluye hipótesis pasadas y datos recientes de estudios larvales o marcas por satélite o la presencia de YOY pequeños. Esto proporciona la información de contexto necesaria para definir mejor las necesidades científicas de prospecciones de larvas ampliadas. Las prospecciones larvales futuras deberían cubrir todo el GOM y el MED, incluidas todas las zonas conocidas, con el fin de contar con una exhaustiva cobertura de todas las zonas de desove conocidas, todas con diferentes condiciones oceanográficas e hidrodinámicas. Las réplicas a lo largo de los años deberían permitir

¹ ICCAT – Calle Corazón de Maria 8, 6 floor, 28002 Madrid (Spain).

proporcionar un índice larvario y muchos elementos para afinar los modelos medioambientales. La investigación debería ser lo más inclusiva posible en términos de países. Además, son necesarias prospecciones de larvas exploratorias para evaluar una posible variabilidad y para confirmar la presencia y/o importancia de zonas de desove adicionales.

KEYWORDS

Bluefin tuna, large pelagic species, reproduction, spawning areas, larval survey, Mediterranean Sea, Atlantic Ocean, Black Sea

1. Introduction

The very first plan of ICCAT GBYP, as it was approved in 2008 by the SCRS (Anon., 2009), among eight different research activities included also the larval surveys in the Mediterranean Sea. The original text stated “The aim would be to carry out larval surveys simultaneously in the western, central and eastern Mediterranean in order to better understand spawning distribution and potentially design a fishery independent survey. Additionally, larvae genotyping would be used for genetic tagging studies”. The multiyear GBYP budget included funds for two surveys in year 2 and year 3.

When the Commission approved the GBYP in the same year, the larval surveys were excluded, and the aerial surveys on spawners were set with the maximum priority for possibly provide fishery-independent indices (Anon., 2009).

As a matter of fact, GBYP larval surveys were set aside, while national scientists continued their activities in this field with national funding, mostly in US and EU-Spain.

After several discussions with the specialist, after a proposal presented to the SCRS (García and Lamkin, 2014) and after the agreement of the GBYP Steering Committee, it was decided to include a “Workshop on bluefin tuna larval studies and surveys” among the GBYP activities in Phase 6. This paper would like to provide the basic background overview about the current knowledge on spawning areas of the Atlantic bluefin tuna (*Thunnus thynnus*) and the scientific needs for a better understanding of the spawning areas using larval surveys.

2. Spawning areas and periods of Atlantic bluefin tuna

The Atlantic bluefin tuna (*Thunnus thynnus*) is a multi-spawner. Marino *et al.* (2005), demonstrated that the same bluefin tuna individual is able to release mature eggs from the ovary spread in several periods, over a certain time, with depositions having one or more days distance one from the other. In the same individual, spawning may occur for more than one month in the same season. Of course, the same happens to male bluefin tunas, even if studies on males are much more limited. Recent information seems to indicate that this spawning period could be much more extended under peculiar and environmentally anomalous circumstances (Piccinetti *et al.*, 2013).

Eggs fecundation is external in bluefin tuna and may occurs after isolated contemporary emissions of couples or more individuals within a school or, more commonly, after the spawning of all or most of the individuals within the same school, as it was clearly documented in many studies in open sea during the spawning season, where also the reproductive behaviour in the wild was observed (Arena, 1963, 1964, 1980, 1981, 1982a, 1982b, 1982c, 1982d, 1985, 1986a, 1986b, 1988a, 1988b, 1990; Arena *et al.*, 1979, Arena & Cefali, 2002).

During the reproductive migrations or the spawning periods, bluefin tuna aggregates in schools which are quite variable in numbers of individuals and age composition. Bluefin tuna schools may have all together individuals immediately close to their first reproduction (around 20-25 kg for the eastern stock, slightly bigger for the western stock) up to very big individuals even over 600 kg.

Each bluefin tuna ovary contains many million of oocytes, and those having the biggest size can be hydrated and released in a very short time following hormonal stimuli, which some predators may also capture (Susca *et al.* 2000, 2001; Schaefer, 2001; Medina *et al.* 2002; Abascal *et al.* 2003, 2004; Corriero *et al.* 2003, 2005; Santamaria *et al.* 2003; Zupa *et al.* 2009). The fecundity for females is reported as proportional to the total body weight: the average is 120 eggs for each gram of body weight (Frade, 1935; Rodríguez Roda, 1967; Baglin, 1976, 1982; Baglin

and Rivas, 1977). Di Natale *et al.*, in press, provided also a review on this matter. Recent data on bluefin tuna maturity, collected three annually by European scientific institutions within the EC Data Collection Framework and regularly provided to EC-DG MARE, confirmed the maturity-at-age.

In the last decades, many other knowledge elements were provided by studies of bluefin tuna in cages (Harada 1973; Ueyanagy *et al.* 1973; Doumenge 1996; Mylonas 2002; Corriero *et al.* 2007; Ottolenghi *et al.* 2008; De Metrio *et al.* 2010) and even in transport cages (Gordoa *et al.*, 2009; Gordoa, 2010) .

Taking into account the various replicates of the bluefin tuna spawning during the same season and the high mobility and natation speed of this species, then the spawning area can be very large and extended, even considering a usual period of about one or one and a half month.

Researches carried out with a daily ichthyoplankton sampling (Sanzo, 1932), with histological analyses of the gonads or with examinations of the bluefin tuna ovaries in tuna traps shows that normally spawning in the Mediterranean Sea occur from the second part of May to July, but in some years mature eggs and larvae were found also in August, demonstrating that occasionally the reproductive season might be extended in some years. The extensive studies conducted on purse-seine fishery in the Tyrrhenian Sea (Arena, 1980, 1981, 1982a, 1982b, 1982c, 1985, 1986a, 1986b, 1988a, 1988b, 1990; Arena *et al.*, 1979; Arena & Cefali, 2002), clearly showed that the main reproductive season in this area is mostly between mid-May to mid-July, with a peak in June and a limited variability, before or after, each year, depending mostly on the oceanographic and environmental conditions. This period was confirmed also by De Metrio *et al.*, (1988, 2003a, 2003b), Block *et al.*, (2001) and Rooker *et al.* (2007) for the central Mediterranean Sea. Again, recent data on bluefin tuna maturity, collected three annually by scientific institutions within the EC Data Collection Framework and regularly provided to EC-DG MARE, confirmed the classic spawning period. Extended spawning periods, linked to particular oceanographic or climate conditions, have been reported several times in the past (De Buen, 1923a, 1923b, 1923c; Biancalana, 1958; Scaccini, 1959; Arena, 1963, 1964; Sarà, 1983, 1998), and other evidences are provided by “anomalous” size frequencies of age 0 and 1 bluefin tunas in spring-summer fisheries (Piccinetti *et al.*, 2013)..

For several centuries, information about bluefin tuna spawning areas was derived from observations on concentrations and movements mostly in the straits and catches in coastal traps. According to what Aristotle (1635, also in Athenaeus, 1653) said in the IV century BC, “*thyngnum esse tradit gregalem ac locum mutare*”, this species is a very migrant one, able to cross the ocean in a short time, and to carefully read the chemical and physical messages from the sea waters for various reasons, including for spawning. He, but also many other classic authors, including Oppianus (177 B.C. in Salvini, 1738) and Plinius (65 AD, re-edited in 1553) considered that possibly the bluefin tuna was migrating from the Atlantic to the Mediterranean Sea in spring, for later going to the Black Sea for spawning. This observation, which was biased by the lack of studies in the Levantine Sea, affected the general knowledge about the spawning areas in the Mediterranean Sea for about 24 centuries. Spawning in the eastern Mediterranean and in the Levantine sea usually occurs slightly earlier, starting in the first part of May (Oray and Karakulak, 1998; De Metrio *et al.*, 2003b), when the sea temperatures in this area increase well before than in all other parts of the Mediterranean Sea and when favourable weather situations allows the formation of an upper stratum with relatively high temperatures and a stable thermocline at the proper depth. According to the evolution of the hot water masses, this situation possibly occurs also along the South-eastern part of the Mediterranean Sea, along the eastern Egyptian coast. The beginning of the bluefin tuna reproduction season in the western Mediterranean area and particularly in the Balearic Sea is usually delayed by one or two weeks compared to the central Mediterranean Sea (Alemany *et al.*, 2010). In very recent years and particularly in 2011, spawning occurred also at the very beginning of May, due to very particular oceanographic event, but this fact can be regarded as an anomaly, because the situation returned to the normality after about 10 days. The spawning period in the various areas of the Mediterranean Sea is also confirmed by the comprehensive review by Aguilar & Lastra (2009)

Most of the hypotheses about the spawning areas of the bluefin tuna in the Mediterranean Sea have been based for many centuries only on the observations made on tuna trap catches. As a matter of fact, the traditional fishery with tuna traps in the Mediterranean and in the adjacent seas allowed for a close examination of the gonads which were and are still used for the production of salty ovaries, a very high-value bluefin tuna product, called “bottarga” in Italian, “huevas de atún” in Spanish and “battarik” in Arabic, possibly deriving from the ancient Greek name “ootàrichon”. Several additional data on this issue are in Piccinetti *et al.*, 2013.

Unfortunately, the earlier observations were not very accurate and frequently pre-spawning gonads were thought to be fully mature gonads and the same happened for post-spawning gonads. Several hypotheses about the vicinity of spawning areas were biased by this poor knowledge of the gonad maturations in these old ages, but also to the

lack of observations of bluefin tuna spawning aggregations in offshore areas, because bluefin tuna fishery was based mostly on traps or very coastal activities. These biased hypotheses resulted in a progressive list of possible spawning areas for the bluefin tuna, which were, in historical order, the Black Sea, the Tyrrhenian Sea, the seas around Sicily, the Libyan sea, the southern Tyrrhenian Sea, the Sardinian Sea, the Strait of Messina, the Algerian Sea, the Tunisian Sea, the Balearic Sea, the Atlantic Spanish-Moroccan area, the waters North of Madeira, the Gulf of Mexico, the Caribbean Sea, the Gulf of Guinea, the eastern US waters including Bahamas, the Azores, the Bay of Biscay, the Strait of Sicily and the southern-central Mediterranean Sea, the Ionian Sea, the Levantine Sea, the sea around the Yucatan peninsula and the Slope Sea.

Just on the XVIII century, but always on the basis of the bluefin tuna travelling towards possible spawning grounds, it was possible to have more reliable, even if still partial information about the spawning areas or those supposed to be at that time: still the Black Sea, but also the Balearic Sea, the Tyrrhenian Sea, the Libyan waters and the Sardinian Sea. Some of these were discarded much later.

Starting from the second part of the XIX century and going to the first part of the XX century, finally there were much broad views and several scientists started to correlate mature tunas with the following presence of very small tunas, in the same areas (D'Amico 1816; Dieuzeide and Roland, 1955; Roule, 1923) and the oldest reports were summarised by Piccinetti and Piccinetti Manfrin, 1970), trying to have a better identification of the spawning areas (also excluding some hypotheses previously provided), but there were still many biases. Slowly, few studies on bluefin tuna larvae refined some ideas about possible areas, while studies on maturity and fecundity were largely developed in some countries (mostly Italy, Spain, France, Algeria and Tunisia) (Cetti, 1777; Lo Bianco 1908; Bounhiol, 1911; Roule, 1917; Parona 1919; De Buen, 1925; Sella, 1929a, 1929b; Frade and Manacas, 1933; Scordia, 1938). The presence of full mature spawners or extremely young bluefin tunas pointed out the presence of potential spawning areas in some parts of the Atlantic Ocean (Mather *et al.*, 1995) (**Figure 1**).

The progressive development of off-shore fisheries after the use of engines in fishing vessels were able to finally directly observe the bluefin tuna spawning behaviour in some areas, like the southern Tyrrhenian Sea, the Balearic Sea and the Gulf of Mexico. Several studies were carried out mostly by Prof. Arena's team (Arena, 1980, 1981, 1982a, 1982b, 1982c, 1985, 1986a, 1986b, 1988a, 1988b, 1990; Arena *et al.*, 1979; Arena and Cefali, 2002) in the southern Tyrrhenian Sea using aircrafts, for accurately study and describe the spawning behaviour of the species; these are the only studies which are able to describe the behaviour of spawning aggregations in the Mediterranean Sea, which is an essential component for better understanding the ethology of this species but also the data which are provided by electronic tags.

