EASTERN BLUEFIN TUNA (*THUNNUS THYNNUS*, L.) REPRODUCTION AND REPRODUCTIVE AREAS AND SEASON

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SUMMARY

This paper provides a general overview on existing scientific knowledge on eastern bluefin tuna reproduction and reproductive areas, based on many published papers and even several recent reports. The various larval campaigns were considered, as well as any additional data source concerning age 0 distribution and evidence of mature gonads in various Mediterranean areas over at least more than a century. According to all the published information, it is evident that most of the Mediterranean Sea is a spawning area, with some areas more documented than others (maps were provided), but it is very clear that spawning aggregations can vary in time and space each year, according to several factors, mostly environmental. Some issues concerning the reproduction are still to be understood, but a lot of knowledge is already available. The possibility of having extra-Mediterranean spawning areas was also discussed, but without any definitive additional element.

RÉSUMÉ

Le présent document donne un aperçu général des connaissances scientifiques actuelles sur la reproduction du thon rouge de l'Est et sur les zones de frai, sur la base de nombreux documents publiés et plusieurs rapports récents. Les différentes campagnes larvaires ont été prises en compte, ainsi que toute autre source supplémentaire de données concernant la distribution d'âge 0, et les éléments de preuve de gonades matures dans plusieurs zones de la Méditerranée pendant, au moins, plus d'un siècle. D'après toutes les informations publiées, il apparaît clairement que la plus grande partie de la Méditerranée constitue une zone de frai, certaines zones étant plus documentées que d'autres (des cartes sont apportées), mais il est très clair que les concentrations de reproducteurs peuvent varier dans le temps et dans l'espace chaque année en fonction de plusieurs facteurs, principalement environnementaux. Quelques aspects de la reproduction restent encore à comprendre, mais de nombreuses connaissances sont déjà disponibles. L'existence possible de zones de frai extra-méditerranéennes a également été abordée, mais sans aucun élément supplémentaire définitif.

RESUMEN

Este documento presenta una visión general del conocimiento científico existente sobre las zonas de reproducción y la reproducción del atún rojo oriental, basándose en muchos documentos publicados e incluso en varios informes recientes. Se consideraron diferentes campañas de larvas, así como cualquier fuente de datos adicionales relacionada con la distribución de la edad 0 y la evidencia de gónadas maduras en varias zonas del Mediterráneo durante al menos más de un siglo. En la información publicada se evidencia que la mayor parte del mar Mediterráneo es una zona de reproducción, con algunas zonas mejor documentadas que otras (se proporcionan mapas), pero queda muy claro que las concentraciones de reproductores pueden variar en el tiempo y en el espacio cada año, en función de varios factores, sobre todo medioambientales. Todavía tienen que comprenderse algunos temas relacionados con la reproducción, pero ya se dispone de una gran cantidad de conocimientos. También se debate la posibilidad de que existan zonas de reproducción fuera del Mediterráneo, pero sin un elemento adicional definitivo.

KEYWORDS

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

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1. Introduction

In the very last years, the SCRS has been requested to provide details about the spawning periods or the spawning areas on the eastern bluefin tuna stock. As a matter of fact, this information exists in many published papers, even if it is sometimes partial, scattered or related so some specific areas. Anyway, several general syntheses are available, even if sometimes they are not in any official ICCAT languages or they are in domestic reports or even difficult to access.

It is important to take into account that data and detailed information about eastern bluefin tuna exist since more than 22 centuries (Di Natale, 2010), and the total number of papers concerning these aspects of the reproductive biology of this important species are included in many hundreds of papers, published in several countries on both sides of the Atlantic Ocean and in the Mediterranean Sea (Corwin, 1929; Aguilar & Lastra, 2009; Di Natale, 2012). It is true that this is one of the species for which data about its biology are among the most numerous and detailed, even if some aspects are still to be further investigated and understood, because this species has a very complex behaviour and it is distributed in all oceans, in various environments. Its historical interest as an important marine resource always stimulated scientific activities and not only fishing.

The scope of this paper is to resume all the information available for the eastern bluefin tuna (*Thunnus thynnus*, L.) and make a clear synthesis to be used for SCRS purposes, also according to our long scientific and field experience on this species.

2. Reproduction in eastern Atlantic bluefin tuna

Whenever we deal with the reproduction of a marine organism, and particularly a fish species, it is basic to know its general reproductive aspects, the age of first maturity, various biological parameters such as fecundity, mortality of the eggs or larvae or juveniles, reproductive period, spawning areas, etc.

As concerns the reproduction, it was finally scientifically clarified something that was reported in many previous papers, mostly as direct field observations at the factories: the reproduction occours in a fractionated manner in each bluefin tuna individual. Marino *et al*. (2005), demonstrated that the same bluefin tuna individual is able to release the 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. Spawning may occur for more than one month in the same season in the same individual,. Recent information seems to indicate that this spawning period could be much more extended under particular circumstances. Of course, it is logical that the same happens to male bluefin tunas, even if studies on males are much more limited.

Eggs fecundation is external in bluefin tuna and may occurs after isolated contemporary emissions of gametes by 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 field studies, where also the 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).

Usually, 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 close to their first reproduction (just over 20 kg) up to very big individuals even over 500 kg. It is difficult to discriminate about the reproductive role or importance of the various age components within the same schools during spawning, beside of the differnt quantity of genetic products they can release according to their dimension, except when it is possible to carry out detailed genetic investigations as it was done during some experiments in cages.

Each bluefin tuna ovary contains various millions of oocytes, and those having the biggest size can be hydratated and released in a very short time following hormonal stimuli, which some predators may also captate (Suska *et al*. 2000, 2001; Schaefer, 2001; Medina *et al.* 2002; Abscal *et al.* 2003, 2004; Corriero *et al*. 2003, 2005; Santamaria *et al*. 2003; Zupa *et al*. 2009).

The surface of the sea area where bluefin tuna spawning is occurring usually became whitish and milky for a few minutes when the contemporary emission of female and male gametes occurs.

In the last decades, many other knowledge elements where provided by studies on 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 speed of this species, then the spawning area can be very large and extended, even considering a period of about one or one and a half month.
For better defyning the bluefin tuna reproduction, we are considering the various researches and observations carried out so far by many authors about the following points:

a) Age or size at first maturation
b) Spawning period when eggs are released
c) Identification of the spawning areas by presence of adult individuals with mature gonads during the spawning season, or presence of bluefin tuna eggs and larvae in the plankton, or presence of very small bluefin tunas of age 0.

