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REVIEW OF THE HISTORICAL AND BIOLOGICAL EVIDENCES ABOUT A POPULATION OF BLUEFIN TUNA (*THUNNUS THYNNUS* L.) IN THE EASTERN MEDITERRANEAN AND THE BLACK SEA

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SUMMARY

*The history of the bluefin tuna (*Thunnus thynnus*) population which was originally distributed in the Black Sea and in the eastern Mediterranean Sea, which is not fully known, is one of the main key points for better understanding the current situation of the bluefin tuna inhabiting the Mediterranean Sea. As a matter of fact, the ancient information from historical periods might help the interpretation of the recent preliminary tagging and genetic data, which show differences between the individuals mostly present in the eastern Mediterranean and those distributed in other Mediterranean and Atlantic areas. This review examines both the ancient evidence and recent data, contributing to improve the scientific knowledge on this species.*

RÉSUMÉ

*L'histoire de la population de thon rouge (*Thunnus thynnus*) qui, à l'origine, était distribuée dans la mer Noire et dans la Méditerranée orientale et qui n'est pas complètement connue, est l'un des points essentiels pour mieux comprendre la situation actuelle du thon rouge qui peuple la mer Méditerranée. Les anciennes informations provenant des périodes historiques pourraient contribuer à faciliter l'interprétation des récentes données préliminaires de marquage et de génétique, lesquelles montrent des différences entre les spécimens essentiellement présents dans la Méditerranée orientale et ceux répartis dans d'autres zones méditerranéennes et atlantiques. La présente étude examine les éléments de preuve anciens et les données récentes, contribuant à améliorer les connaissances scientifiques sur cette espèce.*

RESUMEN

*La historia de la población de atún rojo (*Thunnus thynnus*) que se distribuía originalmente en el mar Negro y el Mediterráneo oriental, y que no se conoce plenamente, es uno de los puntos principales para entender mejor la situación actual del atún rojo que habita en el Mediterráneo. De hecho, la información antigua de periodos históricos podría ayudar a interpretar los datos recientes y preliminares géneticos y de marcado, que presentan diferencias entre los ejemplares principalmente presentes en el Mediterráneo oriental y los distribuidos en otras zonas del Mediterráneo y del Atlántico. Esta revisión examina tanto las pruebas antiguas como los datos recientes y contribuye a mejorar los conocimientos científicos sobre esta especie.*

KEYWORDS

Bluefin tuna, Historical fish distribution, Atlantic Ocean, Mediterranean Sea, Black Sea, Reproduction, Fish populations

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1. Foreword

The identification of population components is an essential part of the studies for a more appropriate management of any natural resource. The Atlantic bluefin tuna is currently managed by ICCAT by two stocks (the western Atlantic and the Eastern Atlantic ones) and this separation is mostly based on the current hypothesis that the western stock is spawning exclusively in the Gulf of Mexico, while the eastern stock is spawning exclusively in the Mediterranean Sea and maybe also in adjacent areas. These hypotheses are supported by evidence, because not a single specimen spawning in one of the two principal areas was ever found spawning also in the other area, but the population situation might be much more complex.

The literature in the last century sometimes reported additional possible spawning areas in the Atlantic and in the adjacent seas (Mathers, 1995; De Buen, 1926; Piccinetti *et al.*, 2012), while recent tagging activities conducted by ICCAT-GBYP showed that some adult and potentially sexually mature bluefin tunas coming accros the Moroccan Atlantic coast do not necessarily enter into the Mediterranean Sea for spawning, going to other Atlantic areas during the usual spawning season.

Recent preliminary genetic studies carried out within the ICCAT-GBYP framework showed differences between the eastern Mediterranean bluefin tunas and those sampled in the central and western Mediterranean Sea, without excluding further differences in other areas, suggesting that the bluefin tuna population structure might be quite complex.

As a matter of fact, the genetic preliminary findings are simply confirming the knowledge about the ancient distribution of bluefin tuna in the eastern Mediterranean Sea, which is logically correlated to the natural distribution of bluefin tuna in the Black Sea until the beginning of the '70s.

Even if the data on the distribution of bluefin tuna in the Black Sea in the last 40 years are very poor (when existing), together with the knowledge about the distribution and biology of bluefin tuna in the eastern Mediterranean area in historical times, the evidence deriving from the literature may help our understanding of this part of the bluefin tuna stock.

2. A very short history of the Black Sea (including the Azov Sea)

The geological history of the Black Sea is quite complex, like for our entire planet, but it is essential to shortly review it for a better understanding of the history of the bluefin tuna in this semi-enclosed basin and in the eastern Mediterranean Sea. Zaitsev and Mamaev (1997) provide a summary of the geological sequence which resulted in the area now occupied by the Black Sea. After the end of the Terziary Period, the Tethys Sea was still extended in a large part of this part of the Earth; in the Miocene period (from 7 to 5 million years ago) the Tethys Sea become divided into numerous large brackish basins and one of them was the Sarmantic Sea, which included, among many other areas, the actual Black Sea and the Azov Sea. Slowly, the salinity of the Sarmantic Sea decreased, due to the inflow of many rivers and the marine fauna and flora endemic from the Tethys Sea became extinct, except for some marine Mammal species.

In the late Miocene and the early Pliocene (from 5 to 3 million year ago), the Sarmantic Sea had a much more reduced area, forming the Maeotic Sea and the connection with the ocean was somewhere re-established, allowing for the settlement of several marine species. In the late Pliocene (from 3 to 1.5 million years ago) the Maeotic Sea was again isolated from the ocean, forming the almost freshwater Pontian Sea-Lake, and the marine species were replaced by brackish and freshwater ones; the descendents of these species are still present in the Caspian Sea, in the Azov Sea and in some areas of the Black Sea having a very low salinity, and they are called Pontian relicts. In the latest part of the Pontian phase, the rise of Caucasus created the Caspian Sea and a large area which included the current Black Sea together with a small area now occupied by the Azov Sea, with a dynamic shaping of these areas, forming the Chaudian Sea-Lake. This dynamic spatial situation continued in the Quaternary period, the Ice age and the late Pliocene (less than 1 million years ago), along with various changes in the salinity.

In the late Mindel Glaciation (about 500,000/400,000 years ago) the Chaudian Sea/Lake, filled with glacial melt waters, turned into the Paleoeuxian basin, which covered the same area where the current Black Sea, the Azov Sea and the Marmara Sea are, and it was connected again for some periods with the Caspian Sea; the salinity was again very low. The Marmara Sea, at that time, was separated from the Mediterranean Sea. During the last Riss-Würm Interglacial period (between 150,000 to 100,000 years ago), following the opening of the Dardanelles for the first time, this huge basin was connected to the Mediterranean Sea, forming the Karangat Sea, having a higher salinity and allowing the settlement of several marine species, which almost entirely replaced the Pontic fauna, limiting it to some marginal areas with a lower salinity.

About 20,000-18,000 years ago, at the end of the Interglacial period, the Karangat Sea became again isolated from the sea, forming the Neoeuxian Sea/Lake and this caused a dramatic change in the local marine flora and fauna and the Pontian species came back again to the area. The moder phase of the Black Sea begun about 10,000 years ago, but it is about 7,000 years ago that finally and again the marine water of the Mediterranean Sea falled into the Black Sea thought the Dardanelles, the Marmara Sea and the Bosphorus and it is suspected that the gradual increase of the salinity brought to the modern situation within a period of about 1,000/1,500 years. Ryan and Pitman (1999) reported that the flood happened in the Black Sea about 5,600 years ago, caused by the raising waters of the Mediterranean falling into the Black Sea was the famous Noah Flood.

In the modern situation, as a result of the increased salinity, about 80% of the fauna is made by Mediterranean species settlers, while the Pontian species are limited to the low salinity areas. In the last decades, many alien species, incidentally imported by ships (either with ballast waters or as fouling) or emigrating from other oceans, had important massive blooms in the Black Sea, sometimes substituting original Mediterranean species.

The Black Sea is an inland semi-enclosed basin, linked to the Mediterranean and the world ocean through the Straits, a water passageway system consisting by the Strait of Bosphorus (Istanbul), the Marmara Sea and the Strait of Dardanelles (Çanakkale) (**Figure 1**). According to Pinardi (2009), which compared very recent and old oceanographic data (Marsili, 1681), the conditions of the Bosphorous remained almost stable for a long time and the accuracy of old oceanography measures is confirmed.

The modern Black Sea has a surface of about 423,000 km^2 and a maximum depth of 2,212 m, while the Azov Sea has a surface of about 39,000 km^2 and an average depth of only 8 m (**Figure 2**).