The bluefin tuna larvae were found in several areas, mostly in or close to the most important spawning areas that were supposed in previous years: the southern Tyrrhenian Sea, the Balearic Sea and the Gulf of Mexico, but also in other areas. Several research cruises, some of them within an international framework, have been carried out so far in the Mediterranean, searching for bluefin tuna larvae having less than 10 days of days of life, and the results of these campaigns have been regularly reported to ICCAT SCRS; the most extended was a multinational cruise organised within the ICCAT BYP framework in 1994 (Nishida *et al.*, 1998) (**Figure 2**). The possibility to find bluefin tuna larvae is linked to the presence of bluefin tuna active spawners in the same area in a period ranging from 5 to 10 days before. In the Mediterranean, due to the extension of the spawning area, the very complex current regime, the peculiar oceanography, and the high mortality of larvae in the early stages, usually the density of larvae is very low, even in the areas having a high concentration of bluefin tuna spawners (Dicenta *et al.* 1979; Piccinetti *et al.*, 2013). More recently, a large scale larval campaign was conducted by Oceana (Aguilar and Lastra, 2009) and bluefin tuna larvae were found in all areas, except for the Aegean Sea. Another important research campaign (TUNIBAL) is carried out since many years in the Balearic area (Garcia *et al.*, 2001, 2002; Alemany *et al.*, 2010), further confirming this area as one of the spawning grounds for the bluefin tuna. Besides the large scale larval surveys, other researches were carried out where some marine research institutes (having a tradition in this field) are based (i.e.: Messina and Palma de Mallorca), collecting bluefin tuna larvae. As a matter of fact, only in a very few areas it was not possible so far to find tuna larvae during the bluefin tuna spawning period (i.e.: Alboran Sea, Gulf of Lion, Northern Adriatic Sea, Northern Aegean Sea) (Alemany *et al.*, 2010, Barrois 1977; Dicenta 1977; Dicenta and Piccinetti, 1977, 1980; Dicenta *et al.*, 1975, 1979; Duclerc *et al.*, 1973; Eherembaum, 1924; Giovanardi *et al.*, 2010; Ingran *et al.*, 2010; Karakulak *et al.*, 2004a, 2004b; Lalami *et al.*, 1973; Malca *et al.*, 2015; Matsumoto *et al.*, 1972; Montolio & Juarez, 1977; Nishida *et al.* 1997; Oray *et al.*, 2005; Padoa, 1956; Piccinetti 1973, 1995; Piccinetti and Piccinetti Manfrin, 1978, 1993; Piccinetti *et al.*, 1995, 1997; Richards, 1976, 1977; Richards and Potthoff, 1979; Scaccini 1966, 1968; Scaccini *et al.*, 1973, 1975; Scott *et al.*, 1993; Sella, 1924; Ueyanagy 1966; Vodionitzki & Cazanava, 1954; Yabe and Ueyanagi, 1962; Yabe *et al.*, 1966; Cavallaro *et al.* 1997; Nishida *et al.* 1997; Tsuj *et al.* 1997).

Finally, only in the second half of the XX century and in the very first part of the XXI century, it was possible to better understand the situation of the spawning areas in the full distribution range of the Atlantic bluefin tuna. The Black Sea was finally excluded (Di Natale, 2015), while the Gulf of Mexico, the Balearic Sea, the southern Tyrrhenian Sea, the central-southern Mediterranean Sea and the Levantine Sea were all confirmed, even taking into account their different characteristics. For the Mediterranean Sea, both the aerial surveys and the electronic tagging carried out by GBYP clearly showed that bluefin tuna spawners are usually concentrating in the four main areas (**Figure 3** and **Figure 4**).

It is very important to notice many recent improvements about the identification of bluefin tuna larvae (Puncher *et al.*, 2015a, 2015b), thanks to the ICCAT GBYP activities.

It is interesting to note that the spawning area in the central-southern Mediterranean Sea showed relevant changes in the last decades. As a matter of fact, bluefin tuna spawning aggregations were not common in that area before 1996, as it was confirmed by some exploratory trips made by several purse-seine vessels during the usual spawning season. Since that year, due to the effects of a modification of the Eastern Mediterranean Transient (EMT) (Incarbona *et al.*, 2016), one of the most important and not-well studied oceanographic factor for the Mediterranean Sea, bluefin tuna spawners concentrated massively in this large area, which apparently attracted also bluefin tunas typically spawning in the Tyrrhenian Sea. This shifting was confirmed by the subsequent changes in the distribution and concentrations of bluefin tuna purse-seiners. In 2006, following another modification of the EMT, several spawning aggregations moved back again to the southern Tyrrhenian Sea, but the spawning area in the central-southern Mediterranean Sea persisted and it is still one of the most important in terms of concentrations. Anyway, in 2011 ICCAT GBYP noticed an important shifting toward the East of the spawning area, for about 10 days, due to the anomalous combination of strong winds in the central Mediterranean and calm condition in a large geographic area between Greece, southern Italy and Cyrenaica. This anomaly was detailed by Piccinetti *et al.* (2013). Apparently, this shifting caused a more intense mixing among the various schools of bluefin tunas, noticed also by the genetic analyses on bluefin tuna YOY.

Occasional and opportunistic spawning outside the four main spawning areas in the Mediterranean Sea was documented also with a serendipity event for 2015 by Di Natale *et al.* (2016) (**Figure 5**), a year characterised by peculiar and hot weather conditions, which also affected in various ways the initial growth of YOY (Di Natale *et al.*, in press b).

The possibility to have opportunistic bluefin tuna spawning areas outside the Mediterranean Sea and the Gulf of Mexico was summarised for the first time by Mather *et al.* (1995) and then by various papers (Piccinetti *et al.*, 2013). Recently, bluefin tuna larvae have been found also in the Slope Sea (Richardson *et al.*, 2016) (**Figure 6**), an area already included in those listed by Mather *et al.* (1995). This opportunistic spawning is possibly the motivation of several bluefin tuna samples, examined with micro-chemical analyses, that were not possible to assign either to the western or the eastern stock, as revealed by the ICCAT GBYP Biological Studies (**Figure 7**) (Di Natale *et al.*, in press c).

A species having a so wide distribution, from the Arctic Sea to the South Atlantic, from the Gulf of Mexico to the Black Sea, must use every opportunity offered by the natural environment. It is its way of surviving over the centuries, even if the human fishing pressure was very high in past and recent times. These are the reasons why the scientific needs that a bluefin tuna larval survey could cover are important to be defined.

3. Development of a bluefin tuna larval index

Reliable indices are needed for better understanding the changes in abundance of the several components of bluefin tuna stocks. Fishery-independent indices have been requested many time by the SCRS, but they are not easy to get. The aerial survey for bluefin tuna spawning aggregations is the methodology chosen by the ICCAT Commission at the beginning of the GBYP and four surveys have been carried out in the Mediterranean Sea, but more years are needed for getting any reliable trend.

Larval indices have been developed for the northern part of the Gulf of Mexico, over an extended period of years, and for the Balearic Sea, over a reduced number of years, mostly thanks to the efforts of both NOAA and IEO and their scientists (Ingram, 2013; Ingram *et al.*, 2007, 2008, 2010, 2015; Scott *et al.*, 1993; Scott and Turner, 1996,

1996, 1997, 1999, 2001, 2003). Now the two surveys work with a standardised methodologies (Habtes *et al.*, 2014), and, thanks to the collection in parallel of environmental and ecological parameters, it has been possible to have also some environmental models for the two areas (Alemán *et al.*, 2006, 2010; Catalán *et al.*, 2011; García *et al.*, 2001, 2002, 2004, 2005, 2013b; Laiz-Carrión *et al.*, 2015; Llopiz *et al.*, 2015; Muhling *et al.*, 2010, 2014; Reglero *et al.*, 2011, 2012, 2014a, 2014b, 2015; Torres *et al.*, 2011).

Being economically impossible carrying out every year the larval surveys over the full potential areas of dispersion², larval indices can be developed for the two main spawning areas (Gulf of Mexico and Mediterranean Sea), possibly taking into account all known main spawning sub-areas within these two large marine areas.

The current larval indices and environmental models cannot be simply extrapolated to the other core spawning areas because of the different oceanographic characteristics of these areas. Furthermore, interannual variability may differ from area to area, as shown by the aerial surveys on spawning aggregations. Therefore, the environmental models developed so far for the two main surveyed areas (GOM and Balearic) should be necessarily further developed for the additional areas, taking into account the possible different oceanographic conditions for better reassessing the models in order to respond to additional or alternative correlations.

The surveys must be planned taking into account the main spawning season in each sub-area, the usual spawning peak, the legal constraints (i.e. limits for operating in various EEZs or coastal waters) and the logistic needs. The recent climate changes and the inter-annual variability might complicate or affect the surveys (García *et al.*, 2013a). Collecting the fundamental oceanographic parameters shall be necessary, as usual.

Surveys should be carried out every year for many years with the same strategy, with small refinements when necessary. Larval surveys would need several vessels used at the same time for covering the various areas using the same plankton nets and agreed techniques.

The large coverage implies a research programme at an international level, in order to have all concerned countries involved. This will help for better covering the various areas and EEZs. Various constraints (limited access areas, political constraints, etc.) may limit some parts of the areas, also complicating the logistic needs.

A project with similar characteristics needs conspicuous multi-year international funding within a cooperation framework.

4. Checking additional spawning areas

The opportunistic bluefin tuna spawning outside the main spawning sub-areas within the same spawning ground happens (Di Natale *et al.*, 2016). Its assessment is not easy (extended surveys are necessary in some years for better detecting it and assess the variability in space and time) and but is not a very first scientific priority, because of the probable minor importance for assessing the bluefin tuna SSB. Therefore, periodic extended larval survey might be also useful for this purpose, if they will be possible from a logistic and economic point of view.

On the opposite, a clear scientific priority is to confirm or exclude the additional potential spawning areas in the Atlantic Ocean, in the eastern, the central and in the western parts. For the central-eastern Atlantic, the potential areas are the zone West of the Strait of Gibraltar on both sides, the area between the southern Morocco and the Canary Islands, the area north of Madeira islands and the area near the Azores (**Figure 8**).

For this purpose, bluefin tuna larval surveys during the most probable potential spawning period should be planned, taking into account the oceanographic characteristics in the various areas.

Most of the areas are within the EEZ of single countries (Spain, Portugal, USA), while others are concerning at least two countries (Spain-Morocco). This implies that some surveys can be planned at a national level, while others needs a bilateral agreement (i.e.: Spain-Morocco).

The scientific results which could be provided by the surveys in the Atlantic Ocean would be able to better define several important scientific aspects of bluefin tuna natural history, research and management.

² Furthermore, the logistic problems would not be very easy to solve in some geographical areas.

5. Conclusions

Larval surveys are very useful for developing another fishery-independent index, which will improve our understanding of the Atlantic bluefin tuna and possibly a more focused management of the stocks.

There are quite large discussions if bluefin tuna larval surveys can be used for assessing the SSB. As a matter of fact, they are already used for this purpose and since many years for the western Atlantic bluefin tuna stock (Ingram, 2013; Ingram *et al.*, 2007, 2008; Scott *et al.*, 1993; Scott and Turner, 1996, 1996, 1997, 1999, 2001, 2003) in the ICCAT assessment which use the VPA.

But most of the doubts are well-based on both ecological and environmental issues that surely bias, in an undefined manner, the results of the larval surveys, making them possibly not exactly representative of the SSB in the areas that have been surveyed. Bluefin tuna eggs have a certain buoyancy and they float and are drifted by surface currents; being a source of proteins, they are predated by several species, including jellyfish, pelagic crustaceans and pelagic fish. The bluefin tuna egg predation is extremely variable, depending on the egg dispersion and the quantity of predators, while the final “survival rate” of the eggs in the wild is almost unknown, because of the high variability for each event.

The same happens with the bluefin tuna larvae at the different stages, but in this case the situation is even more complex, because in addition to the predation by several species and the dispersion caused by hydrodynamic factors, the survival rate is also linked to the availability of the right food chain at the right time in the right place. Even for this life stage of the bluefin tuna, the available information is largely incomplete and many components of the environmental and ecological game are simply not available.

When these real problems are duly taken into account, the potential correlation between a larval index and a SSB seems quite weak, even if the current statistical models provide numbers. This is a point of reflection that should be duly considered.

On the opposite, a bluefin tuna larval index, obtained by a series of surveys carried out on all the main sub-areas³ on both sides of the Atlantic Ocean, using a standard methodology and a common sampling strategy, would be certainly very useful for obtaining trends to be used under an Operative Model (OM) or within a Management Strategy Evaluation (MSE), even developing new tools for keeping a larval index into account. As a matter of fact, even if a model should necessarily simplify the reality, larvae are a real life stage of this species, as the SSB is, while the recruitment is a concept much more linked to the fishery. All various different indices or estimates could be components of a model, if properly developed for taking into account the differences.