2.1 Age or size at first maturation

For centuries, 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". This product is obtained after gutting the bluefin tuna, by keeping the female gonads aside, then accuratelly washing them internally and externally with salty water and then, after cleaning as much as possible all blood traces, pressed, dried and salty for a certain period. This commercial practice allowed for accurate observations of all fenale gonads coming to the tuna factories, about their turgidity, the blood irroration, presence of transparent and isolated oocytes, etc., all elements which are considered important for defyning the state of maturation (Sarà, 1961). These observations were carried out by centuries on many thousand specimens, but the scientific observations were increased a lot in the last 150 years, in many places around the Mediterranean and not only on traps. For istance, when two of us (Arena and Di Natale) carried out many years of observations on purse seine fishery in the '70s and the '80s[4], having the opportunity to check sometimes more than 5,500 adult bluefin tunas during the main spawning season in each year, in a factory in Palermo, these extremely numerous observations provided an incredible number of data of various types, including those on bluefin maturity for the southern Tyrrhenian Sea over a number of years.

Beside these observations, conducted over a great number of specimens and for many years by several scientists, many hystological analyses have been conducted on the ovaries, defyning the stage of maturation by using conventional indices, the beginning of spawning and the estimation of frequency among the subsequent egg releases (Marino et al., 2005). As a matter of fact, the different stages of maturation which are present at the same time in the same ovary confirm the asynchronic maturation of the oocytes in the gonads, mature or immature. The presence of post-ovulatory follicules in mature gonads indicates a previous partial spawning, while other oocytes are becoming mature. In same cases, it was possible to estimate also very short time between one egg release and the following one, close to 24 hours.

Many authors (Frade, 1935; Scaccini, 1965a; Lutcavage *et al*., 1999; Tawill *et al*., 2002; Marino *et al*., 2005; Diaz, 2011) demonstrated that the year of first maturity for the male bluefin tuna of the eastern stock is at age 3, while females are 100% mature at age 4 (many female individuals are mature at age 3). The smallest female bluefin tuna reported so far as mature and spawning was 15 kg RW and 96 cm FL. 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; Rodriguez Roda, 1967; Baglin, 1976, 1982; Baglin *et al*., 1977). Recent data on bluefin tuna maturity, collected three annually by Italian scientific institutions within the EC Data Collection Framework and regularly provided to EC-DG MARE, confirmed those data on the maturity-at-age.

SCRS (Anonymous, 2008) acknowledged that the current age of first maturity in EBFT occurs at a lower size than the one used for establishing the minimum size and recommended revising it accordingly, proposing 25 kg following a prudential approach.

[4] This multi-year programme was carried out by ESPI-Centro Regionale per la Pesca ed i Prodotti del Mare of the Sicilian Region and regularly funded by the Italian Direction General for Fishery and Aquaculture (Italian Ministry of Merchant Marine at that time).

2.2 Spawning period and eggs release

Researches carried out by 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 occurs from the second part of May to July, but sometimes mature eggs and larvae were found also in August, demonstrating that the reproductive season might be extended in some years.

The extensive studies conducted on purse-seine fishery in the Tyrrhenian Sea in recent times (Arena, 1980, 1981, 1982a, 1982b, 1982c, 1985, 1986a, 1986b, 1988a, 1988b, 1990; Arena *et a*l., 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 Italian scientific institutions within the EC Data Collection Framework and regularly provided to EC-DG MARE, confirmed the classic spawning period.

Spawning in the eastern Mediterranean and in the Levantine sea usually occurs slightly earlier, starting in the first part of May (Oray, 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. This spawning area is very well known since many years and recet studies have confirmed its relevance (Garcia *et al*., 2001, 2002, 2005a, 2005b, Alemany *et al*., 2010; Mariani *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 event, because the situation returned to the normality after about 10 days. Presence of spawners in the Balearic areas is usually reported until the very last part of the spawning season.

Spawning in the central-southern Mediterranean is known to occur, but it is poorly documented in offshore areas. The massive displacement of bluefin tuna spawners from the Tyrrhenian Sea to the Central Mediterranean Sea, noticed for the first time in 1996 and put in correlation with anomalies caused by the Eastern Mediterranean Transient (Di Natale, 2008). The presence of bluefin tuna larvae has been confirmed and documented even in very recent times (giovanardi and Romanelli, 2010).

The spawning period in the various areas of the Mediterranean Sea is also confirmed by the comprehensive review by Aguilar & Lastra (2009) while data on spawning periods in the Black Sea, when bluefin tuna was still distributed there, are not exactly defined, even if spawning was reported in late spring and early summer (Akyüz & Artüz, 1957, Piccinetti *et al*., 1995).

In general, it should be always taken into account that annual oceanographic and climate variability in the various areas may result in changes in the bluefin tuna spawning calendar.

In some recent years (2003, 2006 and 2007) spawning bluefin tunas were reported even in the first part of September, possibly because of the very special climatic and oceanographic conditions in these years. On October 5, 2011, a school of spawning bluefin tunas was reported off the eastern coast of Sicily and one female individual that was fished on that day had still mature eggs in the gonads; this very unusual situation was reported in real time. Later on, when oceanographic and climate data have been made available for 2011, the observations in the wild got a strong support: the temperature map for that day at -5 m clearly shows a very anomalous situation not only in waters East of Sicily, but also in a large part of central-eastern Mediterranean Sea (http://gnoo.bo.ingv.it/mfs/analysis_archive.htm) (**Figure 1**).

This anomalous situation in the bluefin tuna spawning period finds a more complete possible explanation observing the sea temperature anomalies in the Mediterranean in 2011, as they are provided by the Italian Operational Oceanography Group (http://gnoo.bo.ingv.it/mfs/B4G_indicators/SST_anomaly.htm), computing the SST data of the real-time Mediterranean Forecasting System (MFS) (http://gnoo.bo.ingv.it/mfs) against the Medatlas climatology (http://www.ifremer.fr/medar/). **Figure 2** shows the SST temperature anomalies in 2011, providing a reasonable overview of the spawning possibilities for bluefin tuna in the Mediterranean Sea.