The Black Sea is well-known among the marine scientist for the peculiarity of its water masses, because a large volume of the water masses (about 87% of the total volume) are anoxic, containing high levels of hydrogen sulphite and they are characterised by the relevant activity of various sulphur bacteria (Sorokin, 2002); this huge anoxic watermass is separated from the surface sea water by a interface layer of "red water", fluctuating at a depth between about 100 to 180 m. A natural "model in scale" of the Black Sea system exists in Sicily (the coastal brakish Lake Faro), where various studies have been conducted by both Russian and Italian scientists, trying to better understand the action of sulfur bacteria in these extreme environments (Sorokin, 1965; Trüpper and Genovese, 1968).

The presence of oxygenated waters only in the upper layer of the Black Sea is a severe limiting factor for all marine species, concentrating both prey and predators in this restrict environment, where all the biological cycles can be carried out by those species encountering there the right environmental conditions. No marine vertebrate species can survive outside the upper oxygenated water in the Black Sea, while only one worm species and several bacteria were found in deeper waters. This particular situation was described as "a surface film of life stretched over an abyss of lifelessness" (Ascherson, 2007).

A comprehensive overview of the Black Sea ecology and oceanography is provided by Sorokin (2002) and Oguz *et al.* (2005).

A recent overview paper the Black Sea fisheries (Popescu, 2010) included a short review of the current oceanographyc conditions in the Basin, along with the environmental problems occurred in the last decades. In addition to the peculiar distribution of oxygen described above, the salinity plays an important role in the marine ecosystem of the Black Sea and the Azov Sea. The upper layer of the basin has a low salinity (about 18‰) separated from the saltier deep waters (about 22‰); this stratification is the result of the input of river waters (at the surface) and the Mediterranean salty water (at the bottom). Very recently, Parson and Peakall (2010) discovered an undersea "river" in the Bosphorus and in the nearest area of the Black Sea, bringing the salty Mediterranean water inside the basin, showing even more the complex oceanography of this particular area. The salinity step (halocline) forms also a density step (pycnocline), both situated about 100-180 m depth. The stratification inhibits the vertical mixing of waters and results in the permanent anoxic water mass described in the previous paragraph, the largest anoxic basin worldwide.

3. The ancient reports on bluefin tuna

In the Aegean area, the bluefin tuna was representing about 80% of the fish food in prehistoric times (Powell, 1966). The bluefin tuna was also distributed in the Black Sea, including the Azov Sea, since ancient times. The first physical evidence of this historical distribution is provided by fish salting factories in Pontikapaion, active in the Azov Sea from III b.C. to IV a.C. (Morales *et al*., 2007), and Chersonesus, active in Crimea from I b.C. to III a.C., where bluefin tuna bones have been found (Morales *et al*., 2004; Di Natale, 2014). Apparently, bluefin tunas in these remote areas were essentially big adults or giant specimens.

The presence and sometimes the fishery of bluefin tuna in the eastern Mediterranean (Levantine Sea and Aegean Sea) was also documented by several authors (Omerus, VII b.C.; Esopo, V b.C. in 1592; Herodotus, V b.C.; Aeschylus, 472 b.C.; Aristoteles, III b.C rep. in 1635; Philostratus de Lemnos, III b.C.; Solinus, III b.C.; Theocritus, III b.C.; Ulpianus, III b.C.; Aelianus, II b.C.; Athaneus de Naucratis, II b.C.; Polibio, II b.C.; Strabonis, I b.C.; Manilus, I AD; Plinius, 65AD? in 1553;Ray and Willughby, 1686; Willughby, 1686; Smidth, 1876; Adams, 1883; Baskett, 1899; Keller, 1913; Radclife, 1921; Athanassoupolus, 1923, 1926; Heldt, 1925, 1926; Corwin, 1929; Thomazi, 1947; Mather *et al*., 1974, 1995; Dumont, 1976-1977, 1981; Mastromarco, 1988; Curtis, 1991, 2005; Powell, 1996; Doumenge, 1998, 1999; Mert *et al*., 2000; Torrente, 2002; Bekker-Nielsen, 2005; Fernández Nieto, 2006; Levine, 2006; Pepe, 2006; Vargas and del Corral, 2010; Vargas *et al*., 2010; Di Natale, 2012a, 2012b), and the description of the tuna trap by Oppianus (177 B.C.) is a clear evidence of the bluefin tuna fishing activity in that area. Documents having description of bluefin tuna fishery or distribution the Levantine Sea, along the southern coasts of the modern Turkey and other eastern Mediterranean areas are less available. At the same time (Di Natale, 2014), bluefin tuna salting factories and garum factories were clearly present in several places in the eastern Mediterranean Sea, providing a further evidence of the bluefin tuna distribution.

The high value of the bluefin tuna fishery in the Bosphorus and in the Marmara Sea from about 6 centuries b.C. is confirmed by the presence on many coins with tuna images in Cyzicus (now Aydıncıķ, Turkey), Byzantion (now Istanbul, Turkey) and Charia Kyndia (Greece) (Savas Lenger, n.d.; Di Natale, 2014), while the local use of bluefin tuna is proved by the *garum* and salting factories in both Byzantion (now Istanbul, Turkey) (Tekin, 2000) and Cyzicus (now Aydıncıķ, Turkey), but also in the northern Aegean Sea, in Charia Kindia (Greece) (Di Natale, 2014) (**Figures 3** to **5**).

The ancient evidence confirms the many classic reports on bluefin tuna migrations in the eastern Mediterranean Sea, in the Bosphorous, in the Marmara Sea and in the Black Sea, but it is still not very clear if the migration to the Black Sea was a feeding migration or a spawning migration or both. As a matter of fact, bluefin tuna was constantly present in the eastern Mediterranean Sea, in the Black Sea and in the adjacent seas for at least 26 centuries and up to the beginning of the ‘70s in the XX century.

This long historical presence and distribution possibly supported the formation of a local bluefin tuna population, because this species was present in the eastern areas even after the usual migration months into the Atlantic, as it happens in other Mediterranean areas (Scordia, 1938; Di Natale *et al*., 2005), where bluefin tuna can stay for more than one year. Evidence of many wintering bluefin tuna in various parts of the Mediterranean Sea is also reported in the comprehensive study by Mather *et al*. (1995).

Occupation of specific habitat for reproduction and the subsequent development of offsprings in isolation from those produced in other areas is one of the mechanisms which can lead to the development of distinctive populations, including those having specific genetic characteristics (MacKenzie and Mariani, 2012).

Aristotles (about 325 B.C.) clearly reported the seasonal migration of bluefin tuna from and to the Black Sea and he was one among the many who reported these movements. Aritotles reported that bluefin tuna was going to the Black Sea in late spring, returning to the Mediterranean Sea in late autumn.

Apparently, if we superficially compare this ancient information to the timing of the spawning migrations from the Atlantic into the Mediterranean, these local movements in the eastern part could be related to a spawning migration to the Black Sea, but not necessarily, because of the difference in months, but also considering the historical ecology and ethology of this species in the eastern Mediterranean basin, which was and is still possibly poorly known and even more poorly understood.

4. More recent bluefin tuna fisheries in the eastern Mediterranean, the Marmara Sea and the Black Sea

In addition to ancient reports, bluefin tuna fisheries in the eastern part of the Mediterranean Sea, in the Marmara Sea and in the Black Sea have been described by several authors and they have been also reviewed by Mathers *et al.* (1991, 1995) up to the '90s. Even if reports from this area are much less numerous when compared to other parts of the Mediterranean Sea, the extistence of an important fishing activity for bluefin tuna is somewhat documented over the past centuries.

Bluefin tuna fishery was carried out in Greece only in few areas and information is extremely difficult to find. Old partial reports are mentioned by Guys (1776) and Fernández Nieto (2006). Athanassoupolus (1923, 1924, 1926a, 1926b) added some general descriptions of the local bluefin tuna fishery at the beginning of the XX century, including a discussion on possible migrations. Vinciguerra (1886) and Nuinni (1922) reported that bluefin tuna fishery was mostly concentrated in the Aegean Sea. More modern data are those concerning the eleven tuna traps reported by Belloc (1961) for the period 1954-1958, but all species are mixed-up in catch reports, making almost impossible to understand the quantity of bluefin tuna by year. Fisheries for young-of-the-year bluefin tuna were reported by Oren *et al.* (1959) in several parts of the Aegean Sea, between September and December.