³ Survey carried out in one single sub-area by each side of the Atlantic Ocean are certainly useful, but they cannot be extrapolated to the other spawning sub-areas, as discussed above, because the bias could be substantial.

Bibliography

- Abascal F.J., Megina C., Medina A., 2003, Histological and stereological assessment of batch fecundity spawning frequency and maturation of female Atlantic northern bluefin tuna around the Balearic Island. *Cahiers Options Méditerranéennes*, 60: 13-14.
- Abascal F.J., Megina C., Medina A., 2004, Testicular development in migrant and spawning bluefin tuna (*Thunnus thynnus* L.) from the eastern Atlantic and Mediterranean. *Fishery Bull.*, 102: 407-417.
- Aguilar R., Lastra P., 2009, Bluefin Tuna Larval Survey. 2008 Oceana MarViva Mediterranean Project, 1-76.
- Alemaný F., Deudero S., Morales-Nin B., López-Jurado J.L., Jansà J., Palmer M., Palomera I., 2006. Influence of physical environmental factors on the composition and horizontal distribution of summer larval fish assemblages off Mallorca Island (Balearic archipelago, western Mediterranean). *J. Plankton Res.*, 28(5): 473-487.
- Alemaný F., Quintanilla L., Velez-Belchí P., García A., Cortés D., Rodríguez J.M., Fernández de Puellas M.L., González-Pola C., López-Jurado J.L., (2010), Characterization of the spawning habitat of Atlantic bluefin tuna and related species in the Balearic Sea (western Mediterranean). CLimate Impacts on Oceanic TOp Predators (CLIOTOP), CLIOTOP International Symposium, Progress In Oceanography Volume 86, Issues 1-2, July-August 2010: 21-38.
- Anonymous, 2009, ICCAT Report of the Standing Committee on Research and Statistics (SCRS), SCI-018/2008, ICCAT Report for the Biennial Period 2008-2009, part I (2008), vol 1 (COM): 284-286.
- Arena P., 1963, Observations dans la partie sud de la mer Tyrrhénienne sur les habitudes et le comportement du thon rouge (*Thunnus thynnus* L.) pendant sa période génétique. *Proc. Gen. Fish. Coun. Medit.*, 7, 39.
- Arena P., 1964, Observation on habits and behavior of the tuna (*Thunnus thynnus*) in the southern zones of the Tyrrhenian Sea during the genetic period. *Proc. Gen. Fish. Coun. Medit.*, 7, (39): 395-411.
- Arena P., 1980, Observation aeriennes sur la distribution et le comportement du Thon rouge, *Thunnus thynnus* (L.), de la Mer Tyrrhenienne. XXVII Congr. Assem. Plen. CIESM, Cagliari.
- Arena P., 1981, Osservazioni sulle concentrazioni e sulla pesca del Tonno e dell'Alalunga nelle zone di mare meridionali. *Quad.Lab.Tecn.Pesca.*, 3(1), suppl.: 77-79.
- Arena P., 1982a, Biologia, ecologia e pesca del tonno (*Thunnus thynnus* L) osservati in un quinquennio nel Tirreno meridionale. *Atti Conv. UU.OO: sottop. Ris.Biol.Inq.Marino*, Roma: 381-405.
- Arena P., 1982b, Caratteristiche delle reti a circuizione per tonno e loro efficienza in relazione alle condizioni ambientali ed ai comportamenti della specie pescata. *Atti Conv. UU.OO. sottop. Ris.Biol.Inq.Marino*, Roma: 407-424.
- Arena P., 1982c, Composizione demografica dei branchi di tonno (*Thunnus thynnus*, L.) durante il periodo genetico, con indicazioni utili alla individuazione dello stock di riproduttori che affluiscono nel Mar Tirreno. *Atti Conv. UU.OO. sottop. Ris. Biol. Inq. Marino*, Roma.
- Arena P., 1982d, La pêche a la senne tournante du thon rouge, *Thunnus thynnus* (L.), dans les bassins maritimes occidentaux italiens. *Collect. Vol. Sci. Pap. ICCAT*, 17(2): 281-292.
- Arena P., 1985, La pesca del tonno in Sicilia. *Atti Conv.Pesca e Trasf. Prod. Itt. Siciliani*, Trapani: 23-28.
- Arena P., 1986a, Sullo stato e le caratteristiche della pesca in Italia dei grandi Teleostei pelagici (Tonno, Alalunga e Pescespada). *Rapp. Min.Mar.Merc.*, miméo: 1-17.

- Arena P., 1986b. Pesca dei grandi Scombroidei e degli Xifioidei nei mari Italiani. *Nova Thalassia*, 8 (3): 657-658.
- Arena P., 1988a, Risultati delle rilevazioni sulle affluenze del tonno nel Tirreno e sull'andamento della pesca da parte delle "tonnare volanti" nel triennio 1984-1986. MMM-CNR, Atti Seminari UU.OO. Resp.Prog. Ric., Roma: 273-297.
- Arena P., 1988b, Rilevazioni e studi sulle affluenze del tonno nel Tirreno e sull'andamento della pesca da parte delle "tonnare volanti" nel quadriennio 1984-1988. Report to: ESPI, Ente Siciliano per la Promozione Industriale, Palermo, 1-55, I-XI.
- Arena P., 1990a, Rilevazioni e studi sulle caratteristiche e lo stato delle risorse di Tonno e sugli andamenti della pesca (Relazione sulla prosecuzione 1987-89). Report to: ESPI, Ente Siciliano per la Promozione Industriale, Palermo, 1-58.
- Arena P., Cefali A., Potoschi A., 1979, Risultati di studi sulla biologia, la distribuzione e la pesca dei grandi scombroidi nel Tirreno meridionale e nello Ionio. *X*(4): 329-345.
- Arena P., Cefali A., 2002, Composizione demografica dei branchi di tonno, *Thunnus thynnus* (L.) durante il periodo genetico, con indicazioni utili alla individuazione dello stock di riproduttori che affluiscono nel Mar Tirreno. Atti Conv. UU: OO. Ris. Biol. Inq. Marino, Roma: 425-442.
- Aristotelis, 1635, De Animalibus. In: Stagiritae peripateticorum. Principis de Historia Animalium. Ed. Theodoro Goza, Venezia: 1-843.
- Atheneus di Naucratis (Ateneo), 1656, Deumosophistae. Hugueton J.A. & Ravan M.A.: 1-812.
- Baglin R.E., 1976, A preliminary study of the gonadal development and fecundity of the western Atlantic bluefin tuna. ICCAT, Coll. Vol. Sci. Pap. 5 (2): 279-289.
- Baglin R.E., 1982, Reproductive biology of western Atlantic bluefin tuna. *Fish. Bull.* 80: 121-134.
- Baglin R.E., Rivas L.R., 1977, Population fecundity of western and eastern Atlantic bluefin tuna (*Thunnus thynnus*). ICCAT, Coll. Vol. Sci. Pap. 6 (2): 361-365.
- Barrois J.M., 1977, Données préliminaires sur quelques larves de Thonidés recueillies en mer Egée (Golfe Saronique et Golfe Euboïque Nord). *Rapp. Comm. Int. Mer Médit.*, 24 (5): 37-39.
- Biancalana T., 1955, Alla pesca vagativa dei giovani tonni. *Il Giornale della Pesca*: 1-32.
- Block, B.A., Dewar, H., Blackwell, S.B., Williams, T.D., Prince, E.D., Farwell, C.J., Boustany, A., 2001, Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. *Science*, 293:1310-1314.
- Bounhiol J.P. – 1911 – Une théorie hydrodynamique des pseudo-migrations du thon commun (*Thynnus vulgaris* C. e V.) dans la Méditerranée. *C.R. Acad. Sciences*, 152.
- Catalán I.A., Tejedor A., Alemany F., Reglero P., 2011, Trophic ecology of Atlantic bluefin tuna *Thunnus thynnus* larvae. *J. Fish Biology*, Volume 78 (5): 1545–1560.
- Cavallaro G., Manfrin G., Lo Duca G., Cavallaro M., 1997, The presence of tuna larvae in the straits of Messina. ICCAT, Rec. Doc. Sc., XLVI (2): 222-224.
- Cetti F., 1777, Storia naturale di Sardegna. III. Anfibi e Pesci. Tip. Giuseppe Piattoli, Cagliari: 1-208.

- Corriero, A., Desantis, S., Deflorio, M., Acone, F., Bridges, C.R., de la Serna, J.M., Megalofonou, P., 2003, Histological investigation on the ovarian cycle of the bluefin tuna in the western and central Mediterranean. *Journal of Fish Biology*, 63: 108-119.
- Corriero A., Karakulak s., Santamaria N., Deflorio M., Spedicato D., Addis P., Desantis S., Cirillo F., Fenech-Farrugia A., Vassallo-Agius R., De La Serna J.M., Oray Y., Cau A., Megalofonou P., De Metrio G., 2005, Size and age at sexual maturity of female bluefin tuna (*Thunnus thynnus* L. 1758) from the Mediterranean Sea. *J. Appl. Ichthyol.* 21: 483-486.
- Corriero A., Medina A., Mylonas C.C., Abascal F.J., Deflorio M., Aragon L., Bridges C.R., Santamaria N., Heinisch G., Vassallo-Agius R., Belmonte Rios A., Fauvel C., Garcia A., Gordin H., De Metrio G., 2007, Histological study of the effects of treatment with gonadotropin-releasing hormone agonist (GnRHa) on the reproductive maturation of captive-reared Atlantic bluefin tuna (*Thunnus thynnus* L.). *Aquaculture*, 272: 675-686.
- D'Amico F.C., 1816, Osservazioni pratiche intorno alla pesca, corso e cammino de' tonni. Società Tipografica, Messina: 1-164.
- de Buen F., 1923a, La pesca marítima en España en 1920. Costa sud-Atlántica y Canarias. Min. Marina, Madrid, 1: 9.
- de Buen F., 1923b, La pesca marítima en España en 1920. Costa sud-Atlántica y Canarias. Min. Marina, Madrid, 10: 35-50. Comm. Expl. Méditerranée, 3 ns: 115-123.
- de Buen F., 1923c, La pesca marítima en España en 1920. Costa sud-Atlántica y Canarias. Min. Marina, Madrid, 73: 155.
- de Buen F., 1925, Biología del Atún *Orcynus thynnus* (L.) Resultado de las campañas realizadas por acuerdos internacionales. Instituto Español de Oceanografía, Madrid, 1: 1-118.
- De Metrio G., Filanti T., Megalofonou P., Petrosino G., 1988, Valutazione sull'entità, la composizione strutturale e la dinamica biologica degli stock dei grandi scombroidi (*Thunnus thynnus* (L.), *Thunnus alalunga* (Bonn.), *Xiphias gladius* (L.), nel Golfo di Taranto. Relazione preliminare. C.N.R., Roma 10/11 Settembre 1986. Atti Seminario UU.OO. responsabili dei progetti di ricerca promossi nell'ambito dello schema preliminare di Piano per la Pesca e l'Acquacoltura. MMM-CNR, 1: 219-255.
- De Metrio G., Arnold G.P., De la Serna J.M., Yannopoulos C., Labini G.S., Deflorio M., Buckley A., Ortiz De Urbina J.M., Megalofonou P., Pappalepore M., Block B., 2003a, Where do Atlantic bluefin tuna (*Thunnus thynnus* L.) spread after the spawning in the Mediterranean Sea? Workshop on farming, Management and conservation of bluefin Tuna, 2003, Istanbul: 96-101.
- De Metrio G., Corriero A., Desantis S., Zubani D., Cirillo F., Santamaria N., Aprea A., Deflorio M., De la Serna J.M., Megalofonou P., Bridges C.R., 2003b, Gonadal cycle of wild bluefin tuna in the western Mediterranean and central Mediterranean. Workshop on farming, Management and conservation of bluefin Tuna, 2003, Istanbul: 89-95.
- De Metrio G., Caggiano M., Deflorio M., Mylonas C.C., Bridges C.R., Santamaria N., Caprioli R., Zupa R., Pousis C., Vassallo-Agius R., Gordin H., Corriero A., 2010, Reproducing the Atlantic bluefin tuna in captivity: the Italian experience. ICCAT, Coll. Vol. Sci. Pap., 65 (3): 864-867.
- Dicenta A., 1977, Zonas de puesta del atun (*Thunnus thynnus* L.) y otros tunidos del Mediterraneo occidental y primer intento de evaluación del "stock" de reproductores de atun. Bol. Inst. Espa. Oceanogr., 2: 111-135.
- Dicenta A., Piccinetti C., 1977, Desove del atun (*Thunnus thynnus* L.) en el Mediterraneo occidental y evaluación directa del stock de reproductores basado en la abundancia de sus larva. ICCAT, Rec. Doc. Sc., VII: 389-395.