According to the graphic data, there is a clear increase of the temperatures in mid-May[5], which was the beginning of the spawning period for bluefin tuna in several areas of the Mediterranean, with a remarkable decrease at the end of the first part of June, when strong winds mixed the upper layer of the sea in many areas, followed by a new rapid increase of the temperatures up to mid-July, when spawning usually terminates and the bluefin tuna physiology starts reabsorbing not-used gonad products for recovering energy. It is possible that the following high SST temperatures between the very end of July and mid-August delayed the process and some bluefin tuna schools, having still a sufficient quantity of gonad products, possibly had a revival of the maturation process when the temperatures increased again from the last part of August up to the first week of October, when finally the situation at sea prevented any further spawning. **Figure 3** shows the mean SST values for the same period of time; it is clear that SST in the Mediterranean were high for many months, from spring up to fall 2011.

Similar extended spawning periods 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. As we previously mentioned, bluefin tuna spawning in some recent years was more intense and expanded in time (and maybe also in space) in the Mediterranean Sea, resulting in a higher recruitment particularly in 2003, 2006, 2007, 2009, 2010 and 2011. All these year classes showed-up in the same years at very small sizes (200-600 gr) late in August and up to early September in many areas, while the very strong 2003 class is now evident in almost all fisheries in the Atlantic Ocean and in the Mediterranean Sea, as a result of the climate anomalies in that year. The other year classes are now showing up in several fisheries. Even in this case, the analysis of the SST anomalies helps in "reading the sea" and **Figure 4** shows the sequence of high temperature periods in the Mediterranean Sea over the last 12 years. Larger periods of high temperature anomalies usually are correlated with higher spawning opportunities, while time-concentrated high temperature anomalies might not be necessarily correlated with higher spawning opportunities. It is also important to note that very high temperatures (>26°C) stabilised in some areas usually prevent further bluefin tuna spawning.

According to the environmental and behaviour observations at sea, particularly during the aerial surveys carried out in the '80s and the '90s in the southern Tyrrhenian Sea, a stable warm (at least >20°C) sea upper layer having a proper minimum depth (over 10 m) and a well-established thermocline having a negative gradient of at least 3°C, seem all together some of the useful environmental factors for allowing bluefin tuna spawning. Strong or time-prolonged winds prevent some of these factors to be established. Spawning at lower temperatures (even at 17°C) was reported in several tuna traps in the past (Scordia, 1931, 1933, 1934, 1936, 1937, 1939, 1940; Lozano Cabo, 1957, 1958; Sarà, 1964, 1983, 1998), even if stress factors correlated with the fishery might be also taken into account.

The bluefin tuna behaviour is another important factor for better defining a spawning activity, because this species clearly shows specific behaviours. One of them, maybe the most evident from the surface, is the "shining" behaviour, when the individuals rapidly turn on a side at the surface or immediately below, reflecting the natural sun light and releasing eggs or sperm. Another specific behaviour, observed several times, is the fast vertical or diagonal swimming from the surface to deeper areas, crossing the thermocline up and down. It supposed that this behaviour may help in activating some physiological factors linked to the spawning activity.

It should be considered that bluefin tuna has a very complex reproductive behaviour that we only partly know and that most of the physical factors which are apparently useful for supporting spawning are anyway not individually essential, because spawning may also occur in deeper parts of the water columns and under different oceanographic conditions. Bluefin tuna eggs have well defined buoyancy, helped by a small drop of natural oil, and this fact helps the fertilised eggs to stay at the right depth, independently from the depth of release.

It is always to be taken into account that physiological factors directly linked to the maturity might be predominant on other external factors and mature females meeting mature males can spawn under various environmental conditions (within certain limits) due to a prevalent hormonal and physiological pressure. It is not known if each adult bluefin tuna can spawn every year and how many times the same mature individual can spawn is one season.

Recent models developed for predicting the presence of bluefin tuna spawners in the Mediterranean (Cañadas *et al.*, 2010, 2011; Druon, 2010; Druon *et al.*, 2011; Anonymous, 2012a) need further developments and refinements because, at the moment, they are taking into account mostly the SST (and the chlorophyll

[5] The same graph shows also an isolated peak at the early beginning of May, which possibly justify the anticipated spawning in the Balearic area for about two weeks in that period in 2011.

concentrations for those including also juvenile tunas); the false-positive and false-negative outputs in some of these models may be caused by the complexity of the possible variables existing in the wild and by the use of a very few variables in the models, but also in some conceptual problems. Conducting a contemporary extensive survey for studying the bluefin tuna reproduction in the various parts of the Mediterranean over the whole period will be helpful for clarifying several issues.

2.3 Identification of the spawning area

According to what Aristotelis (1635, also in Athaeneus, 1653) said, "*thynnum 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.

The spawning area of the eastern bluefin tuna is always reported as the Mediterranean Sea but it is important to further define this general knowledge according to the huge literature existing on this subject and on some specific indicators, such as the well-identified presence of adult spawners with mature gonads during the spawning period, the presence of bluefin tuna eggs and/or larvae in the plankton and the presence of very young bluefin tunas of age 0.

2.3.1 Presence of adult spawners with mature gonads during the spawning period

Since historical times and at least for more than 26 centuries, tuna traps were able to intercept the passage of pre-spawners (these traps were identified as "tonnare di corsa" in Italian and "almadraba de derecho" or "almadraba de paso" in Spanish) and of mature tunas and/or postspawners (called "tonnare di ritorno" in Italian and "almadraba de retorno" or "almadraba de revés" in Spanish). The logic correlation with the reproduction has been made always by the presence of full ovaries which were often well vascularised and turgid male gonads in those traps intercepting pre-spawners, and by partly empty gonads in traps intercepting spawners or post-spawners. The different number of eggs in the gonads sampled in the Atlantic traps (Morocco, Portugal and Spain) is different from those samples taken in the Mediterranean (Sicily, Sardinia, Libya, etc) where there is a temporal progression in the catches (Sarà, 1998).