Even for bluefin tuna total catch reports from 1950 to 1970 the statistics are not clear, because some authors (Hamre *et al*., 1966; Miyake and Manning, 1975; Miyake and Tibbo, 1972) reported a range between less than one ton to a maximum of 1,220 tons, while ICCAT data base shows a range between 400 and 1,200 tons, but quantities seem estimated. Statistics from 1971 to 1982 are missing, while Mathers *et al.* (1995) reports that bluefin tuna catches in Greece were negligeable after 1970. In more recent years (1982 to 2012) total bluefin tuna catches ranged from 5 tons to 1,217 tons.

Bluefin tuna fishery in Turkey is much more documented, because the fishery in the Marmara Sea was described or reported by several authors (Guys, 1776; Pavesi, 1887, 1889; Deveciyan, 1915, 1926; Karahís,, 1915; Parona, 1919; Ninni, 1922, 1923; Marti, 1941; Carp, 1951; Lebedev and Lapin, 1954; Iyigungos, 1957; Sarikaya, 1980; Karakulak, 1999, 2000; Mert *et al*., 2000; Kahraman and Dağlı, 2003; Karakulak *et al*., 2004; Oray and Karakulak, 2004; Oray *et al*., 2005; Karakulak and Oray 2009; Anon., 2012; Di Natale, 2012a, 2012b).

Very recently (Örenc *et al.*, in press) ICCAT GBYP tentively explored various old Turkish archives, including the Ottoman Archives, trying to find any additional evidence about fishery and trade of bluefin tuna in old times in the various part of the eastern area. It is very clear that bluefin tuna fishery was very important for many years, generating high-value trade, but bluefin tuna data are difficult to isolate from those of other products, because they were all registered under the common name of "salt fish".

Bluefin tuna fishery in Turkey was carried out by several methods: tuna traps, seines, harpoons and lines (**Figure 6** and **Figure 7**). Tuna traps (locally called "dalians") were the most important possibly since historical classic times and they were set mostly in the Bosphorous and in the nearest areas including the Black Sea at least until the '50s (Belloc, 1961; Karakulak and Oray, 2009), even if the distribution of the salting factories suggest that they have been distributed also deeply in the Black Sea, including the Azov Sea. **Figure 8** shows the distribution of the tuna traps in the Bosphourus in the last part of the XIX century (Pavesi, 1889), while **Figure 9** shows the official distribution of traps in the Marmara Sea in 1913 (Örenc *et al*., in press). It is very clear that the traps were set for intercepting migratory or passage movements along the Straits and in the Marmara Sea. Trap cacth data in the ICCAT bluefin tuna data base exists up to 1984. Bluefin tuna was and it is still a common species for the Turkish market in Istanbul (**Figure 10**), even if bluefin tuna meat was not part of the Turkish seafood tradition for a long period (Örenc *et al*., in press).

According to Deveciyan (1915, 1926), Ninni (1923) and Örenc *et al.* (in press) the bluefin tuna fishery peaked in March, April, July, August and December in 1915, in January in 1916, in May and July in 1921, in April, July, August and December in 1922, in March, April and July in 1923, but catches were reported on every month on the Istanbul market, which was the most important in Turkey. These statistical data, the only clearly referred to bluefin tuna among the historical Turkish data, are not very helpful for defining any clear migration, due to their variability and to the presence of bluefin tuna in the area all year round.

Sport fishing for bluefin tuna was also important in Turkey since many years, according to the old report from Lebedev (1936), Lebedev and Lapin (1954), also mentioned by Iyigüngör (1957), while recent information is not available. Harpoon fishery was certainly carried out in the last century, but even here recent information is not available. Hand line for bluefin tuna is also practiced in Turkey, but data are not available or maybe included in the total catches. Gill nets were also catching tunas, but just a negligeable quantity was reported in 2010.

The Turkish catches of bluefin tuna from 1927 to 1960 differ again between the sources: according to Hamre *et al*. (1966), Miyake and Manning (1975) and Miyake and Tibbo (1972) ranged from less than a ton to over 1,500 tons, while according to the ICCAT data base they ranged from 100 tons to a maximum of 1488 tons in the same years. Mathers *et al*. (1995) reports that bluefin tuna catches in Turkey were negligeable after 1970, but the ICCAT data base shows catches from 1971 to 2012 ranging from 22 tons in 1971 to a maximum of 5,899 tons in 1999. Bluefin tuna catches from the Black Sea or the Marmara Sea or the Straits were never distinguished from all other Turkish catches obtained in the Mediterranean Sea, and the statistics are comprehensive. This fact prevents any analysis about the effects of the bluefin tuna disappearance from the Black Sea in the last decades.

Information on fisheries in other parts of the eastern Mediterranean Sea is very scarce and scattered. Some informarion exists for Syria, with catches ranging from 15 to 34 tons between 2007 and 2010 (obtained by LL and PS, see ICCAT bluefin tuna data base), Lebanon (Di Natale *et al*., 2006; Gucu, 2006; STECF, 2006), Cyprus (with reported catches from 1980 to 2012, ranging from 1 to 147 tons, obtained mostly by LL but also by PS and handlines) and Egypt (reporting 64 tons in 2012 by PS, but without any previous data available).

The sport and recreational fishery for bluefin tuna is very common in all countries in the eastern Mediterranean Sea (Greece, Turkey, Cyprus, Lebanon, Israel, Egypt) and many videos or short documents are easily available on the web, but data on these fisheries are not available. Some of the video clearly show bluefin tuna spawners at the surface in May in front of the Lebanese coast.

Bluefin tuna fishery in the Black Sea is much less documented in recent years. Esipow (1928) made a general overview, but without relevant details. Popescu (2010) made a comprehensive report of all fisheries in the Black Sea, including the bluefin tuna one, reporting that bluefin tuna was abundant prior to the ‘70s but it almost disappeared at the beginning of the ‘70s; he reported that bluefin tuna disappeared from the Romanian catches in the Black Sea in the ‘60s (Dumont *et al*., 1999), but that catches were reported from Turkey up to 1986. It is not clear if those Turkish catches were originating from the Black Sea or from the Bosphorous. He referred to the drastic reduction of the bluefin tuna stock reported by Zaitsev and Mamaev (1997), who reported that small schools of bluefin tuna were sighted at the surface from aircrafts in the 1950s, but then the population fallen dramatically in the ‘80s in Bulgarian waters. This information is not in line with the graph included in the National Bulgarian National Report in 1995, which is the same included in the papers by Zaitsev and Mamaev (1997) and Kideys (2004); according to the official figure, bluefin tuna fully disappear from the Bulgarian catches in 1970 (**Figure 11**).

The disappearance of top predators in the Black Sea was apparently caused by dramatic environmental changes, possibly induced also by the heavy industrial pollution, but it also caused a chain of changes in the Black Sea throfic chains (Di Natale, 2010). At the same time, a chain of drastic changes have been reported in the biological components in the upper stratum of the Black Sea since the ‘70s, with various blooms of plancktonic species, mostly alien and invasive. Detailed data are provided by the comprehensive work by Sorokin (2002).

According to recent anecdotic and local press reports, sporadic catches of bluefin tuna individuals have been reported again in the Turkish Black Sea waters, in the vicinity of the Bosphorous. These sporadic catches, all concerning single fish, which were mostly reported in very late summer and autumn, are possibly showing a slow reappearance of bluefin tuna in the Black Sea, maybe linked to more acceptable environmental conditions. A strick monitoring of incidental catches, including sport fishery, in this area is certainly highly wished.

5. Spawners, feeders or both?

A very recent paper (MacKenzie and Mariani, 2012) deeply analysed the hypothesis of a bluefin tuna spawning in the Black Sea since historical periods, studying the necessary physiological adaptations of adults, eggs and larvae for allowing the reproduction in the very particular marine environment of the Black Sea. This paper theoretically examined the necessary adaptation of bluefin tuna eggs for their successful development in the particular environment of the Black Sea, where salinity and oxygen are clearly different from all other spawning areas in the Mediterranean Sea.

If spawning somewhere occurred in the Black Sea when the bluefin tuna was still present there, then this was theoretically possible (even if it is extremely difficult), as a result of a long adaptation of the local population to the particular marine environment. MacKenzie and Mariani (2012) conclude that, if this was the case, therefore it will be unlikely that bluefin tuna can go back again in the Black Sea for spawning in a short time, because it will need many years for the physiological adaptation to the local marine conditions.

In the same paper, MacKenzie and Mariani (2012) did not exclud the possibility that bluefin tuna was migrating to the Black Sea for feeding purposes only and, in this case, the possibility of having again a bluefin tuna presence in the Black Sea can be more likely.