- Dicenta A., Piccinetti C., 1980, Comparison between the estimated reproductive stocks of the bluefin tuna (*Thunnus thynnus*) on the Gulf of Mexico and western Mediterranean. ICCAT, Coll. Vol. Sci. Pap., 9 (2): 442-448.
- Dicenta A., Piccinetti C., Piccinetti Manfrin G. – 1975 – Observaciones sobre la reproducción de los túnidos en las islas Baleares. Bol. Inst. Espa. Oceanogr., 204: 25-37.
- Dicenta A., Piccinetti C., Sarà R. – 1979 – Recherches sur la mortalité des oeufs et larves de thonidés. Rapp. Comm. Int. Mer Médit., 25-26 (10): 191-192.
- Dieuzeide R., Roland J., 1955, Contribution à la connaissance des formes jeunes de *Thunnus thynnus* Linné. Bull. Sta. Aquic. Peche Castiglione, n.s. 7.
- Di Natale, 2015, Review of the historical and biological evidences about a population of bluefin tuna (*Thunnus thynnus* l.) in the eastern Mediterranean and the Black Sea. Collect. Vol. Sci. Pap. ICCAT, 71(3): 1098-1124.
- Di Natale A., Tensek S., Pagá García A., 2016, Is the bluefin tuna facing another 2003? Collect. Vol. Sci. Pap. ICCAT, 72(6): 1614-1630.
- Di Natale A., Tensek S., Pagá García A., (in press a), Studies on eastern Atlantic Bluefin tuna (*Thunnus thynnus*) maturity – Review of old literature. Document SCRS/2016/141: 17 p.
- Di Natale A., Tensek S., Celona A., Garibaldi F., Oray I., Pagá García A., Quilez Badía G., Valastro M., (in press b), A peculiar situation for YOY of Bluefin tuna (*Thunnus thynnus*) in the Mediterranean Sea in 2015. Document SCRS/2016/140: 12 p.
- Di Natale A., Tensek S., Pagá García A., (in press c), ICCAT Atlantic-wide Research Programme for Bluefin Tuna (GBYP). Activity report for the last part of Phase 5 and the first part of Phase 6 (2015-2016). Document Document SCRS/2016/193: 1-98.
- Doumenge F., 1996, L'aquaculture des thons rouges. Biol. Mar. Medit., 6 (2): 258-288.
- Duclerc J., Sacchi J., Piccinetti C., Piccinetti Manfrin G., Dicenta A., Borrois J.M., 1973, Nouvelles donne sur la reproduction du thon rouge (*Thunnus thynnus* L.) et d'autres espèces de Thonidés en Méditerranée. Rev. Trav. Inst. Peches marit., 37,2.
- Ehrenbaum E., 1924, Scombriformes. Report on the Danish Oceanographical Expeditions. 1908-10 to the Mediterranean and adjacent seas. Vol. II Biology, p. 42.
- Frade F., 1935, Recherches sur la maturité sexuelle du Thon rouge. C.R. Congr. Int. Zool., 12.
- Frade F., Manacas S., 1933, Sur l'état de maturité des gonades chez le thon rouge génétique (note préliminaire). C.R. Assoc. Anat.: 15 p.
- García A., J.M. de la Serna Ernst J.M., López Jurado J.L., Alemany F., Rodríguez Marin E., 2002. Bluefin tuna egg and larval survey in the Balearic Sea, June 2001 (TUNIBAL 06/01). ICCAT, Coll. Vol. Sci. Pap., 54 (2): 425-431.
- García A., Alemany F., Rodríguez J.M., 2002, Distribution of tuna larvae in the Balearic Sea: preliminary results of the Tunibal 0600 larval survey. ICCAT, Coll. Vol. Sci. Pap., 54 (2): 554-560.
- García, A., Alemany F., Velez-Belchí P., López Jurado J.L., Cortés D., de la Serna J.M., González Pola C., Rodríguez J.M., Jansá J., Ramírez T., 2004, Characterization of the bluefin tuna spawning habitat off the Balearic archipelago in relation to key hydrographic features and associated environmental conditions. CGPM/ICCAT 7th Joint Ad-hoc meeting, May, Málaga, 2004.

- García A., Alemany F., Velez-Belchí P., López-Jurado J.L., Cortes D., de la Serna J.M., Rodríguez J.M., Jansá J., Ramirez T. 2005, Characterization of the bluefin tuna spawning habitat off the Balearic Archipelago in relation to key hydrographic features and associated environmental conditions. Col. Vol. Sci. Pap. ICCAT 58: 535–549.
- García A., Cortes D., Quintanilla J., Ramirez T., Quintanilla L., Rodríguez J.M., Alemany F. 2013a, Climate-induced environmental conditions influencing interannual variability of Mediterranean bluefin (*Thunnus thynnus*) larval growth. Fish Oceanogr. 22: 273–287.
- García A., Laiz R., Quintanilla J., Corregidor F. and Alemany. F. 2013b. Report on abundant bluefin larval concentrations (*Thunnus thynnus*) found off the shelf/slope area in the Spanish Levantine coasts. Signs of recovery? Collect. Vol. Sci. Pap. ICCAT, 69(1): 292-296.
- García A., Lamkin, J., 2014, Advances in research of larval bluefin ecology: Workshop proposal towards establishing future research actions. Collect. Vol. Sci. Pap. ICCAT, 70(2): 511-517
- Giovanardi O., Romanelli M., 2010, Preliminary note on tuna larvae in samples from the coasts of the southern central Mediterranean sea collected by the MV Arctic Sunrise in June/July 2008. ICCAT, Coll. Vol. Sci. Pap., 65 (3): 740-743.
- Gordoa A., 2010, The Atlantic bluefin tuna: study on the temporal pattern of spawning in the western Mediterranean region and reproductive capacity in captivity. ICCAT, Collect. Vol. Sci. Pap. ICCAT, 65(3): 837-847.
- Gordoa, A., Olivar, M.P., Arevalo, R., Viñas, J., Molí, B., Illas, X. 2009, Determination of Atlantic bluefin tuna (*Thunnus thynnus*) spawning time within a transport cage in the western Mediterranean. ICES Journal of Marine Science, 66: 2205-2210.
- Habtes S., Muller-Karger F. E., Roffer M. A., Lamkin J. T., Muhling, B. A. (2014). A comparison of sampling methods for larvae of medium and large epipelagic fish species during spring SEAMAP ichthyoplankton surveys in the Gulf of Mexico. Limnol. Oceanogr.: Methods, 12, 86-101
- Harada T., 1973, Artificial breeding of tuna and raising larvae. Mem. Fac. Agr. Kinki Univ., 6: 109-112.
- Incarbona A., Martrat B., Mortyn P.G., Sprovieri M., Ziveri P., Gogou A., Jordà G., Xoplaki E., Lutherbaker J., Langone L., Marino G., Rodríguez Sanz L., Triantaphyllou M., Di Stefano E., Grimalt G.O., Tranchida G., Sprovieri R., Mazzola S., 2016, Mediterranean circulation perturbations over the last five centuries: relevance to the past Eastern Mediterranean Transient-type events. Scientific Reports, 6, 29623, doi:10.1038/srep29623.
- Ingram W.G., 2013, Annual indices of Atlantic bluefin tuna (*Thunnus thynnus*) spawning biomass in the Gulf of Mexico. Collect. Vol. Sci. Pap. ICCAT, 69 (2): 980-991.
- Ingram W.G., Richards W.J., Porch C.E., Restrepo V., Lamkin J.T., Muhling B., Lyczkowski-Shultz J., Scott G.P., Turner S.C., 2008, Annual indices of bluefin tuna (*Thunnus thynnus*) spawning biomass in the Gulf of Mexico developed using delta-lognormal and multivariate models. SCRS/2008/086: 1-30.
- Ingram G. W., Richards W. J., Scott G. P., Turner S. C., 2007, Development of indices of bluefin tuna (*Thunnus thynnus*) spawning biomass in the Gulf of Mexico using delta-lognormal models. Collect. Vol. Sci. Pap. ICCAT, 60(4): 1057-1069
- Ingram G.W., Richards W.J., Lamkin J.T., Muhling B.A., 2010, Annual indices of Atlantic bluefin tuna (*Thunnus thynnus*) larvae in the Gulf of Mexico developed using delta-lognormal and multivariate models. Aquatic Living Resources 23: 35-47.
- Ingram G.W., Alvarez-Berastegui D., García A., Pollack A. G., López-Jurado J.L., Alemany F., 2015, Development of indices of larval bluefin tuna (*Thunnus thynnus*) in the western Mediterranean Sea. Collect. Vol. Sci. Pap. ICCAT, 71(3): 1279-1296.

- Karakulak S., Oray Y., Corriero A., Deflorio M., Santamaria N., Desantis S., De Metrio G., 2004a, Evidence of a spawning area for the bluefin tuna (*Thunnus thynnus* L.) in the Eastern Mediterranean. J. Appl. Ichthyol., 20: 318-320.
- Karakulak S., Oray Y., Corriero A., Spedicato D., Suban D., Santamaria N., De Metrio G., 2004b, First information on the reproductive biology of the bluefin tuna (*Thunnus thynnus*) in the eastern Mediterranean. ICCAT, Coll. Vol. Sci. Pap., 56 (3): 1158-1162.
- Kocked W., Hattour A., Alemany F., García A., Zarrad R., 2012, Distribution of tuna larvae in Tunisian east coasts and its environmental scenario. Cahiers de Biologie Marine 53(4):505-515.
- Kocked W., Hattour A., Alemany F., García A., Said K., 2013, Spatial distribution of tuna larvae in the Gulf of Gabes (Eastern Mediterranean) in relation with environmental parameters. Mediterranean Marine Science, <http://dx.doi.org/10.12681/mms.314>
- Kocked W., Alemany F., Ben Ismail S., Benmessaoud R., Hattour A., Garcia A., 2015, Environmental conditions influencing the larval fish assemblage during summer in the Gulf of Gabes (Tunisia: South central Mediterranean). Mediterranean Marine Science, DOI: <http://dx.doi.org/10.12681/mms.1158>.
- Kocked W., Alemany F., Rimel B., Hattour A., 2016, Characterization of the spawning area of tuna species on the northern Tunisian coasts. Scientia Marina, Vol 80, No 2, doi:10.3989/scimar.04332.27A.
- Laiz-Carrión R., Gerard T., Uriarte A., Malca E., Quintanilla J.M., Muhling B., Alemany F., Privoznik S., Shiroza A., Lamkin J.T., García A., 2015, Larval bluefin tuna (*Thunnus thynnus*) trophodynamics from Balearic Sea (WM) and Gulf of Mexico spawning ecosystems by stable isotope. Collect. Vol. Sci. Pap. ICCAT, 71(3): 13
- Lalami Y., Tellai S., Barrois J.M., Piccinetti C., Piccinetti Manfrin G., 1973, Observations sur les oeufs et larves des thonides des cotes algeriennes. Pelagos, IV (2): 54-65.
- Llopiz J.K., Muhling B.A., Lamkin J.T., 2015, Feeding dynamics of Atlantic bluefin tuna (*Thunnus thynnus*) larvae in the Gulf of Mexico. Collect. Vol. Sci. Pap. ICCAT, 71(4): 1710-171
- Lo Bianco S., 1908/1909, Notizie biologiche riguardanti specialmente il periodo di maturità sessuale degli animali del Golfo di Napoli. Mitt. Zool. Sta. Neapel, 19.
- Malca E., Muhling B., Lamkin J., Ingram W., Gerard T., Tilley J., Franks J., 2015, Age and growth of larval Atlantic Bluefin tuna, *Thunnus thynnus*, from the Gulf of Mexico. Collect. Vol. Sci. Pap. ICCAT, 71(4): 1728-1735.
- Marino G., Candi G., Di Marco P., Longobardi A., Priori A., 2005, Supporto scientifico per la riproduzione controllata di grandi pelagici: *Seriola demerilii* e *Thunnus thynnus*. ICRAM, Relazione finale VI Piano Triennale della Pesca e dell'Acquacoltura. Progetto di Ricerca 6C83.
- Mather F.J.III, Mason J.M., Jones A.C., 1995, Historical document: Life History and Fisheries of Atlantic Bluefin Tuna. NOAA Tech. Mem., NMFS-SEFSC, 370: 1-165.
- Matsumoto W.M., Ahlstrom E.H., Jones S., Klave W.L., Richards W.J., Ueyanagi S., 1972, On the clarification of larval tuna identification particularly in the genus *Thunnus*. U.S. Fish Wildl. Serv., Fish. Bull., 70.
- Medina A., Abascal F.J., Megina C., Garcia A., 2002, Stereological assessment of the reproductive status of female Atlantic northern bluefin tuna during migration to Mediterranean spawning grounds through the Strait of Gibraltar. Journal of Fish Biology, 60: 203-217.
- Montolio M., Juarez M., 1977, El desove de *Thunnus thynnus thynnus* en el Golfo de México – Estimado preliminary de la magnitud de la poblacion en desove a partir de la abundancia de larvas. ICCAT, Coll. Vol. Sci. Pap. 6: 337-344.