There is also a difference between male gonads, which are found fluent for a longer period of time compared to female gonads, as well as for the individual size, because it seems that larger tunas mature earlier in the season compared to smaller individuals. The growth of the ovaries along with the maturation of oocytes anticipate spawning sometimes up to one month and, as a consequence, the only presence of adult bluefin tuna genetically mature, without the corresponding analyses of the ovaries, cannot be considered sufficient for defining a spawning area. At the same time, the presence of adult bluefin tunas in pre-spawning or spawning periods is not sufficient for defining a spawning area, taking into account the speed capacity of this species and the presence of adult bluefin tuna during the spawning period in Atlantic areas clearly defined as non-spawning grounds (Cetti, 1777; Lo Bianco 1908; Bounhiol, 1911; Roule, 1917; Parona 1919; De Buen 1925; Sella, 1929a, 1929b; Frade & Manacas, 1933; Scordia, 1938; Rivas, 1954; Akiuz *et al*. 1957; Arena 1959; Furnestin *et al*. 1962; Tiews, 1962; Aloncle, 1964; Rodriguez Roda, 1964; Piccinetti & Piccinetti Manfrin, 1980, 1992; de la Serna & Alot, 1992; Mather *et al*., 1995; Corriero *et al*., 2003; Hattour 2003; El Tawill *et al*. 2004).

In the last 30 years, the major diffusion of the various bluefin tuna fishing activities in many areas allowed for a better knowledge of the distribution of the spawning areas, improving our understanding and enlarging the spawning area to many offshore areas, where the fishery was not operating before. Real time information provided by fishermen and confirmed by scientific observers on board or at landings better defined those large areas in the framework of many research programmes.

2.3.2 Presence of bluefin tuna eggs or larvae in the plankton

It is important to note that simply collecting tunas eggs at sea, without the possibility to further control the development stages of the same eggs or without a genetic analysis, does not allow for a sure and certain identification of eggs belonging to *Thunnus thynnus* (L.). As a matter of fact, there are several fish species which have eggs with a diameter between 1 and 1.15 mm, with a big oil drop and many other characteristics similar to *Thunnus thynnus* and then it is necessary to keep alive these eggs for hours or even one day for following their development and then more precisely identify the species.

Sanzo (1932), Sella (1924), Scaccini (1961,1966,1968,1973,1975) and Yabe (1962), among other scientists, well defined that bluefin tuna larvae bigger than 3 mm (having 1 or 2 days of life) can be perfectly identified and the

also indicate spawning events in a the area around the collection place in a period between 5 to 7 days before. The position of the spawning area can be more precisely defined because the bluefin tuna larvae at these stages are not able to actively swimming contrasting the currents, but they are moved by the surface currents. 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: one with the RV "Cornide de Savedra" in the western Mediterranean and two in most of the Mediterranean Sea, with Japanese and Italian research vessels, and the results of these campaigns have been regularly reported to ICCAT-SCRS. The general conclusion is that bluefin tuna larvae were found in most of the Mediterranean areas. 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; due to the extension of the spawning area, the very complex current regime in the Mediterranean 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). More recently, a large scale larval campaign was conducted by Oceana with the RV Marviva Med (Aguilar & 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, 2005a, 2005b), confirming the relevance of this area for the bluefin tuna reproduction. 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) (Barrois 1977; Dicenta 1977; Dicenta & Piccinetti, 1977, 1980; Dicenta *et al.*, 1975, 1979; Duclerc *et al*., 1973; Eherembaum, 1924; Giovanardi and Romanelli, 2010; Karakulak *et al.*, 2004a, 2004b; Lalami *et al.*, 1973; Matsumoto *et al*., 1972; Montolio & Juarez, 1977; Nishida *et al*. 1997; Oray *et al*., 2005; Padoa, 1956; Piccinetti 1973, 1995; Piccinetti & Piccinetti Manfrin, 1978, 1993; Piccinett *et al.*, 1995, 1997; Richards, 1976, 1977; Richards & Potthoff, 1979; Scaccini 1966, 1968; Scaccini *et al.*, 1973, 1975; Sella, 1924; Ueyanagy 1966; Vodionitzki & Cazanova, 1954; Yabe & Ueyanagi, 1962; Yabe *et al.*, 1966; Cavallaro *et al.* 1997 ; Nishida *et al*. 1997; Tsuj *et al*. 1997).

2.3.3 Presence of age 0 fish

The presence of small bluefin tuna of age 0 (less than 20 cm) was always considered as clear evidence of the proximity of a spawning area. Taking into account the mobility of young bluefin tuna and the time laps between the date of birth and the time of capture, sometimes more one month, there might be a certain distance between the spawning area and the area were very young bluefin tuna were fished. Very small fish (less than 50 gr) are usually inside or very close to the spawning areas, but concentrations of very early stages of age 0 is also dependent on the availability of a proper trophic chain (Lutcavage, 2011). Many authors reported areas where bluefin tunas of early stages of age 0 were present (D'Amico 1816; Dieuzeide & Roland, 1955; La Mesa *et al*., 2005; Logan *et al*., 2011; Oren *et al*., 1959; Roule, 1923; Santamaria *et al*. 2003b; Scaccini 1953, 1959, 1961a, 1961b, 1961c, 1965b) and the most old reports are summarised on **Table 1** (Piccinetti and Piccinetti Manfrin, 1970), while the most updated were recently mapped by ICCAT-GBYP (Anonymous, 2012).

3. Conclusions

Taking into account the already existing vast knowledge, the available data and information, various criteria reported above and their precision limits, it is reasonable to consider most of the Mediterranean Sea as a spawning area for bluefin tuna, except the Alboran Sea (where further investigations may help for claryfing some residual doubts in some coastal areas of Mediterranean morocco and Algeria), the Gulf of Lion and the Liguro-Provençal basin, the northern Adriatic Sea and the northern Aegean Sea. There are some areas where research and/or fishing activities, with details on bluefin tuna spawning, are more documented than others, and a previous paper (Di Natale, 2006) reported most of these areas. **Figure 5** shows the various areas, with the different typologies.

In several areas, every time bluefin larvae were searched for with a proper equipment and sampling strategy during the spawning period they were find, confirming that these areas are frequently used by bluefin tuna for spawning. The low presence of larvae in some other areas may also depend by the low number of sampling stations, the time of sampling and the irregular distribution of spawners in that area or period of time.

It should be also considered that bluefin tuna has very sophisticated behaviour and we understand only some of the factors which may potentially affect the behaviour and individual or school choices in the various life stages.

Vertical or horizontal variability in bluefin tuna aggregations of spawners may be noticed almost every year, in the varuious parts of the Mediterranean Sea, depending of various factors, which are again only partly known.