As a matter of fact, a lot of confusion does exist in both ancient and moder reports dealing with bluefin tuna movements in the eastern Mediterranean and in the Black Sea.

All classical authors (Aristoteles, Oppianus, Aetaneus, Plinius, Strabonis, Omerus, etc.) always mentioned the migrations of bluefin tuna from the Atlantic Ocean to the Mediterranean Sea for spawning, even with little or none knowledge of the bluefin tunas staying in the Mediterranean Sea for more than one year (the so-called "resident" fraction of the stock). The massive migrations were so well known since the Phoenician times that the idea of having also "resident" tunas was not so evident for those classical authors. So, linking all the informations and the timing for the coastal fishery of bluefin tuna along the coasts, the conclusion that some of these tunas were finally migrating to the Black Sea for spawning was a logical one at that time.

This conclusion was supported and confirmed in the last century by the identification of bluefin tuna eggs and even larvae in the Black Sea (Zaitzev, 1959, 2003; Akyüz and Artúz, 1957; Vodyanizky, 1936; Vodianisky and Kazianova, 1954; Vinobradov, 1948; Oven, 1959). Among these authors, Vodyanizky (1936) and Oven (1959) reported eggs from Sevastopol and Karadag (in Crimea) in late July and August. Mathers *et al.* (1995), acknowledging all these reports, were very sceptical about the correct identification of both eggs and larvae, also considering that no images were available. More recently, Piccinetti-Manfrin *et al.* (1995) did not find any bluefin tuna larvae in the Black Sea, possibly due to the full absence of this species from the area. In very recent times, only the genetic analyses were able to find some mis-identification mistakes in bluefin tuna larvae in the eastern Mediterranean and this fact might raise well-based doubts about the correct classification of both eggs and larvae attributed to bluefin tuna in the samples collected in the Black Sea, which were possibly confused with other tuna species.

More recently, Zaitsev and Mamaev (1997) again reported the theory that bluefin tuna from the Black Sea was migrating to the Marmara Sea for wintering, then going back to the Black Sea for spawning and feeding, but this report seems strongly biased by historical descriptions and does not consider the movements to and from the eastern Mediterranean Sea.

Some authors (Hovasse, 1927; Akyüz and Artúz, 1957; Iyigüngör, 1957) mentioned passages of maturing fish through the Bosphorus into the Black Sea and of spent fish in the opposite direction, but in different periods: Hovasse (1927) and Iyigüngör (1957) st the northward migration between March and April and the beginning of the southward one in July, while Akyüz and Artúz (1957) described a from April to September (peaking in July) and the southward one between October and November.

According to all available reports, newborn juvenile tunas were never reported in the Black Sea and in the Marmara Sea, besides an intense fishing for small tuna species is historically carried out in these areas. If juvenile (age 0) bluefin tuna were there, they will be concentrated in areas where other tuna species of the same size were concentrated for taking advantage of the same trophic chain and logically these catches should be reported somewhere, but this is not the case.

Ninni (1922) discussed the possibility of having several Mediterranean populations of bluefin tuna in great detail. He believed that the Tyrrenian population was independent of the Adriatic one, that both were independent from the Aegean Sea population and that the last was atleast partly independent from the one in the Marmara Sea. According to Ninni (1922), all populations had their independent wintering areas, possibly in deep waters, as proposed by Pavesi (1887). According to Ninni (1922), there were two wintering areas for the eastern Mediterranean bluefin tuna: one around Crete and another one between Crete and Alexandria. He also believed that some bluefin tunas wintered in the Marmara Sea.

Sarà (1963, 1964, 1973, 1983, 1998) also considered the information provided by classic historical authors and the more recent Turkish authors, and included the Black Sea among the potential spawning areas for bluefin tuna. He was still not aware of any bluefin tuna spawning area in the eastern Mediterranean.

As a matter of fact, the official knowledge about bluefin tuna spawning in the easter Mediterranean (mostly in the Levantine Sea) was almost nil until the late '80s, when the fishery for adult bluefin tuna became more relevant in these areas and when studies were carried out more extensively and intensively, even if some large areas are still not properly studied.

The oceanography studies and particularly the satellite observations revealed a peculiar and very early increasing of the sea surface temperatures in the eastern Mediterranean, well before the other areas of the Mediterranean Sea, with stable hot water masses in the upper stratum, and bluefin tuna usually spawns much early in this part of the basin, sometimes even one month before than in other Mediterranean areas.

Usually, from then last part of April, the SST start increasing in the SE part of the Mediterranean basin, particularly along the eastern Egyptian coasts, the Nile delta area, the Palestinian coasts and the Israelian coasts, where the SST may easily go over 21°C. In the very last days of April and the first part of May, the SST increases further in all the eastern Mediterranean area, particularly in the Levantine Sea between Israel and Turkey, up to the Greek island of Rhodes, and including the waters around Cyprus; the SST can easily go well over 23°C in this period, with an almost stable stratification of the upper water masses and a well-established thermocline. These temperature conditions will further increase during the final part of May and the full June, while the SST in the northern Aegean Sea will stay usually below 20°C[2]. SST and stratification differences between years in the same period can be remarkable and the last two years, 2013 and 2014, provide an example (**Figures 12 to 15**).

This evidence, clearly induced by the average climate conditions and not by other modification of the oceanographic factors in the area (mostly the opening of the Suez Canal and the effects induced by the Assuan dam), was never considered in various papers, while it is extremely relevant for better evaluating the potential biological status of the bluefin tuna migrating into the Black Sea.

As a matter of fact, it is very reasonable and logic that the bluefin tunas living in the eastern part of the Mediterranean Sea are usually spawning in the Levantine Sea at the early beginning of the season[3], then continuing the spawning in a larger area in late spring. Studies and reviews carried out since the '90s (Piccinetti and Piccinetti-Manfrin, 1997; Nishida *et al.*, 1998; Oray and Karakulak, 1998, 2005; Karakulak, 1999; Corriero *et al*., 2003; Karakulak *et al.,* 2004; Oray *et al*., 2005; Di Natale *et al*., 2006; Gucu, 2006) finally confirmed the logical presense of important spawning areas in the eastern Mediterranean Sea, including the Levantine Sea. This knowledge also exists among fishermen, because opportunistic fishery activities on bluefin tuna spawners have been conducted in the last decade (prior to the seasonally closure adopted by ICCAT) by large purse seiners off the Nile Delta and SE of Cyprus at the early beginning of May.

The fact that some papers reported that the ovaries of bluefin tunas caught in the Marmara Sea were mature (Ninni, 1923; Hovasse, 1927; Akyüz and Artüz, 1957) but not fully expanded during the northward migration can be read in two ways: (1) the bluefin tunas moving to the Black Sea were moving from the eastern Mediterranean just after the main local spawning season and then ovaries were still containing almost mature eggs but they were not fully expanded because of the previous spawning activity and the sexual products will be slowly reabsorbed (post-spawning situation); or, (2) the bluefin tunas were potential spawners, moving from the Aegean Sea or even from the Marmara Sea to the Black Sea for spawning, and their gonads were still not fully mature or they were continuing the spawning already initiated in the eastern Mediterranean area.

[2] Only in some years, as it happened in spring 2014, these hot water masses are able to reach also most of the northern Aegean Sea, creating a broader favourable area for bluefin tuna spawning. Anyway, the suitable conditions for bluefin tuna spawning in the northern Aegean Sea are certainly unusual.

[3] See the typical spawning behaviour of bluefin tuna off the Lebanese coast in May 2012 on http://www.youtube.com/watch?v=zf-GrUveV2U and on http://360tuna.com/index.php?/topic/25483-bluefin-tuna-report-from-the-lebanese-coast/page-2#entry283448

According to the oceanographic conditions in the eastern Mediterranean and in the Black Sea in spring, the first condition appears as the most probable, implicitely informing about a post-spawning feeding migration of those tunas into the Marmara Sea (and, in the past, up to the '70s, into the Black Sea). The first hypothesis is also confirmed by Karakulak *et al.* (2004), because tunas sampled in last part of May and at the beginning of June in the Levantine Sea were post-spawners, while Hovasse (1927) simply reported that gonads were full in spring while they were empty in July; Akyüz and Artüz (1957) reported that, according to their observations on ovaries, spawning should occur between July and September.

Piccinetti *et al.* (2013), reviewing all published data on bluefin tuna reproduction in the Mediterranean area, confirmed the clear existence of bluefin tuna spawning areas in the eastern Mediterranean Sea and in the Levantine Sea, keeping the Black Sea under a very doubtful situation.