- Muhling B.A., Lamkin J.T., Roffer M.A., 2010, Predicting the occurrence of Atlantic bluefin tuna (*Thunnus thynnus*) larvae in the northern Gulf of Mexico: building a classification model from archival data. *Fisheries Oceanography* 19: 526-539.
- Muhling B.A., Reglero P., Ciannelli L., Alvarez-Berastegui D., Alemany F., Lamkin J.T., Roffer M.A., 2013, Comparison between environmental characteristics of larval bluefin tuna *Thunnus thynnus* habitat in the Gulf of Mexico and western Mediterranean Sea.
- Mylonas C., 2002, Hormonal induction of spawning with reference to the bluefin tuna. Proceedins of the first international symposium of domestication of bluefin tuna *Thunnus thynnus thynnus*. Cartagena (Spain), 3-8 February 2002.
- Nishida T., Tsuji S., Segawa K., 1998, Spatial data analysis of Atlantic bluefin tuna larval surveys in the 1994 ICCAT BYP. ICCAT, Coll. Vol. Sci. Pap. 48(1): 107-110.
- Oppianus, 177 b.C., Alieuticon. In: Salvini A.M., 1738, Della Caccia e della Pesca. Firenze : 1-510.
- Oray I.K., Karakulak S., 1998, Investigations on the reproductive biology of bluefin tuna (*Thunnus thynnus*, L. 1758) in the North Aegean Sea. ICCAT. Collect. Vol. Sci. Pap., ICCAT (49): 120-125.
- Oray I., Karakulak F.S., Garcia A., Piccinetti C., Rollandi L., De la Serna J.M., 2005, Report on the Mediterranean BYP Tuna larval meeting. ICCAT, 58 (4): 1429-1435.
- Ottolenghi F., Cerasi S., 2008, Il tonno rosso nel Mediterraneo. Biologia, Pesca, Allevamento e Gestione. UNIMAR, Roma 2008.
- Padoa E., 1956, Uova, larve e stadi giovanili di Teleostei: Scombridae e Thunnidae. Fauna und Flora Naeples, Monographie 38.
- Parona C., 1919, Il Tonno e la sua pesca. R. Comit. Talass. Ital., Venezia, Mem. LXVIII: 1-259.
- Piccinetti C., 1973, Stades larvaires et juveniles des thons en Adriatique. *Ichtyologia*, 5.
- Piccinetti C., 1995, Activités réalisées en Italia en accord au programme “ICCAT BYP Survey of Bluefin Larvae”. ICCAT, Rec. Doc. Sc., XLIV (1): 330-332.
- Piccinetti C., Piccinetti Manfrin G., 1970, Osservazioni sulla biologia dei primi stadi giovanili del tonno (*Thunnus thynnus* L.). *Boll. Pesca Piscic. Idrobiol.*, 25 (2).
- Piccinetti C. , Piccinetti Manfrin G. – 1978 – Presence des oeufs et larves de thonides en fonction des conditions hydrologiques en Mediterranee. *Actes de Colloques du C.N.E.X.O*, 8: 79-86.
- Piccinetti C., Piccinetti Manfrin G., 1993, Distribution des larves de thonides en Méditerranée. *FAO, Fish. Rep.* 494: 186-206.
- Piccinetti Manfrin G., Marano G., De Metrio G., Piccinetti C., 1995, Aree di riproduzione di *Thunnus thynnus* nel mar Nero. *Biol. Mar. Medit.* 2 (2): 503-504.
- Piccinetti C., Piccinetti Manfrin G., Soro S., 1966, Larve di Tunnidi nel mediterraneo. *Biología Marina Mediterránea*, 3(1): 303-309.
- Piccinetti C., Piccinetti Manfrin G., Soro S., 1997, Résultats d’une campagne de recherche sur les larves de thonidés en Méditerranée. ICCAT, Rec. Doc. Sc. XLVI (2): 207-214.
- Piccinetti C., Di Natale A., Arena P., 2013, Eastern bluefin tuna (*Thunnus thynnus*, L.) reproduction and reproductive areas and seasons. *Collect. Vol. Sci. Pap. ICCAT*, 69(2): 891-912.

- Plinius C.S., 65 AD? (re-edited in 1553), *Historia Mundi. Naturalis Historia*. Ed. Antonio Vicentino, Ludguni: 1-882.
- Puncher G.N., Arrizabalaga H., Alemany F., Cariani A., Oray I.K., Karakulak S.F., Basilone G., Cuttitta A., Mazzola S., Tinti F., 2015, Molecular Identification of Atlantic Bluefin Tuna (*Thunnus thynnus*, Scombridae) Larvae and Development of a DNA Character-Based Identification Key for Mediterranean Scombrids. *PLOS One*, <http://dx.doi.org/10.1371/journal.pone.0130407>
- Puncher G.N., Alemany F., Arrizabalaga H., Cariani A., Tinti F., 2015, Misidentification of bluefin tuna larvae: a call for caution and taxonomic reform. *Rev Fish Biol Fisheries*, 25(3): DOI 10.1007/s11160-015-9390-1.
- Reglero P., Urtizberea A., Torres A. P., Alemany F., Fiksen O., 2011, Cannibalism among size classes of larvae may be a substantial mortality component in tuna. *Marine Ecology Progress Series*, 433: 205-219.
- Reglero P., Ciannelli L., Alvarez-Berastegui D., Balbin R., Lopez-Jurado J. L., Alemany F., 2012, Geographically and environmentally driven spawning distributions of tuna species in the western Mediterranean Sea. *Marine Ecology Progress Series*, 463: 273.
- Reglero P., Ortega A., Blanco E., Fiksen O., Viguri F. J., De La Gandara F., Seoka M., Folkvord A., 2014a, Size-related differences in growth and survival in piscivorous fish larvae fed different prey types. *Aquaculture*, 433: 94-101.
- Reglero P., Tittensor D. P., Alvarez-Berastegui D., Aparicio-Gonzales A., Worm B., 2014b, Worldwide distributions of tuna larvae: revisiting hypotheses on environmental requirements for spawning habitats. *Marine Ecology Progress Series*, 501: 207-224.
- Reglero P., Blanco E., Ortega A., Fiksen O., De La Gandara F., Seoka M., Viguri F. J., Folkvord A., 2015, Prey selectivity in piscivorous bluefin tuna larvae reared in the laboratory. *Journal of Plankton Research*, 37: 2-5.
- Richards W.J., 1976, Spawning of bluefin tuna (*Thunnus thynnus*) in the Atlantic Ocean and adjacent seas. *ICCAT, Coll. Vol. Sci. Pap.*, 2: 267-278.
- Richards W.J., 1977, Distribution and abundance of bluefin tuna larvae in the Gulf of Mexico. *ICCAT, Coll. Vol. Sci. Pap.*, 7.
- Richards W.J., Potthoff T., 1979, Distribution and abundance of bluefin tuna larvae in the Gulf of Mexico in 1977 and 1978. *ICCAT, Coll. Vol. Sci. Pap.*, 9: 433-441.
- Richardson D.E., Marancik K.E., Guyon J.R., Lutcavage M.E., Galuardi B., Lam C.H., Walsh H.J., Wildes S., Yates D.A., Hare J.A., 2016, Discovery of a spawning ground reveals diverse migration strategies in Atlantic bluefin tuna (*Thunnus thynnus*). *Proc. Natl. Acad. Sci., U S A.*, 113 (12): 3299-3304. doi: 10.1073/pnas.1525636113.
- Rodriguez-Roda J., 1967, Fecundidad del atún, *Thunnus thynnus* (L.) de la costa sudatlantica de Espana. *Inv. Pesq.* 31:33-52.
- Rooker, J.R., Alvarado Bremer, J.R., Block, B.A., Dewar, H., De Metrio, G., Corriero, A., Kraus, R.T., et al., 2007, Life history and stock structure of Atlantic bluefin tuna (*Thunnus thynnus*). *Reviews in Fisheries Science*, 15: 265-310.
- Roule L., 1917, Etude sur les aires de ponte et les déplacements périodiques du thon commun (*Orcynus thynnus* L.) dans la Méditerranée occidentale. *Annales Inst. Océan.*, Paris, VII-7.
- Roule L., 1923, Considérations sur l'écologie abyssale des alevins de thon. *Communication au Congrès de Paris, Comm.Int.Expl.de la Mer*.

- Santamaria N., Corriero A., Desantis S., Zubani D., Gentile R., Sciscioli V, De La Serna J., Bridges C.R, De Metrio G., 2003a, Testicular cycles of Mediterranean bluefin tuna (*Thunnus thynnus* L.). Cah. Options Mediterr., 60: 183-185.
- Sanzo L., 1932, Uova e primi stadi larvali di Tonno (*Orcynus thynnus*) Linneo). Mem. R. Com. Talassogr. Ital., mem. 198 : 1-16.
- Scaccini A., 1959, Bio-écologie des jeunes thons des mers italiennes. Proc. Gen. Fish. Coun. Medit., 5,70.
- Scaccini A., 1966, Studio dei caratteri differenziali dei primi stadi in alcune specie di tinnidi. Archivio Zoologico Italiano, Vol. LI, parte II: 1053-1061.
- Scaccini A., 1968, Etude des caractères différentiels des premiers stades dans différentes espèces de thonidés. Rapp. Comm. Int. Mer Médit., 19(2): 311-312.
- Scaccini A., Sarà R., Piccinetti C., Manfrin G., 1973, Données préliminaires sur une campagne d'étude sur les oeufs et larves des thonidés. Rapp. Comm. Int. Mer Médit., 21, 10.
- Scaccini A., Sarà R., Piccinetti C., Piccinetti Manfrin G., 1975, Uova e larve di tonno pescate nella Sicilia occidentale e loro allevamento. Ministero della Marina Mercantile, Direzione Generale della Pesca Marittima. Memoria n. 39.
- Schaefer K.M., 2001, Reproductive biology of tunas, in Block, B.A., Stevens, E.D. (Eds), Tuna: Physiology, ecology and Evolution. Academic Press, San Diego, 225-270.
- Scordia C., 1938, Per la biologia del tonno (*Thunnus thynnus*, L.). XV. Le migrazioni dei tonni entro il Mediterraneo. Mem. Biol. Mar. Ocean., Messina, 1938.
- Scott, G.P., Turner, S.C., Grimes, C.B., Richards, W.J., Brothers, E.B. 1993. Indices of larval bluefin tuna, *Thunnus thynnus*, abundance in the Gulf of Mexico: Modeling variability in growth, mortality, and gear selectivity: Ichthyoplankton methods for estimating fish biomass. Bulletin of Marine Science 53: 912-929.
- Scott G.P., Turner S.C., 1994. An updated index of west Atlantic bluefin spawning biomass based on larval surveys in the Gulf of Mexico. Collect. Vol. Sci. Pap. ICCAT, 42(1): 211-213.
- Scott G.P., Turner S.C., 1996, Updated index of bluefin tuna (*Thunnus thynnus*) spawning biomass from Gulf of Mexico ichthyoplankton surveys. Collect. Vol. Sci. Pap. ICCAT, 45(2): 220-221.
- Scott G.P., Turner S.C., 1997, Updated index of bluefin tuna (*Thunnus thynnus*) spawning biomass from Gulf of Mexico ichthyoplankton surveys. Collect. Vol. Sci. Pap. ICCAT, 46(2): 274-275.
- Scott G.P., Turner S.C., 1999, Updated index of bluefin tuna (*Thunnus thynnus*) spawning biomass from Gulf of Mexico ichthyoplankton surveys. Collect. Vol. Sci. Pap. ICCAT, 49(2): 34-346.
- Scott G.P., Turner S.C., 2001, Updated index of bluefin tuna (*Thunnus thynnus*) spawning biomass from Gulf of Mexico ichthyoplankton surveys. Collect. Vol. Sci. Pap. ICCAT, 52(3): 1067-1069.
- Scott G.P., Turner S.C., 2003, Updated index of bluefin tuna (*Thunnus thynnus*) spawning biomass from Gulf of Mexico ichthyoplankton surveys. . Collect. Vol. Sci. Pap. ICCAT, 55(3): 1123-1126
- Sella M., 1924, Caratteri differenziali dei giovani stadi di *Orcynus thynnus* Ltkn, O. alalonga Risso, Auxis bisus Bp. Rendiconti. Acc. Naz. Lincei, 33.
- Sella M., 1929a, Migrazioni ed habitat del tonno (*Thunnus thynnus* L.) studiati col metodo degli ami, con osservazioni sull'accrescimento, sul regime delle tonnare, ecc. Mem. R. Com. Talass. Ital., 16: 3-24.