Recent oceanographic anomalies in the Mediterranean Sea (like the variability in the Eastern Mediterranean Transient in 1996 and 2006) caused different concentrations of bluefin tuna spawners in some central Mediterranean areas, with a partial and temporary shifting of the major concentrations from the southern Tyrrhenian Sea to the southern Mediterranean Sea. More recently, in 2011, the presence of a stable warm water mass between East Libya and southern Italy and Greece, during the spawning period, coupled with the contemporary strong winds in the central Mediterranean Sea South of Malta (which mixed-up the upper layer of the sea, preventing the thermocline to be well established) caused eastward movements of bluefin tuna schools of spawners, noticed also by the ICCAT-GBYP aerial survey for bluefin tuna spawning aggregations in 2011. This fact further confirms how bluefin tuna can opportunistically use most of the Mediterranean Sea for spawning. These considerations imply a lot of caution when spatial model are developed, because variables can be many and important.

The never-clarified issue concerning the possibility that eastern bluefin tuna may have additional spawning areas outside the Mediterranean Sea, in the Atlantic Ocean, also needs attention. Some authors (de Buen, 1925, 1926; Mather *et al*., 1995) reported the possibility that bluefin tuna can sometimes use some Atlantic areas for spawning (**Figure 6**), even if bluefin tuna larvae were never detected in most of these areas and particularly along the Atlantic Morocco coast (Rodriguez Roda, 1975). The only exception seems to be the Gulf of Guinea, where Mather *et al*. (1995) report that some bluefin tuna larvae were found, but the details about these larvae are within an internal NMFS report which is not available. Aloncle (1964, 1975) reports the abundat presence of very small bluefin tuna of age 0 along the Atlantic Morocco coast and in some Atlantic Spanish coasts, but these facts alone cannot be considered as a sufficient evidence of a spawning area there, even if their presence in recent times should be better investigated.

Recently, some electronic tagging activities carried out by ICCAT-GBYP in a tuna trap in Morocco in 2011 and 2012, just before the spawning period, showed that some bluefin tunas went to some of the areas mentioned before (Azores isles, Canary isles) during the usual spawning period (Anonymous, 2011, 2012b; Quílez-Badia *et al*., in press a, in press b). Even if the SST was over 20°C in those areas in most of the cases, this factor alone is not sufficient and further details are needed for better claryfing if these individuals were simply swimming in the various areas or if they were spawning there. At the moment, the possibility to have spawning areas for the Eastern bluefin tuna outside the Mediterranean is still laking of any evidence, even if specific researches may help for better defining if this potential possibility migh be real or not. Further trials with electronic tags implanted on adult bluefin tuna pre-spawners in Morocco or in other Atlantic areas will also improve our knowledge and understanding.

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Table 1a. Presence of juvenile bluefin tunas of age 0 in various areas of the Mediterranean Sea (from Piccinetti & Piccinetti Manfrin, 1970).

AUTORI	AREA	PERIODO	TAGLIA E/O PESO	NUMERO
D'Amico, duca D'Ossada (1816)	Acque di Palermo	giugno	42 g	?
		agosto	122 g	?
		ottobre	840 g	?
Damiani 1909 (cit. Parona 1919)	Camogli (Liguria)	5-7-1895	3.000/5.000 g	500
Bourge (1908) (cit. da Roule 1917)	Tunisia	agosto	150 g	?
		settembre	900 g	?
		ottobre	oltre 1.000 g	?
Lo Bianco (1909)	Golfo di Napoli	luglio-settembre	11-18 cm	abbondanti
		agosto-ottobre	25-32 cm	abbondanti
Ehrenbaum (1924)	Parte Sud Jonio	24-7-1910	7,3 mm	1
	W. di Rodi	1-8-1910	4,7 mm	1
	W. di Rodi	2-8-1910	7,1 mm	1
	Jonio, W. di C. Spartivento	18-8-1910	9,3 mm	1
	Jonio, S. di C. Spartivento	19-8-1910	6,8 mm	1
	Presso Messina	20-8-1910	6,8-9,4 mm	2
	40 M a S. di Capri	22-8-1910	8,4 mm	1
	W. della Sardegna	28-8-1910	9,2 mm	1
	NW. di Pityusen	31-8-1910	8,0 mm	1
	NW. di Malta	5-8-1912	7,8 mm	1
De Buen (1925)	Ceuta – Tres Forcas	maggio	1.000/2.000 g	grandi quantità *
		autunno	4.000/5.000 g	grandi quantità
Sella (1929)	Ceuta – Melilla	settembre	300/500 g	abbondanti
Anonimo (1931)	Tripolitania	settembre	200/500 g	circa 1.500
De Buen (1932)	Aguilas (Spagna)	?	170 mm circa	abbondanti

Table 1b. Presence of juvenile bluefin tunas of age 0 in various areas of the Mediterranean Sea (from Piccinetti & Piccinetti Manfrin, 1970).

AUTORE	AREA	PERIODO	TAGLIA E/O PESO	NUMERO
Sanzo (1932)	Stretto di Messina	giugno–prima metà luglio	uova	abbondanti
Dieuzeide (1951)	Algeria	giugno–luglio 1950	5–8 mm	4
Scaccini (1953)	Coste Adriatiche (fra le foci del Po e Ancona)	fine agosto–inizio settembre 1952	11–20 cm/40–100 g	frequenti
Dieuzeide e Roland (1955)	Baia di Castiglione e Philippeville	ottobre 1948–49–52	800-2.000 g/40-50 cm	molti abbondanti
Dieuzeide e Roland (1955)	Baia di Castiglione	settembre 1954	20–30 cm/135–300 g	abbondanti
Akiüz e Artüz (1957)	Mar Nero	fine luglio - agosto - inizio settembre	uova e larve	numerose
Biancalana (1958)	Golfo di Napoli	settembre 1953	1.500/2.000 g	diversi
	Adriatico	1–15 settembre 1954	4.000 g	876
Oren, Ben Tuvia e Gottlieb (1959)	Coste di Cipro	8–11–1952	45–53 cm	5
	Golfo di Adalia	9–11–1952	45–53 cm	50
	Fuori 7 Capi	10–11–1952	45–53 cm	18
	Fuori Rodi	13–11–1952	45–53 cm	1
	Fuori Kalimno	14–11–1952	45–53 cm	2
	Kios	15–11–1952	45–53 cm	4
	Tenedos	16–11–1952	45–53 cm	diversi
	Imbros	23–11–1952	45–53 cm	diversi
	Lemnos	24–11–1952	45–53 cm	3
	Skiros	25–11–1952	45–53 cm	5
	Golfo di Atene	26–11–1952	45–53 cm	3
	Thermia	29–11–1952	45–53 cm	3
	Stampalia	1–12–1952	45–53 cm	5
	Kastelorizo	2–12–1952	45–53 cm	3
	Golfo di Alexandretta	9–12–1952	45–53 cm	8
	Fuori la Syria	10–12–1952	45–53 cm	16

Table 1c. Presence of juvenile bluefin tunas of age 0 in various areas of the Mediterranean Sea (from Piccinetti & Piccinetti Manfrin, 1970).