Ehrenbaum (1924) reported that he found several larve of *Orcynus thynnus* L. off Cape Matapan (or Cape Tenaro, Ακρωτήριον Ταίναρον), on the western edge of the Aegean Sea, but both Sella (1929a) and Richards (1976) considered that these larvae were not bluefin tuna; on the contrary, one larval specimen found by Ehrenbaum (1924) in the same expedition and originally identified as *Orcynus germo* Lacepede, was considered by Richards (1976) as a possible bluefin tuna. These disputes confirmed again the serious difficulties for correctly identifying the bluefin tuna larvae, which sometimes exists even now. Piccinetti and Piccinetti Manfrin (1994) reported some bluefin tuna larvae in the Levantine Sea, while the larval campaign carried out in summer 1994 by Piccinetti *et al.* (1997), which included the eastern Mediterranena Sea, did not find any bluefin tuna larvae in the eastern part of the basin, possibly due to the late timing. Nishida *et al*. (1998) found bluefin tuna larvae in the Levantine Sea and in the south-eastern Mediterranean in 1994, but without any specification about the date or month. More recent larval samplings in the NE Aegean Sea in summer time (Vassilopoulou *et al*., 2008) and in the whole Aegean Sea by Oceana (Pastor *et al.*, 2009), again did not find any egg or larvae of bluefin tuna, but the timing of the campaign was late. It is not clear if any bluefin tuna larvae was found off Crete by a larval campaign carried out in 2008 (Giovanardi and Romanelli, 2009), because the data are not presented by location.

All these data combined, along with purse-seine fishery anecdotical or VMS information, seem quite clearly supporting an early bluefin tuna spawning period in the eastern Mediterranean and the Levantine Sea, confirming also the findings by Karakulak *et al*. (2003).

Fishing for young-of-the-year bluefin tuna in the entire Mediterranean Sea is a very common ancient practice, and it includes also the eastern Mediterranean area; it is usually carried out by artisanal or recreational fishermen in coastal areas when age 0 bluefin tuna feeding aggregations are there and those fish are almost essentially used for personal consumption or for local markets. This fishery, which became illegal in all the ICCAT area in recent years but which is extremely difficult to control, is diffused also in the SE part (Palestine and Israel), in Lebanon, in some parts of the Turkish coast and in the norther Aegean Sea since times; besides the catches obtained by artisanal fishermen, sometimes catches are made by purse-seiners fishing for other pelagic fish species of the same size. Official reports on these fisheries are not available, and even recent Turkish papers about the bluefin tuna size distribution in these areas are not including young-of-the-year catches, not even as incidental by-catch.

On the contrary, old documents and recent information (Oren *et at*., 1959; Di Natale *et al*., 2006; Gucu, 2006; STECF, 2006) confirm the presence of small bluefin tunas in these eastern Mediterranean waters and particularly in the Levantine Sea and in the NE Aegean Sea, a clear indication about the logic distribution of local offsprings in the vicinity of a spawning area. Again, no information or reports are available about the presence of young-of-the-year in the Black Sea, in the Bosphorus and in the Marmara Sea[4], further confirming the preference of this juvenile part of the bluefin tuna population for the eastern Mediterranean area only. Due to the high availability of food resources for small and medium pelagic in the Black Sea, catches of young-of-the-year should be somewhere reported if spawning may have ever occurred in this basin.

[4] During a personal visit at the Istanbul Galatasaray fish market on 14 November 2007 I found six specimens of bluefin tuna, having a weight of about 700/800 g each, along with many specimens of *Sarda sarda*; according to the fishmonger, these fish were caught all together in the Aegean Sea, very close to the Strait of Dardanelles, in the previous day; the size of the small bluefin tunas was quite unusual for that time of the year (usually, in mid-November, bluefin tuna is around 1.7/2 kg in the other parts of the Mediterranean Sea) and this small size might be caused by: a) a very late spawning somewhere in the area; b) a very late reproduction and a poor proper food chain for the bluefin tuna juveniles at that time of the year.

6. Current evidence about a possible eastern Mediterranean bluefin tuna population

The fact that bluefin tuna fishery was carried out for many centuries in the Marmara Sea and in the neares areas by coastal tuna traps, even in months not strictly correlated with the bluefin tuna massive movements in and out the Straits (Bosphorous and Dardanellis) is a clear evidence that the presence of bluefin tuna in the eastern Mediterranean area was largely independent from any migratory support. Devecyan (1915, 1926), Karahís (1915) Ninni (1922, 1923), Marti (1941), Carp (1951), Lebedev and Lapin (1954), Iyigüngör (1957), Sarikaya (1980), Karakulak (1999, 2000), Mert *et al.* (2000), Kahraman and Dağlı (2003), Karakulak *et al.* (2004); Oray and Karakulak (2004), Oray *et al.* (2005), Karakulak and Oray (2009), clearly demonstrated that bluefin tuna stay in Turkish areas and particularly in the Marmara Sea all year round, with different numerosity from month to month and different peaks among the years. Di Natale (2010), taking into account the previous literature and the clear presence of bluefin tuna all year round (without excluding also the presence of migratory specimens), drafted a first idea of an eastern Mediterranean bluefin tuna population, which was including also the former Black Sea component.

The aerial surveys for bluefin tuna spawners carried out by ICCAT GBYP in several Mediterranean areas including the eastern Mediterranean, between 2010 and 2013, confirmed both the presence of very important concentrations of bluefin tuna schools of spawners in the area between Cyprus and Turkey, mostly concentrated at the beginning of June[5], with much lower concentrations later in the season and with very few bluefin tuna at the surface at the early beginning of July (Cañadas *et al.*, 2010, Di Natale, 2011; Cañadas and Vâsquez, 2013; Di Natale *et al.*, in press). These first aerial surveys confirm an anticipated bluefin tuna spawning season in the eastern Mediterranean Sea.

The preliminary studies carried out by ICCAT GBYP in the first four Phases, which included microchemistry and genetic analyses, are showing genetic differences between the bluefin tuna collected in the eastern Mediterranean and the other Mediterranean specimens (Arrizabalaga *et al.*, 2013; Di Natale *et al.*, in press). The analyses, which clearly separated bluefin tuna larvae collected in the eastern Mediterranean from the other samples, showed a problem for age 0 fish from the eastern Mediterranean, because there was a western Mediterranean genetic component (**Figure 16**).

In this case, the broader view of GBYP was able to provide a possible justification, thanks to the GBYP aerial survey carried out in 2011 and the environmental data collected daily in the same period. According to these field observations, an anomalous oceanographic condition, coupled with strong winds south of Malta[6], caused the presence of a large area of stable hot waters in the western part of the eastern Mediterranean Sea, comprised between Cyrenaica, the southern part of Italy and the western part of Greece. In this area, the stable conditions of hot surface waters allowed for a deep surface thermocline; this fact induced a considerable number of bluefin tuna spawners, usually spawning in the central Mediterranean, to move eastward and possibly spawn in a large area north of Cyrenaica (**Figure 17**), where they possibly mixed with the "eastern Mediterranean" individuals, which were close to their western distribution area. This opportunity possibly caused the presence of some mixed "western-eastern Mediterranean" age 0 fish in 2012 in areas where "eastern Mediterranean" tunas were usually distributed (the right food chain is anyway available for juvenile bluefin tuna also in that area, where there is traditionally a high availability of small pelagic species).

Preliminary microchemistry data revealed differences among the various areas in central-western Mediterranean bluefin tunas (Rooker *et al.,* 2003). On the contrary, microchemistry preliminary analyses carried out in the ICCAT GBYP framework are not showing any specific differenciation among samples collecte in the various eastern Atlantic and Mediterranean areas, which are anyway differenciated from the western Atlantic specimens (Rooker *et al*., 2014). Of course, even in this case, further samplings and analyses are necessary for more deeply investigate the natal origin of bluefin tuna in the various areas.

Other data, derived from tagging activities, are indicating a discrete separation between the eastern Mediterranean bluefin tuna and those distributed in the central and western Mediterranean and in the Eastern Atlantic area.

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[5] Due to the yearly organisation of GBYP, the contracting procedures, the very complex procedures for obtaing the various flight permits in the different air spaces and the complicated political situation in the last years, it was impossible so far to carry out the aerial survey in May or to extend the aerial survey to the SE part of the Levantine Sea.

[6] The strong winds in the central-southern Mediterranean Sea not only caused problems to the purse-seine activity, delaying the fishery, but they also prevented the formation of a stable thermocline and a suitable upper stratum with warm temperatures, inducing bluefin tuna to move eastward. This desk assumption of the GBYP staff was confirmed in real time both by vessels and by the aerial survey.