- Sella M., 1929b, Biologia e pesca del tonno (*Thunnus thynnus* L.). Atti Conv. Biol. Mar. Appl. Pesca, Messina, Giugno 1928: 3-32.
- Susca V., De Florio M., Corriero A., Breidges C.R., De Metrio G., 2000, Sexual maturation in the bluefin tuna (*Thunnus thynnus*) from the Central Mediterranean Sea. In: Prooc. 6th intern. Symp. On the Reproductive Physiology of Fish (Noberg B., Kiesbu O.S., Taranger G.L., Andersson E., Stefansson S.O., eds.), pp 105. Bergen: Fish. Symp. 99.
- Susca V., Corriero A., Bridges C.R., De Metrio G., 2001, Study of the sexual maturity of female bluefin tuna: purification and partial characterization of vitellogenin and its use in an enzyme-linked immunosorbent assay. H. Fish. Biol., 58: 815-831.
- Tsuji S., Nishikawa Y., Segawa K., Hiroe Y., 1997, Distribution and abundance of *Thunnus* larvae and their relation to the oceanographic condition in the Gulf of Mexico and the Mediterranean Sea during May through August of 1994. ICCAT Coll. Vol. Sci. Pap. 46 (2): 161-176.
- Torres A. P., Reglero P., Balbin R., Urtizberea A., Alemany F., 2011, Coexistence of larvae of tuna species and other fish in the surface mixed layer in the NW Mediterranean. Journal of Plankton Research, 33: 1793-1812.
- Ueyanagi S., 1966, On the red pigmentation of larval tuna and its usefulness in species identification. Rep. Nankai Reg. Fish. Res. Lab., 24.
- Ueyanagi S., Keiichiro M., Nishigawa Y., Suda A. (Ed.), 1973, Report on experiments on the development of tuna culturing techniques (April 1970 – March 1973). Far Seas Fish. Res. Lab., S Series, 8.
- Vodionitzkii B.A., Kazanova J.J., 1954, Determination key to the eggs and larvae of the pelagic fishes of the Black Sea. Marine Fish Economy and Oceanography Research Institute, VNIRO, 28: 240-323.
- Yabe H., Ueyanagi S. – 1962 – Contributions to the study of the early life history of the tunas. Rep. Nankai Reg. Fish. Res. Lab., 1.
- Yabe H., Ueyanagi S., Watanabe H. – 1966 – Studies on the early life history of bluefin tuna *Thunnus thynnus* and on the larva of the southern bluefin tuna *T. maccoyii*. Rep. Nankai Reg. Fish. Res. Lab., 23.
- Zupa R., Corriero A., Deflorio M., Santamaria N., Spedicato D., Marano C., Losurdo M., Bridges C.R., De Metrio G., 2009, A low percentage of non-reproductive bluefin tuna (*Thunnus thynnus* L. 1758) in the Mediterranean Sea.. J Fish Biology 75 (6): 1221-122

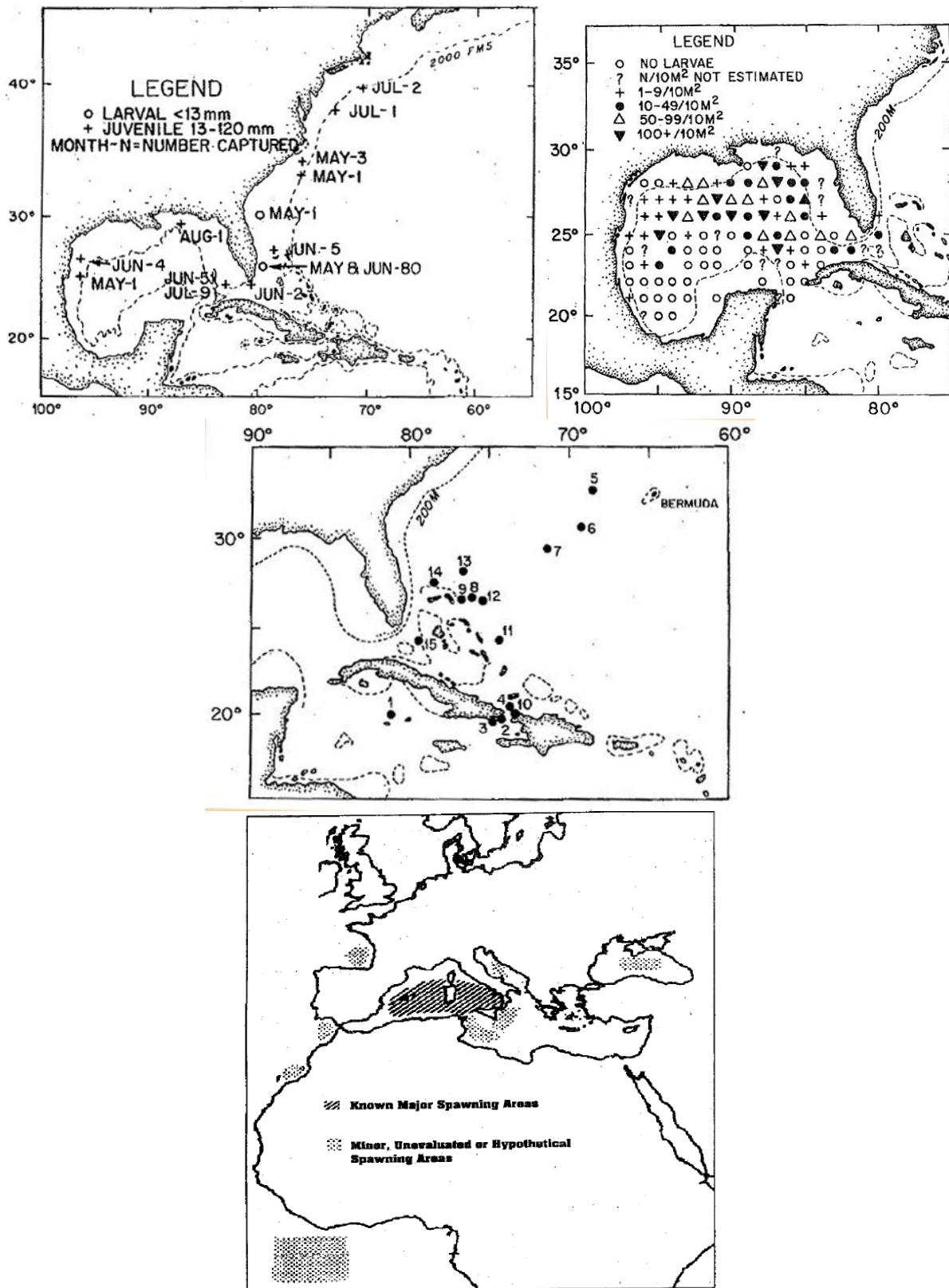


Figure 1. Distribution of bluefin tuna larvae in the Western Atlantic and the Gulf of Mexico, and potential and known bluefin tuna spawning areas in the Mediterranean Sea (bottom) as reported by Mather *et al.*, 1995.

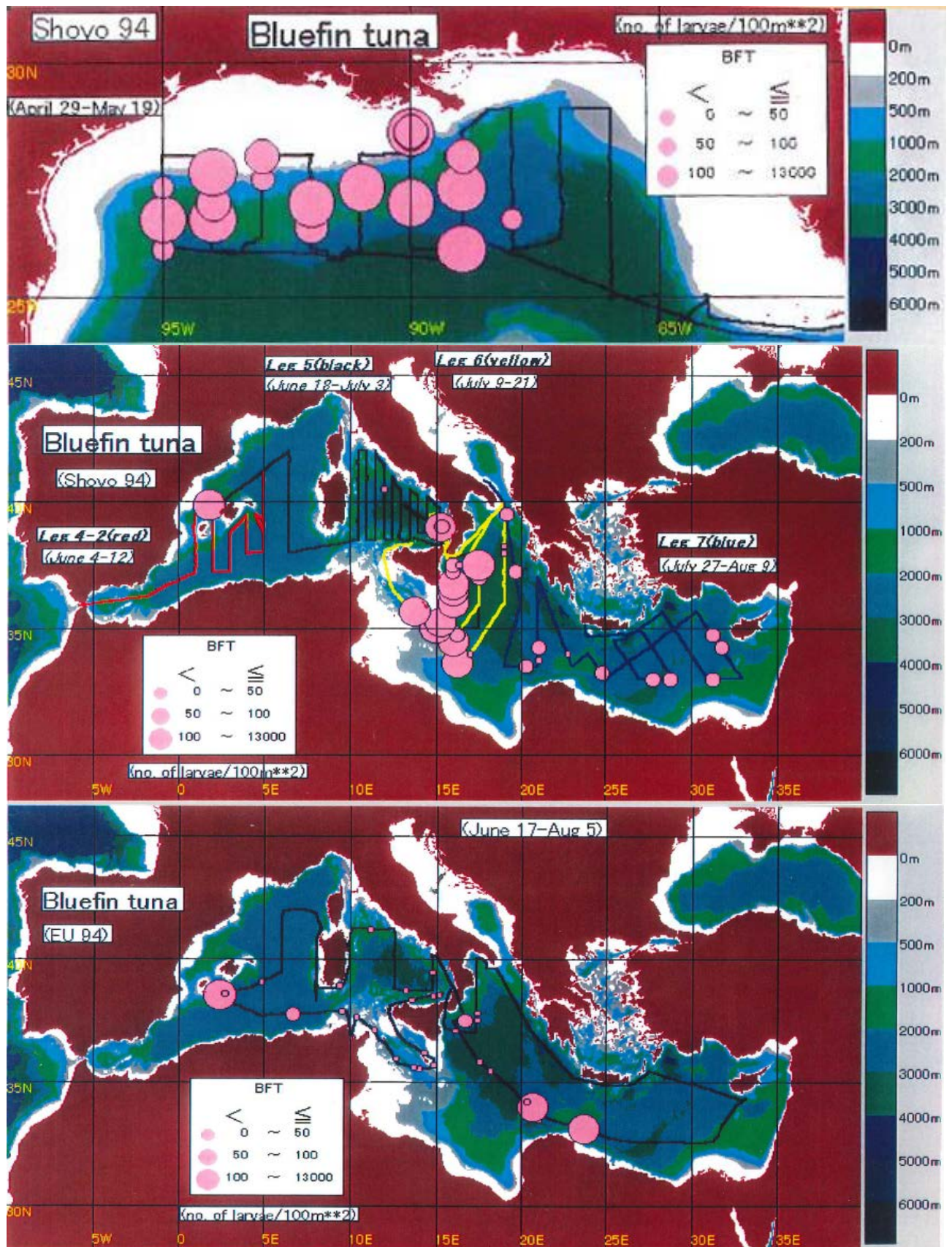


Figure 2. Distribution and abundance of bluefin tuna larvae as detected by the cruises conducted within the ICCAT BYP in 1994 (Nishida *et al.*, 1998).