AUTORE	AREA	PERIODO	TAGLIA E/O PESO	NUMERO
Arena (1959)	Isole Eolie	autunno	10 cm/1.000–2.000 g	abbondanti
Scaccini (1959)	Coste Adriatiche	estate	8–20 cm/40–100 g	frequenti
	Coste Adriatiche e Tirreniche	da aprile a giugno	1.500–2.500 g	frequenti *
Scaccini (1961*a*)	Zona costiera tra Rimini e Ancona	estate	11–20 cm/40–100 g	frequenti
	Dalle foci del Po ad Ancona	da aprile a settembre	40–60 cm/3–5 kg	frequenti
Scaccini (1961*b*)	Stretto di Messina e al largo di Gaeta	ultimi giorni di giugno, primi di luglio	0,5 cm	?
		agosto-settembre-inizio ottobre	fino a 9 cm	?
Sarà (1961)	A N. della Sicilia e isole Eolie	da metà settembre a fine novembre	1.000/2.000 g	numerosi
Sarà (1963)	W. di Gibilterra; Sardegna, Corsica; Tirreno; Jonio; Adriatico; Canale di Sicilia, a S. di Lampedusa e presso le isole Kerkenah; triangolo Haifa–Alexandretta–Cipro; Bosforo Egeo; golfo di Adalia	agosto–metà novembre	200/3.000 g	frequenti
Arena (1963)	Golfo di Patti (Sicilia)	15–20 maggio 1961	45–48 cm 2.000–2.500 g	diversi *
Rodriguez-Roda (1964)	Stretto di Gibilterra	metà luglio–metà settembre	24–25 cm/225 g	abbondanti
		settembre 1960	24 cm	500.000
		settembre 1963	24 cm	2.000

Table 1d. Presence of juvenile bluefin tunas of age 0 in various areas of the Mediterranean Sea (from Piccinetti & Piccinetti Manfrin, 1970).

AUTORE	AREA	PERIODO	TAGLIA E/O PESO	NUMERO
Sarà (1965)	Golfo di Castellammare–Basso Tirreno – Canale di Sicilia	luglio	5 cm	alcuni
		metà luglio	30 g	abbondanti
		metà agosto	120–150 g	abbondanti
		inizio settembre	250 g	abbondanti
		fine settembre	500 g	abbondanti
		inizio ottobre	700 g	abbondanti
		fine ottobre	oltre 1.000 g	abbondanti
		metà novembre	1.500 g	scarsi
Sarà (1965)	Coste occiden. Sicilia	luglio–inizio agosto	5 cm/250 g	abbondanti
		metà agosto–metà novem.	250–1.500 g	abbondanti
Scaccini (1965)	Stretto di Messina	giugno	1–2 cm	numerosissime
	Stretto di Messina, Gaeta, Asinara	fine giugno–inizio luglio	2,5–5 cm	abbondanti
		fine luglio–agosto–settem.	8–15 cm	abbondanti
	Coste Tirreniche	fine luglio	30–40 g	frequenti
		agosto	120 g	frequenti
		ottobre	500–800 g	frequenti
Arena e Sarà (1967)	Basso Tirreno	fine settembre 1965	22–25 cm	abbondanti
		inizio ottobre 1965	30 cm	abbondanti
		10 ottobre 1965 circa	38 cm	abbondanti
		20 ottobre 1965 circa	42 cm/1.500 g	pochi
Patania (1967)	Davanti a Mentone	fine luglio 1967	3.000/4.000 g	9
	25–30 M fuori Nizza	8 agosto 1967	3.000 g	110 *
Sarà (1968)	Ceuta	giugno–luglio 1963	peso inferiore a 2 kg	182 *
	Capo Granitola	giugno 1965	peso inferiore a 2 kg	63 *
	Oliveri (Sicilia nord-orientale)	giugno 1966	peso inferiore a 2 kg	25 *
	Ceuta	giugno 1966	peso inferiore a 2 kg	143 *