Sella (1929) reported two Turkish lures used only in the vicinity of Istanbul (Turkey, recovered in eastern Tunisia and southwestern Sardinia, and four of these lures, each on a different fish, were recovered in a tuna trap near Bengazi (Libya). So far, this is the only available evidence of bluefin tunas moving from the Marmara Sea to central Mediterranean areas in the first part of the XX century.

On the contrary, in the more recent decades, none of the few bluefin tuna tagged in the eastern Mediterranean (De Metrio et al., 2001, 2004, 2005; Rooker *et al*., 2007) moved into the central or western Mediterranean, while none of the many bluefin tunas tagged both with electronic tags or with conventional tags in the eastern Atlantic or in the western or central Mediterranean was never recovered in the eastern Mediterranean (Fromentin, 2010; de la Serna *et al*., 2011, 2013; Medina *et al*., 2011; Tudela *et al*., 2011; Cermeño *et al*., 2012; Aranda *et al.,* 2013). Recent intense tagging activities carried out in the framework of ICCAT GBYP with both electronic (miniPATs) and conventional tags in the eastern Atlantic or in the western or central Mediterranean never resulted with any recovery in the eastern Mediterranean Sea (Quilez-Badia *et al*., 2013a, 2013b; Di Natale *et al*., 2014a, 2014b).

This sort of separation between the eastern Mediterranean bluefin tuna and the rest of the Mediterranean and the Atlantic ocean, as it appears by the lack of recent recoveries in the eastern part, is maybe too extreme to be real, because at least a partial mixing is logic and possible as it has been shown by the preliminary genetic analyses in 2012, but it is anyway a sign of the possible existence of a bluefin tuna population or sub-population, which was probably the ancient Black-Sea/eastern Mediterranean one.

Although if further analyses are necessary for confirming and more precisely define the possible bluefin tuna populations or sub-populations in the Mediterranean Sea amd in the Atlantic Ocean, it is clear that the availability of real-time information from many different sources (aerial survey, environmental data, genetics, microchemistry, conventional and electronic tagging) can certainly contribute to increase our understanding of the results of sophisticated analyses.

7. Discussion

All the many sources of both historical and recent information reported and discussed above are telling us that our knowledge about the natural history and the present distribution seriously improved in the last two decades and it is further improving through the ICCAT GBYP activities in very recent years.

At the same time, it is very clear that many aspects, which are extremely important not only for the scientific knowledge but also for a more focused management of this species, still need to be further clarified. While an eastern Mediterranean population or subpopulation of bluefin tuna seems very possible, some important research issues should be studied in the next years and we propose the following recommendations, taking advantage of the ICCAT GBYP framework:

a) Develop a conventional and electronic tagging activity on bluefin tuna in the eastern Mediterranean Sea;
b) Improve the sampling programme for bluefin tuna in the eastern Mediterranean Sea;
c) Improve the number of analyses (both genetic and for microchemical components) in all the Mediterranean area;
d) Extend the aerial survey for bluefin tuna spawners for at least other 6 years;
e) Carry out field missions, contacting all scientific institutes working on fishery in both ICCAT CPC and non-ICCAT CPC, with the objective to collect information about the presence and distribution of both young-of-the-year or juveniles and adult bluefin tuna in the various areas of the eastern Mediterranean Sea;
f) Monitor as much as possible all available sources of information about possible catches (including sport fishery) in the Black Sea.

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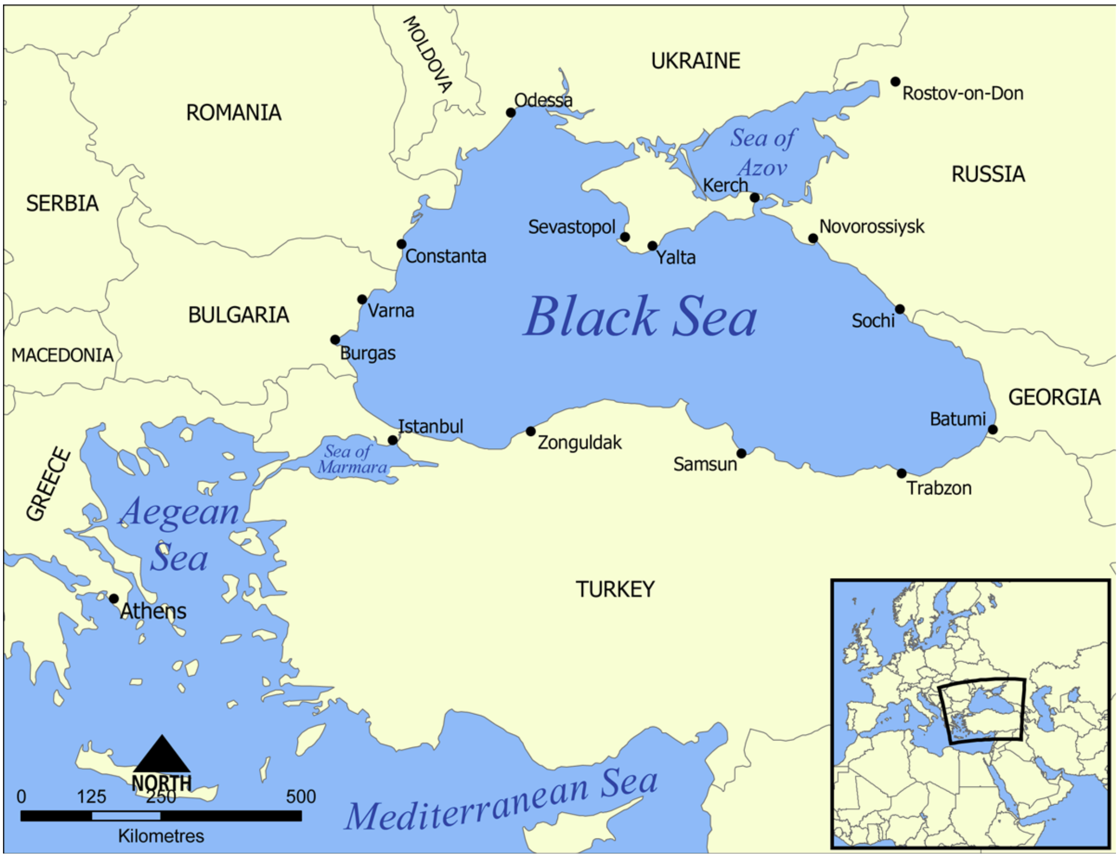

Figure 1. Geographical location of the Black Sea and the Sea of Azov (image from Wikipedia).

Figure 2. Image of the Black Sea bottom (source: NASA World Wind).

a  b 

Figure 3. Distribution of a) the main tuna salting and *garum* factories in classic historical times and, b) the ancient towns where coins with tuna were mint in classic historical times (from V b.C. to V a.C.) in the eastern Mediterranean Sea and in the adjacent seas.


a

b

c

Figure 4a (left). Trihemiobol, Greek coin in silver from Cyzicus, c. 480 b.C. (0.82 g); forepart of boar running left, tuna fish upwards behind; reverse: head of roaring lion left. **4b (center)**: El 1/48 Stater, Greek coin in silver from Cyzicus, c. 600 b.C. (0.39 g); obverse: head of tuna right; reverse: incuse square with swastika. **4c (right).** El Hemihekte, Greek coin in electrum from Cyzicus, c.500 b.C. (1.3 g); obverse: Triton left holding wreath in left hand, on tuna left; reverse: quadripartite incuse square.


a

b

c

Figure 5a (left). El Hekte, Greek coin in electrum from Cyzicus, V b.C. (16.7 g); obverse: Head of Attis right, wearing Phrygian headdress; below, tuna right; reverse: quadripartite incuse square. **Figure 5b (center).** El Stater, Greek coin in electrum from Cyzicus, c.500 b.C. (16.1 g); obverse: Head of Apollo, wearing laurel wreath, facing slightly right; below, tuna right; reverse: quadripartite incuse square. **Figure 5c (right)**: El Stater, Greek coin in electrum from Cyzicus, c. 500 b.C. (16.23 g); obverse: Panther standing left on tuna, raising right forepaw; reverse: quadripartite incuse square.

Figure 6. Old painted illuminated drawings of bluefin tuna fishery in the Turkish-Ottoman area: fishing with nets, which were maybe seines or traps.

