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STUDY OF LARGE PELAGIC FISH LIFE-HISTORY TRAITS IN THE CENTRAL MEDITERRANEAN SEA AND PROJECTION UNDER FUTURE CLIMATE CHANGE SCENARIOS

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List of papers, poster, and talk

Russo, S., Torri, M., Patti, B., Sarà, G., Cuttitta, A. (2022). Oral presentation: Tuna fish life-history traits in the Central Mediterranean Sea and ecological effects on early life stage. ICES PICES Early Career Scientist Conference (ECSC4) will be held on July 17 - 21, 2022.

Russo, S., Torri, M., Patti, B., Musco, M., Masullo, T., Di Natale, M. V., Sarà, G. & Cuttitta, A. (2022). Environmental Conditions along Tuna Larval Dispersion: Insights on the Spawning Habitat and Impact on Their Development Stages. Water, 14(10), 1568. https://doi.org/10.3390/w14101568.

Russo, S., Torri, M., Patti, B., Reglero, P., Álvarez-Berastegui, D., Cuttitta, A., & Sarà, G. (2021). Unveiling the relationship between sea surface hydrographic patterns and Tuna larval distribution in the central Mediterranean Sea. Frontiers in Marine Science, 1116. https://doi.org/10.3389/fmars.2021.708775.

Russo S., Patti B., Torri M., Reglero Barón P., Álvarez Berastegui D., Sarà G., Cuttitta A. (2019). Characterization of the larval habitat of Tuna species in the central Mediterranean Sea. (Poster). 43rd Annual Larval Fish Conference. Spanish Institute of Oceanography, Palma, Balearic Islands, Spain. May 21-24th, 2019, S8: Ecological and evolutionary processes affecting fish, Page 176.

List of acronyms and abbreviations

ABT	Atlantic bluefin Tuna
AIS	Atlantic Ionian Stream
AL	Anus Length
B.40	Bongo40 net
B.90	Bongo 90 net
BDA	Body Depth at the Anus
BDC	Body Depth at the Cleithrum;
Chl-a	Chlorophyll a
CNR	Italian National Research Council
COB	Centro Oceanográfico de Baleares
ED	Eye Diameter
FAO	Food and Agriculture Organization of the United Nations
FL	Fork length
GAM	Generalized additive model
HL	Head Length
ICCAT	International Commission for the Conservation of Atlantic Tunas
IEO	Spanish Institute of Oceanography
IOTC	Indian Ocean Tuna Commission
ISSF	International Seafood Sustainability Foundation
IUCN	International Union for Conservation of Nature and Natural Resources
LH	life-history
Lj	Lower jaw length
RCP	Representative Concentration Pathway

SL	Standard length
SSB	Spawning stock biomass
TL	Total Length
UniPa	University of Palermo

Abstract

One of the most sensitive life periods for each species is the first life stages (larval and juvenile). The survival rates of pelagic fish larvae in these phases are usually very low and subject to fluctuations, consequently affecting the recruitment of new generations.

From historical Tuna landing data, the stock's abundance is fluctuating, especially for Atlantic Bluefin Tuna (ABT) (*Thunnus thynnus*, Linnaeus, 1758) Eastern stock. It is the largest Mediterranean Tuna and one of the most expensive species in the world. Some studies hypothesized that the fluctuations are dictated by environmental factors, particularly in the early life stages.

Therefore, it appears necessary to better understand the reproductive biology, structure of the reproductive population, and the recruitment processes related to the environmental drivers of the Mediterranean Sea. In fact, it is a crucial area for maintaining ABT at a global level. It is essential for the other two species too, which share time and space during spawning in summer and their first life stages: ABT, Albacore Tuna (*Thunnus alalunga*, Bonnaterre, 1788)*,* and Bullet Tuna (*Auxis rochei*, Risso, 1810). They carry out an essential ecological role as top predators, are fisheries targets globally, and have a fundamental influence on the structure and function of marine communities. Therefore, their sustainable management is essential and depends on a better understanding of the fish ecology, especially early life-history (LH) traits.

Despite the Strait of Sicily (Central Mediterranean Sea) being an important spawning site for Tuna species, little is known about the relationship between the environmental variables and their larval spatial distribution in this area. Using historical larval collections from seven yearly surveys, in-situ environmental measurements, and satellite data, in this thesis, the interaction between ecological conditions and Tuna LH traits, horizontal distribution and spatial structure of the three Tuna species at the larval stage has been modelled.

A multidisciplinary approach involving lagrangian simulations, species distribution modelling, and morphometric analysis has been used to verify the influence of environmental conditions on the distribution pattern of Tuna spawning and its effects on larval development and, consequently, on their survival probability.

Finally, the potential impacts of the identified main driver have been investigated through forecasting models to determine the Tuna larval habitat patterns under possible future climate change scenarios. Understanding what influences the growth and survival of individuals among large pelagic fish populations, particularly during the larval stage, is crucial because these factors will eventually determine recruitment variability and the number of survivors that reach the adult stage.

This work aims to improve knowledge of the growth and survival of Tuna larval stages, casting the complex system of the Central Mediterranean Sea in a new light. A site where upwelling, gyres, and fronts play a key role and observations could indicate a meta-population structure in Tuna populations.

Chapter 1 provides an introduction with a background on the topic of study.