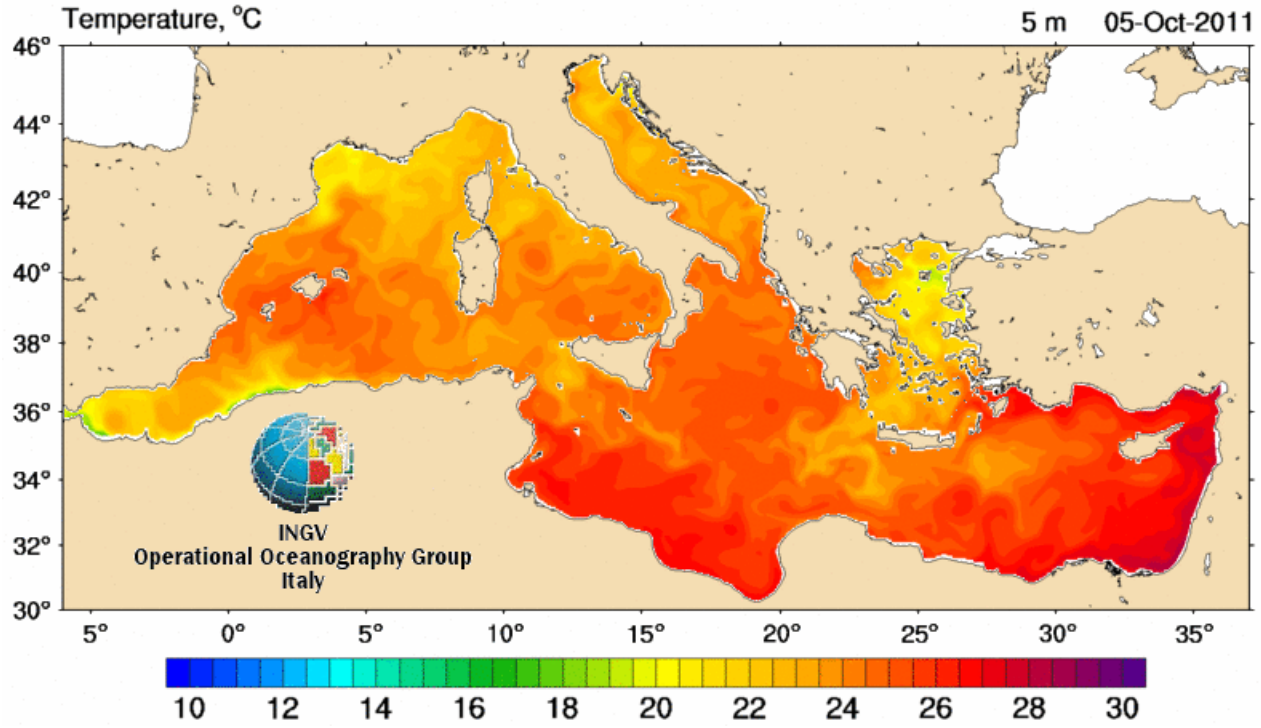

Figure 1. Sea temperature at -5m on October 5, 2011, showing temperatures higher than 20°C in most part of the Mediterranean Sea. The area where bluefin tuna spawning was noticed on that day is off the eastern Sicilian coast.

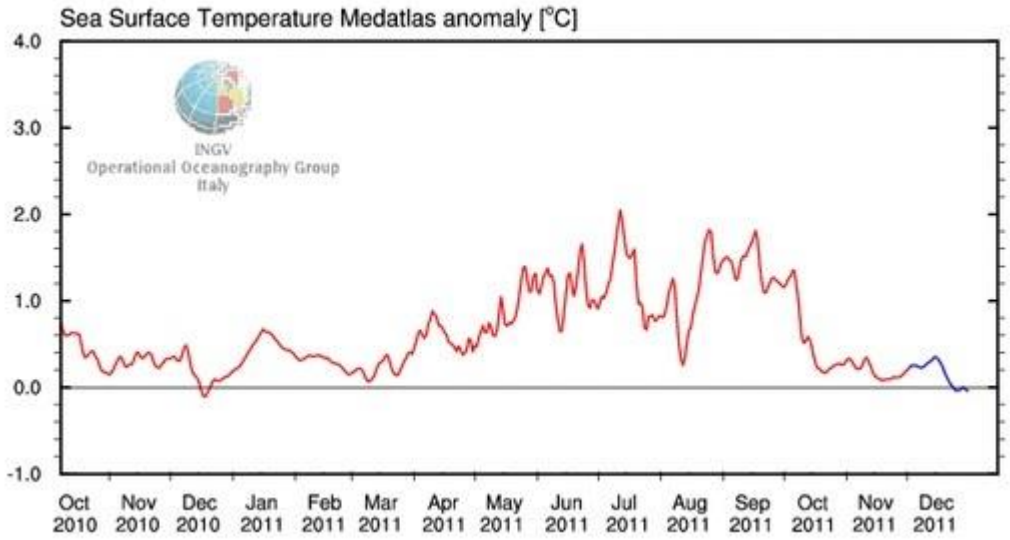

Figure 2. SST anomalies in the Mediterranean Sea form October 2010 to December 2011. The highest peaks are correlated with spawning activities in several areas of the Mediterranean Sea in 2011.

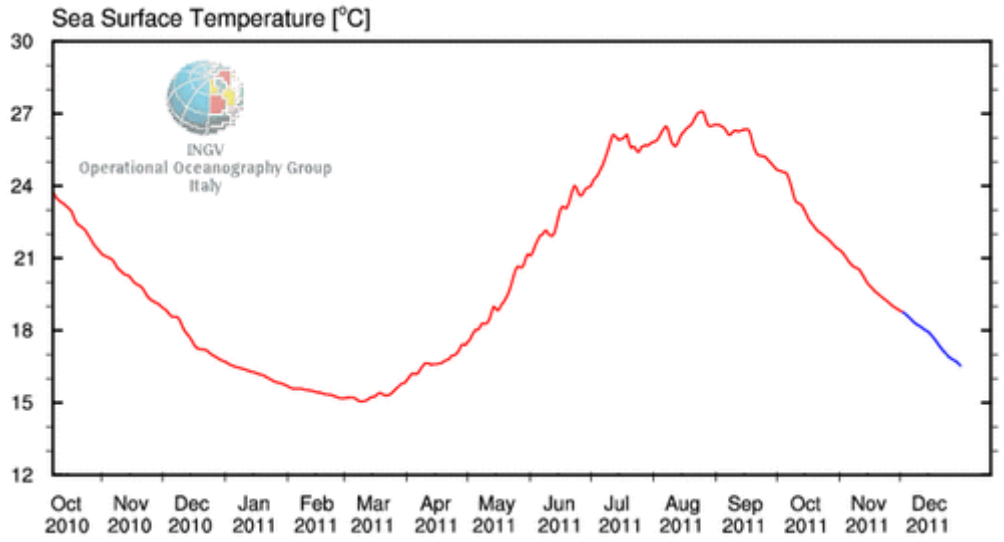

Figure 3. SST mean values in the Mediterranean Sea form October 2010 to December 2011.

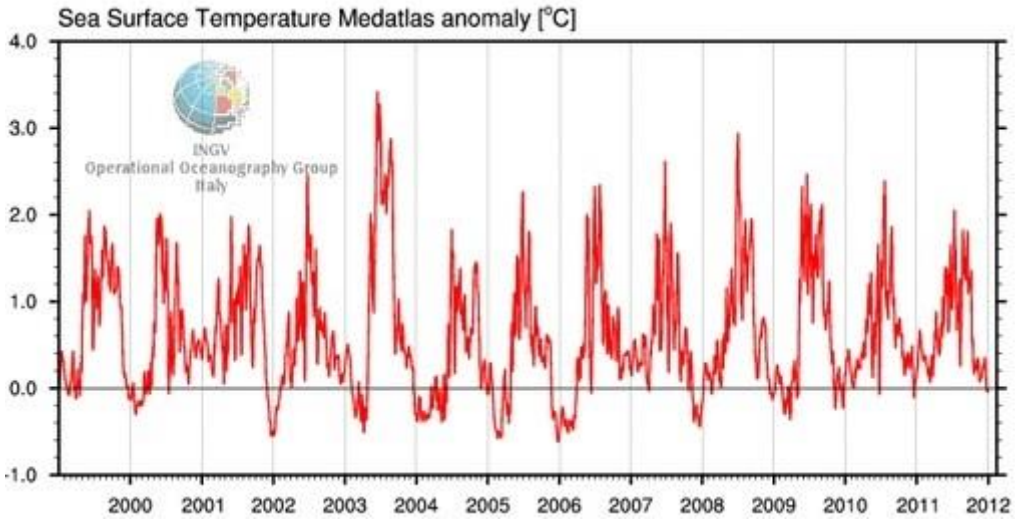

Figure 4. SST anomalies in the Mediterranean Sea for the years 1999 to 2012. The anomaly in 2003 was particularly relevant, but it is clear that this area is facing a sequence of years with anomalous hot springs and summers.