Figure 7. Old painted illuminated drawings of bluefin tuna fishery in the Turkish-Ottoman area: harpoon fishing or maybe the final harvesting of bluefin tuna in a bluefin tuna trap.

Figure 8. Map of the various traps for bluefin tuna existing in the Bosphorous at the end of the XIX century (Pavesi, 1889). The number increased to 25 traps at the beginning of the XX century (Parona, 1919), then decreasing to about 10 in the '50s (Belloc, 1961).

Figure 9. Map of the Marmara Sea, the Gulf of Bandırma, Erdek, and Imralı Island, which was attached to an application for granting a bluefin tuna fishery permit, showing the various locations for installing tuna traps (Document DH. İD, no. 17-65, December 8, 1913) (Örenc *et al*., in press).

Figure 10. A bluefin tuna transported to the fish market in Istanbul in 1933 (Photo courtesy by Prof. Alí Fuat Örenc).

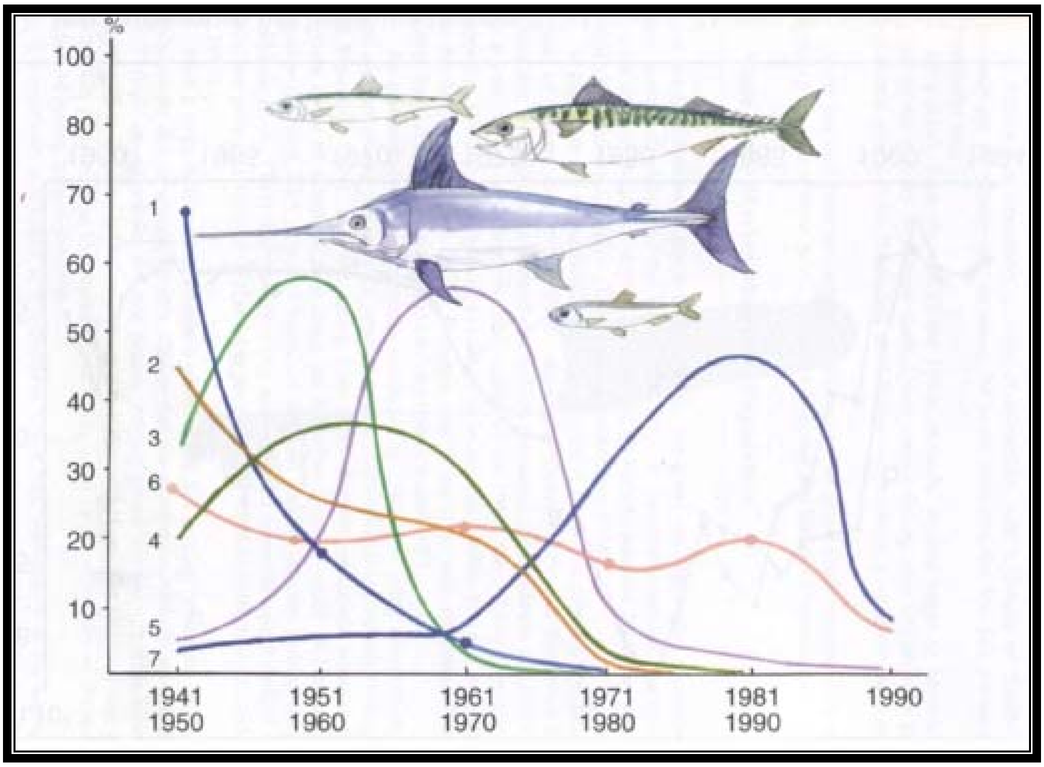

Figure 11. Trends (%) of mean catches per decade of pelagic top predators and forage fish (anchovy and sprat) in 1941-1990 in the Bulgarian Black Sea waters. 1: bluefin tuna; 2: mackerel; 3: swordfish; 4: bonito; 5: bluefish; 6 anchovy; 7: sprat (from Bulgarian National Report 1995, Zaitsev and Mamaev, 1997, and Kideys, 2004)

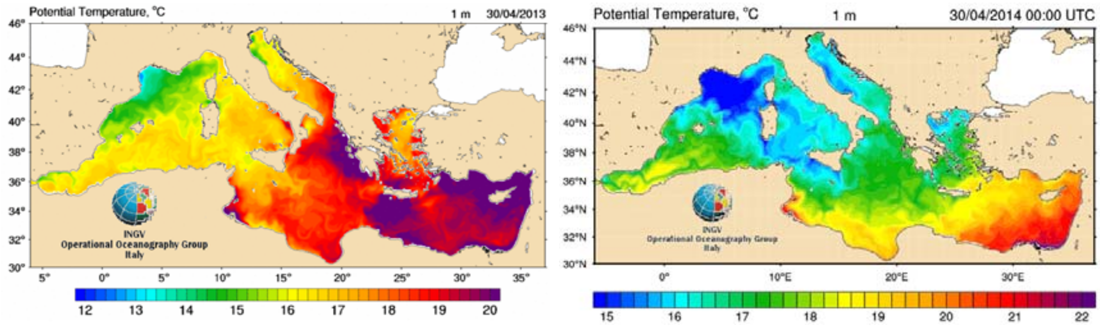

Figure 12. Figure 12a (left) SST image of the Mediterranean Sea on 30 April 2013, clearly showing the warmest area in the SE Mediterranean Sea (t scale 12°-20°C). **Figure 12b** (right) SST image of the Mediterranean Sea on 30 April 2014, even more clearly showing the warmest area in the SE Mediterranean Sea (t scale 15°-22°C).

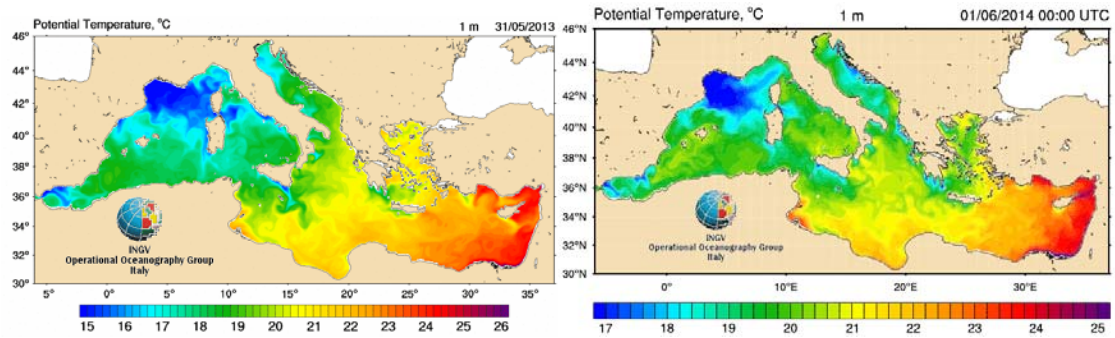

Figure 13. Figure 13a (left) SST image of the Mediterranean Sea on 31 May 2013, confirming the stability of the warmest area in the SE Mediterranean Sea (t scale 15°-26°C). **Figure 13b** (right) SST image of the Mediterranean Sea on 31 May 2014, showing the colder situation of the Mediterranean, with warm water in the eastern side (t scale 17°-25°C).

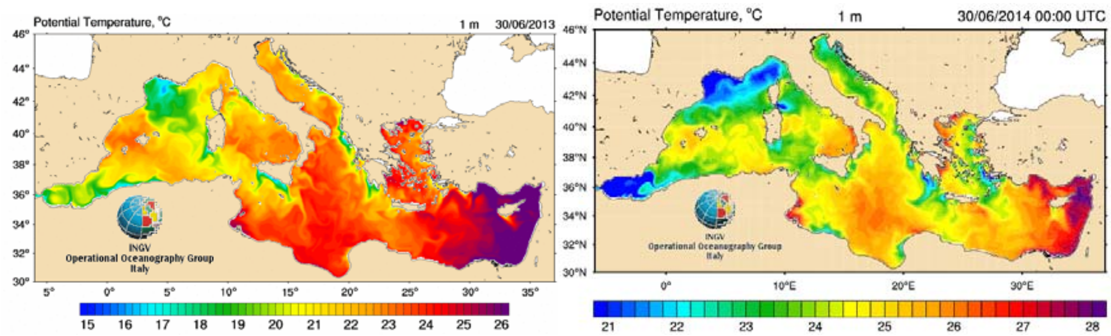

Figure 14. Figure 14a (left) SST image of the Mediterranean Sea on 30 June 2013, with hot water masses in the SE Mediterranean Sea (t scale 15°-26°C). **Figure 14b** (right) SST image of the Mediterranean Sea on 30 June 2014 (t scale 21°-28°C).