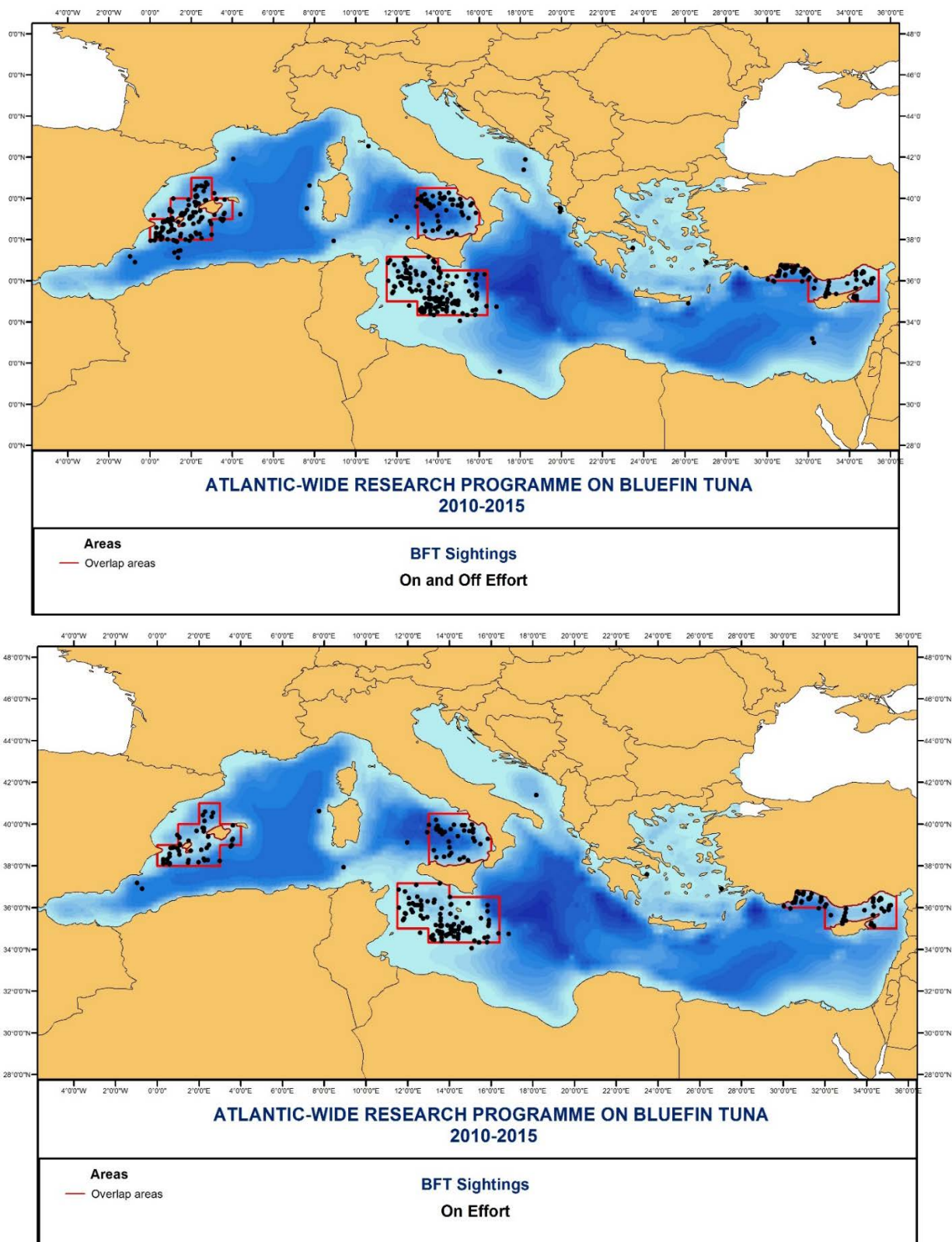


Figure 3. Distribution of bluefin tuna spawning aggregations from the cumulative data obtained by the ICCAT GBYP aerial survey in 2010, 2011, 2013 and 2015. The last two surveys were extended over most of the Mediterranean Sea. Even if the coverage between the four main spawning areas and the other areas was different, it is quite clear that spawning aggregations have been poorly encountered outside the four core areas.

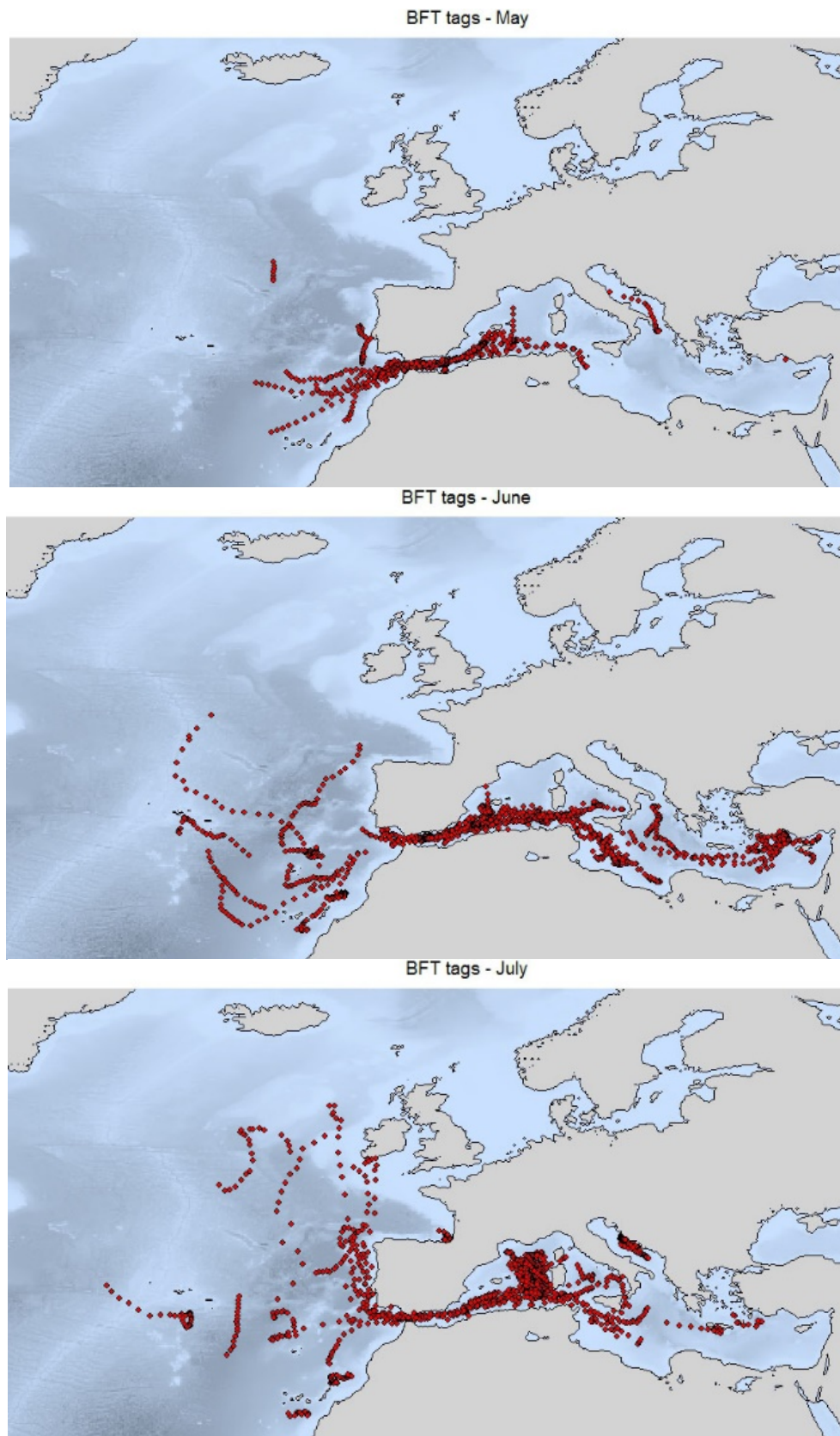
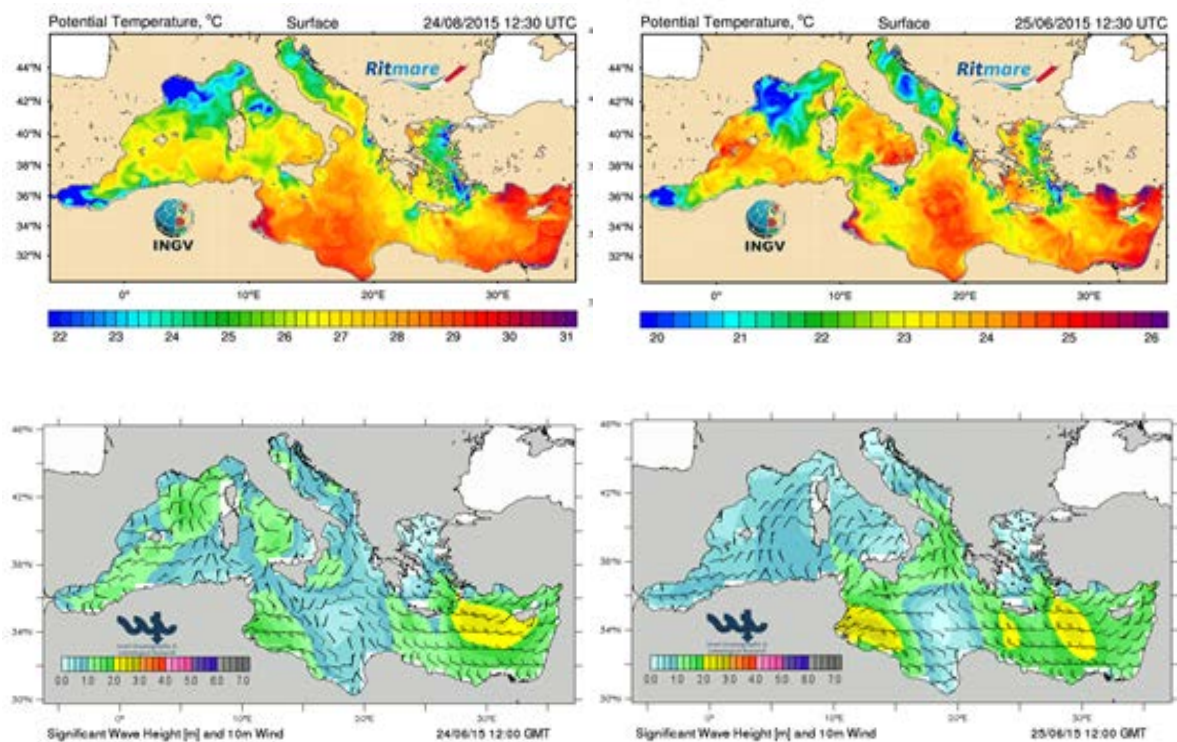


Figure 4. Distribution of bluefin tuna during the three main spawning months: May (prespawners and spawners), June (mostly spawners) and July (spawners and post-spawners); the fish were electronically tagged by ICCAT GBYP. The tracks in the Adriatic Sea are related to immature bluefin tunas.



Estimated Track Tag: 150293

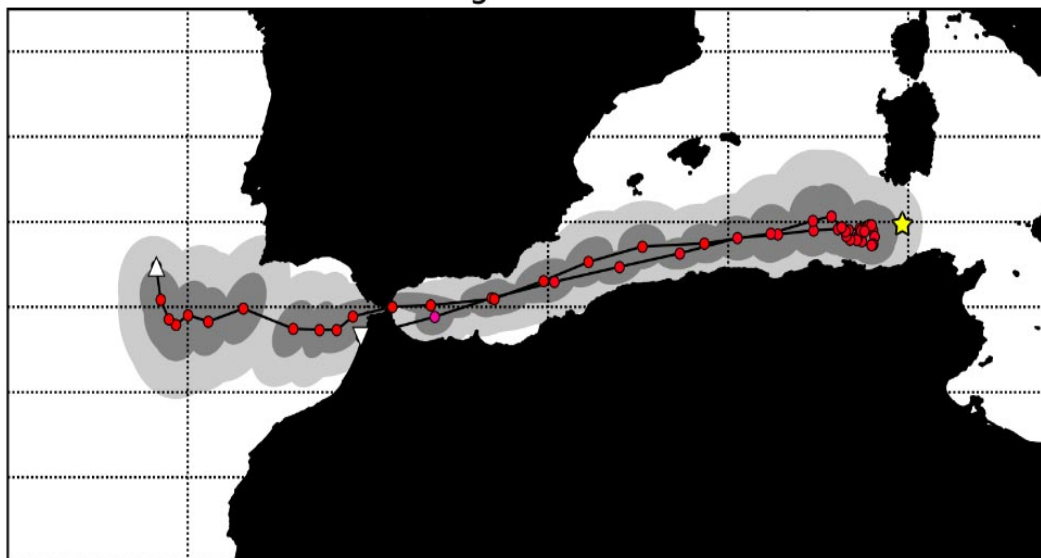


Figure 5. The opportunistic spawning event documented in a serendipity way by ICCAT GBYP in June 2015: the area North of the Tunisian coast had right meteorological and oceanographic conditions for bluefin tuna spawning. A large school of bluefin tuna spawners was spotted by one of the aircrafts acting on behalf of GBYP in that area (yellow star in the lower image), while a bluefin tuna individual, that was tagged in a Moroccan trap by GBYP (dark grey track in the lower image) showed a spawning behaviour in the same zone on the same days (Di Natale *et al.*, 2016).