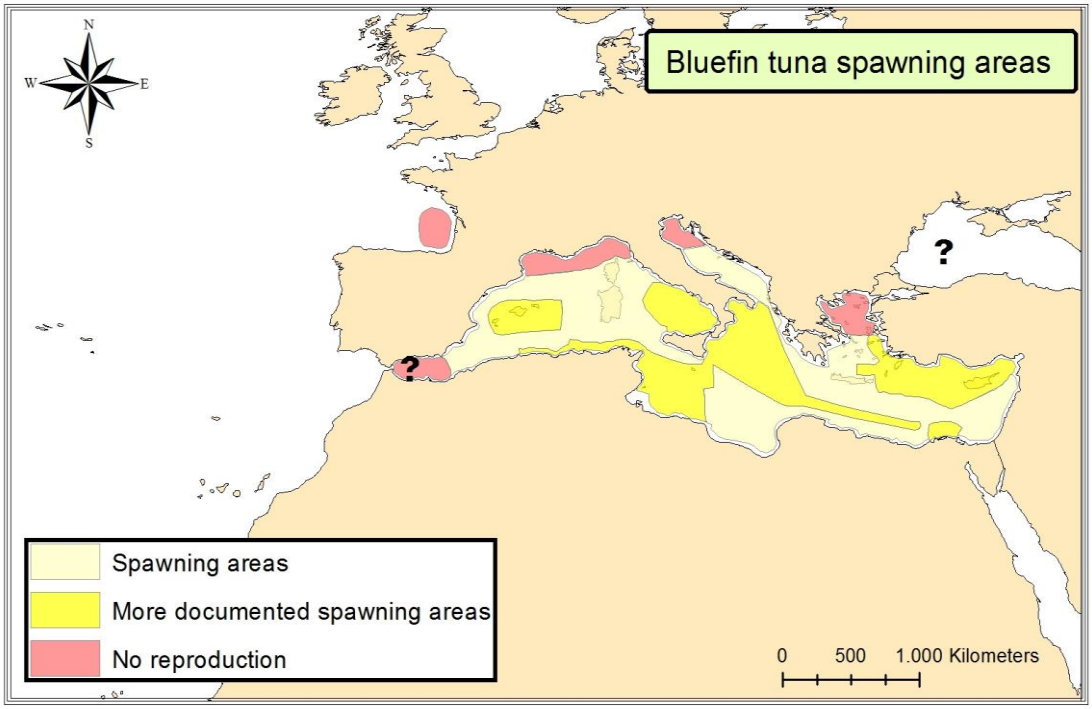

Figure 5. Bluefin tuna spawning and non-spawning areas in the Mediterranean Sea.

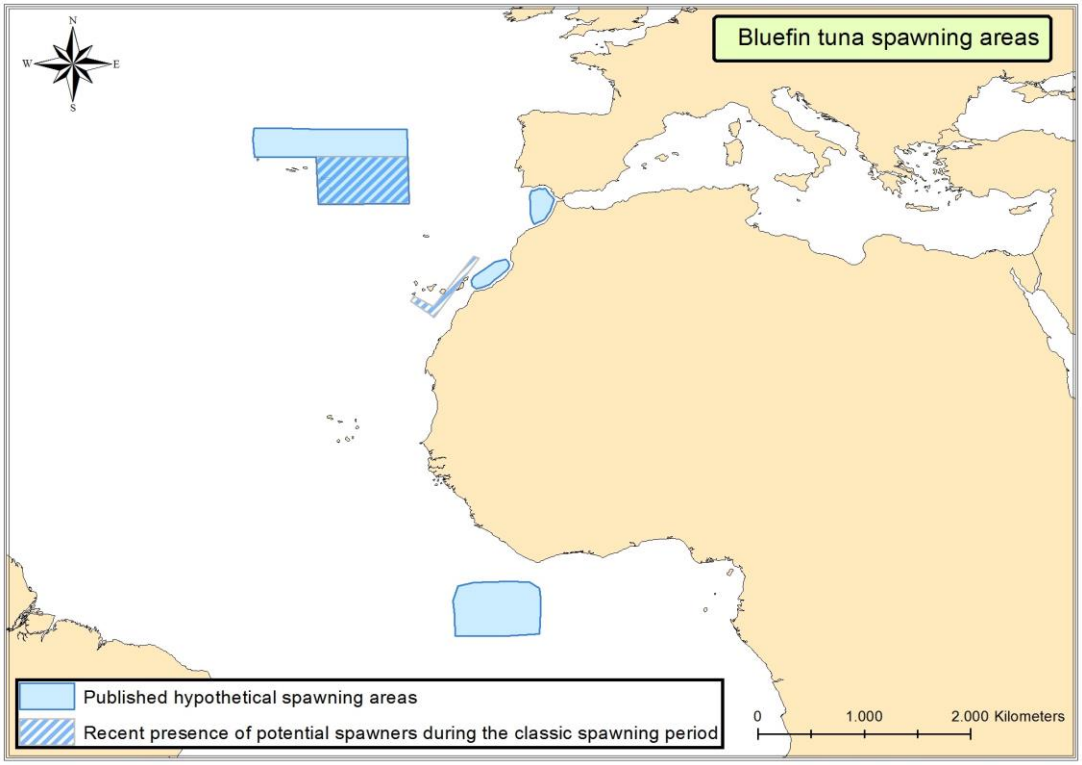

Figure 6. Map of the potential areas where bluefin tuna spawning outside the Mediterranean Sea was considered possible, showing also areas where some of the bluefin tuna tagged by ICCAT-GBYP moved during the usual spawning period in 2011 and 2012.