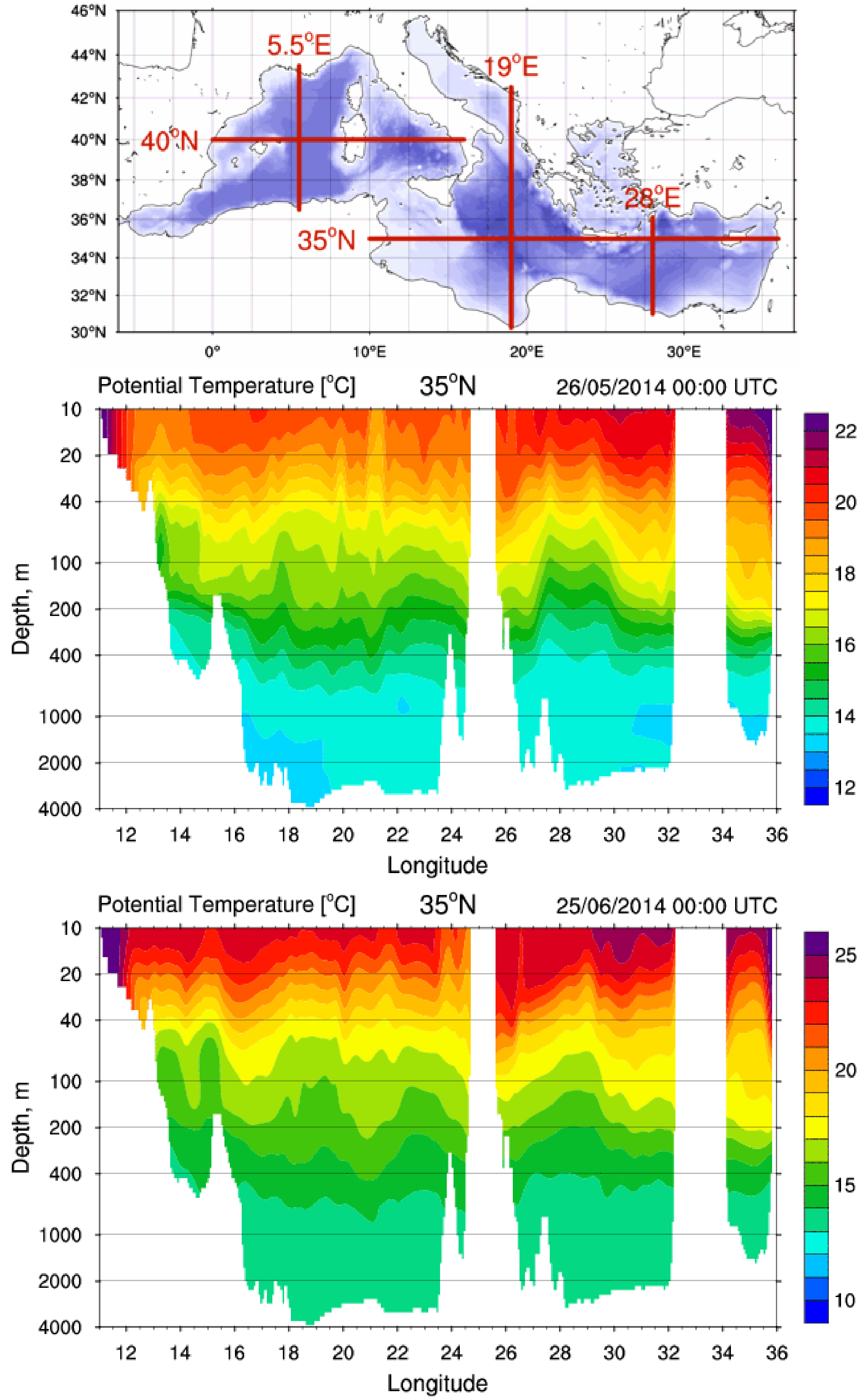

Figure 15. Vertical transect available for the Mediterranean water temperatures (top); situation of the vertical temperatures at 40°N on 25 May 2014, showing few areas with well-established thermocline (center); situation of the vertical temperatures at 40°N on 25 June 2014, showing the eastern Mediterranean waters with hot water (bottom).

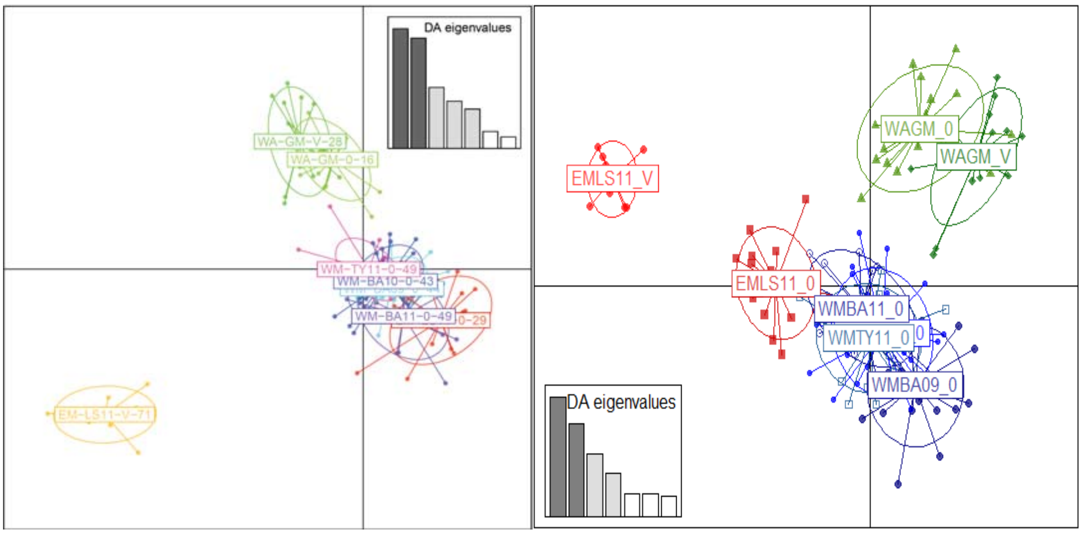

Figure 16. Figure 16a (left). Clustering analysis using DAPC based on the eight reference samples. Three clusters can be seen, roughly coinciding to the expected spawning groups. Note the exception of the Eastern Mediterranean Age-0 sample (red samples) that clustered together with the Western Mediterranean reference samples and not with the Eastern Mediterranean Larvae sample. **Figure 16b (right)**. Clustering analysis using DAPC based on the eight reference samples and a restricted subpanel of 96 SNP. Three clusters can be seen, roughly coinciding with the expected spawning groups, with an improved separation of the Eastern Mediterranean Age-0 sample fro**m the Western Mediterranean reference samples, even if yet not clustering with the Eastern Mediterranean Larvae** sample.

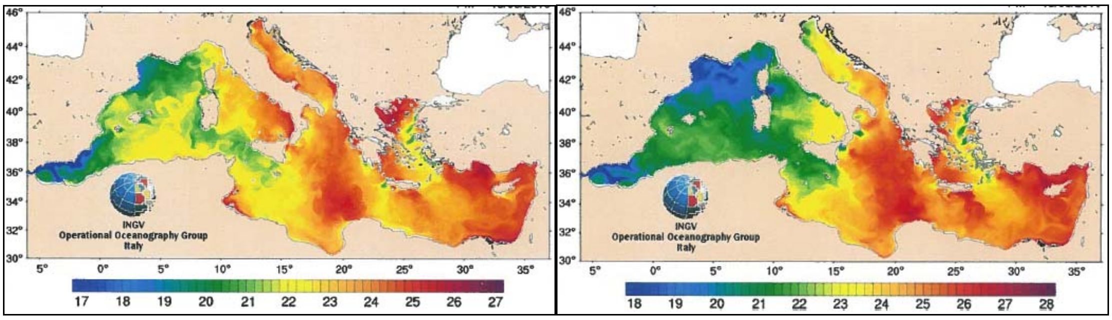

Figure 17. Figure 17a (left) SST image of the Mediterranean Sea on 15 June 2011, with hot water masses in the area between Cirenaica and western Greece (t scale 17°-27°C). **Figure 17b** (right) SST image of the Mediterranean Sea on 19 June 2011 (t scale 18°-28°C); note the mass of very hot water between Cirenaica and western Greece and the anomalous colder water mass in the Strait of Sicily, caused by the strong winds persisting in the western and western-central Mediterranean Sea, which prevented bluefin tuna spawning in these areas in these days.