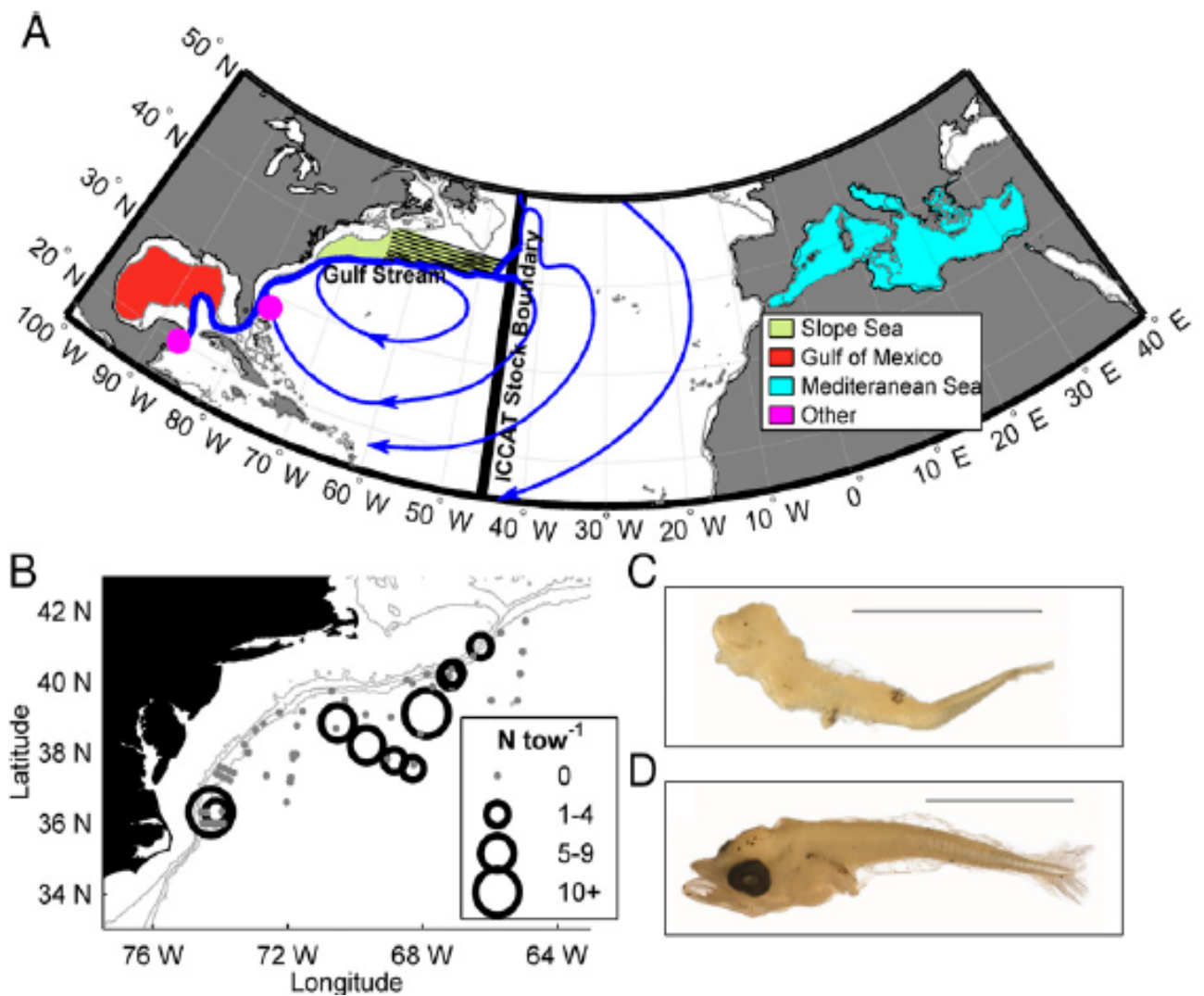


Figure 6. Distribution of bluefin tuna larvae recently discovered in the Slope Sea by Richardson *et al.*, 2016. Due to the age of the larvae, it is excluded that they could be transported by the currents from the Gulf of Mexico, while the possibilities that they were born in the same Slope Sea or even in the northern part of the Bahamas are quite consistent.

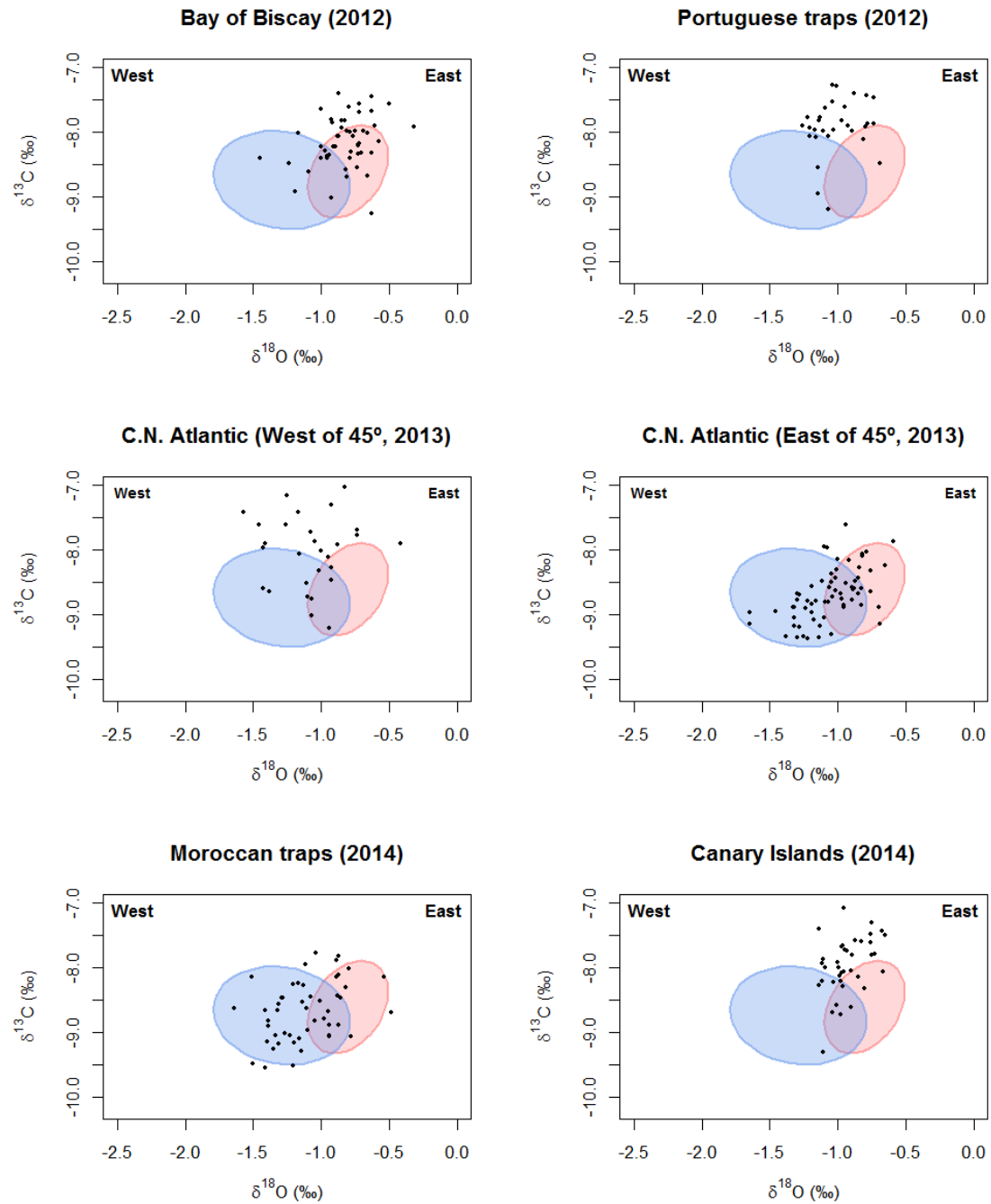


Figure 7. Results of the micro-chemical analyses carried out by the Consortium in charge of the Biological Studies for the ICCAT GBYP: the two ellipses represent the possibilities that the individual belongs to the western (light blue) or the eastern (light red) stock. The points outside the two ellipses are not-assigned individuals that might potentially come from other oceanic areas.

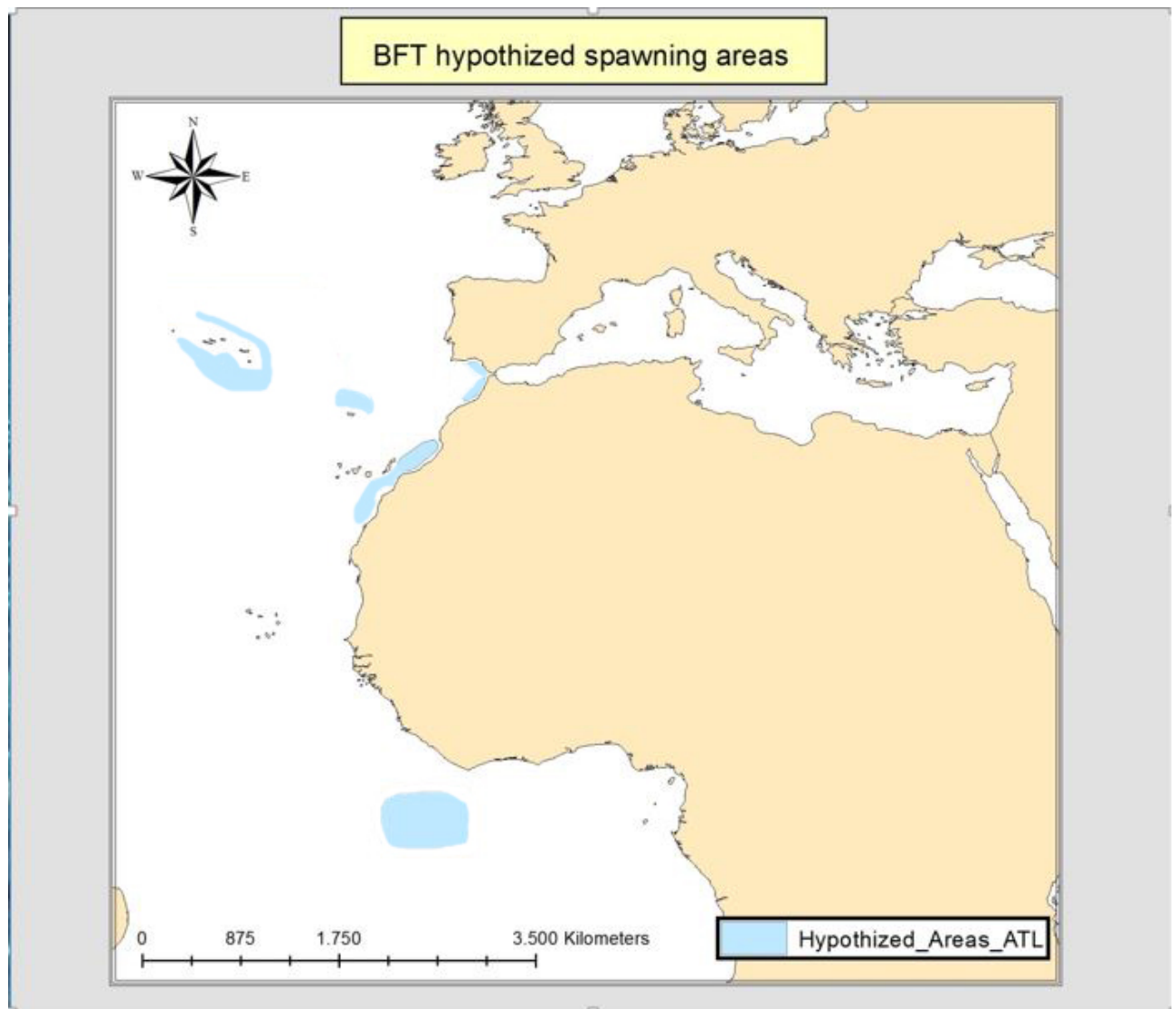


Figure 8. Potential additional spawning areas outside the Mediterranean Sea in the eastern Atlantic Ocean. Bluefin tuna YOY were reported in the area West of the Strait of Gibraltar and in the Ibero-Moroccan area, including the Isle of Tenerife (Canary Islands). Bluefin tuna larvae were found in the Gulf of Guinea in the '60s. Several potential bluefin tuna spawners, tagged in Morocco in various years, went to the Canary Islands, the area North of Madeira and in the area close to the Azores during the spawning period with proper environmental conditions.