Chapter 2 is a work published during my PhD candidate career. It is the basis for the characterization and knowledge of the central Mediterranean larval habitat. The abundance and presence-absence of larval specimens for the three Tuna species were modelled to examine their relationships with environmental factors. The results suggest that temperature, salinity, and day of the year are the key factors for understanding these species' ecological mechanisms and geographical distribution in this area.

In Chapter 3, we look back in time and evaluate the conditions experienced by larvae, starting from their hatching to the point at which we found them. In this way, it was possible to better know the larval habitat and the spawning areas selected by the adults. We hypothesized that the environments experienced along the passive transport period would affect the development of these organisms. It seems helpful to describe not the difference in morphometric measurement but an early or late development. This work also highlighted different spatial patterns between the species.

Finally, in Chapter 4, predictive models have been structured to model recruitment under different future climate change scenarios. Thanks to these models, it was possible to verify how the proportion of species could change. Despite the potential habitat seeming to expand, the abundances do not always follow the same trend. The results of this study can help manage future adult stocks and understand recruitment patterns under different future climate change scenarios. In fact, thermal sensitivity is a fundamental physiological attribute and one of the main reasons for induced changes in natural communities.

Chapter 1. **General introduction**

1.1 Preface

The life-history of marine fishes is fundamental to understanding population dynamics. The baseline for sustainable use and management of the fisheries' target species can be determined by how such species respond to fisheries' exploitation, and climate changes. Often, in the past, the study of fish populations has focused only on the adult stages. Instead, studying the distribution and abundance of early life-history fish stages can help indicate spawning locations and the environmental requirements of important fish species like Tuna. In fact, the survival of fish larvae may directly influence the future abundance of adult fish stocks (Rodríguez et al., 2017) since the success of recruitment events is not determined from the single particular ontogenetic stage but many factors such as species, populations, and environmental conditions (Houde, 2008). Studying all these aspects is fundamental for knowing and understanding stock fluctuations, and change drivers in early life stages are a crucial element. Many early life-history processes that define larval survival appear environmentally driven (Harford et al., 2017).

For this reason, many species make long migrations to reproduce and select areas with perfect conditions for the survival of their offspring. Among these are Tuna, which differ in strategies, size, and habitat, but often have common spawning grounds despite their different life strategies (Reglero et al., 2012). Three Tuna that reproduce in the Mediterranean overlap their ranges during the delicate reproductive phase, and these Tuna are Atlantic Bluefin Tuna (ABT) (*Thunnus thynnus*, Linnaeus, 1758), Bullet Tuna (*Auxis rochei*, Risso, 1810) and Albacore Tuna (*Thunnus alalunga*, Bonnaterre, 1788). However, these areas remain understudied; there is still much to know about the habitats selected by adults in the Mediterranean for spawning, and habitats in which larvae develop and grow.

Adult fishes are able to decide where and when to reproduce based on different signs, including those provided by environment and geography. (Ciannelli et al., 2007). Some prefer geographical areas that are stable over time, with constant environmental conditions. Others are more opportunistic and follow only environmental signals, reproducing wherever these ideal conditions seem to be satisfied.

ABT, Bullet Tuna, and Albacore Tuna are top predators, and they have an important ecological role. They are targets of fisheries globally and have a fundamental influence on the structure and function of marine communities (Kitagawa & Kimura, 2015). Their life strategies are complex and differ, but they have in common certain key traits such as overlapping spawning areas or larval habitats.

This thesis represents the first approach to the study of larval ecology of these species in an area little explored until now from this point of view, the central Mediterranean Sea. This work tries to identify the ecological drivers that have guided the reproductive success of three different Tuna, but which have the choice of reproductive areas in common. Understanding the ecology of the early life stages of these fishes is central to understanding their population dynamics and the functioning of marine ecosystems.

1.2 History

Science, history and fisheries are constantly linked by an interest in large pelagic fish. Tuna are some of the most important species in fishing, not only in the Mediterranean but globally, and have remained a significant economic and cultural pillar for centuries.

They were commercialized during the Roman era (Lleonart et al., 1998), but evidence shows that their importance precedes even these examples by millennia. In the iconographic field, there are prehistoric testimonies (Eneolithic or Cuprolithic period) in the cave of the Genovese in Levanzo with the presence of pictorial representations on the walls of the cave, the outline of the Tuna stands out unequivocally (Bombace, 2017). Their catch became a fundamental activity in the social structure of the Phoenician people. It even seems that Tuna routes in the Mediterranean may have dictated the location of Phoenician colonies (Bombace, 2019). In this regard, the testimonies of the ancient authors are innumerable. Aeschylus, in "The Persians", describes the slaughter of Tuna. Strabone tells us that the Phoenicians intercepted Tuna up to the Pillars of Hercules. Homer, Pliny, Plutarch, Aristotle and other authors also became interested in Tuna from a scientific point of view. Tuna became so crucial in their economy that they even minted coins with depictions of Tuna, a symbol of prosperity (Bombace, 2017).

Historically, from a scientific perspective, the first annotated bibliography of Tunas is dated 1929 and written by Mrs. Helen Genevieve Corwin, a marine scientist. This text was the first to systematically review Tuna, analyzing and studying texts across multiple languages, including Latin and Greek (Di Natale et al., 2020).

1.3 Tuna phylogeny

The Scombridae family, which includes Mackerels, Tunas, and bonitos, comprises 15 genera and 51 species (Fig. 1). It is divided into two subfamilies, Gasterochismatinae and Scombrinae, in turn, composed respectively of a single and 50 species (Froese & Pauly, 2021).

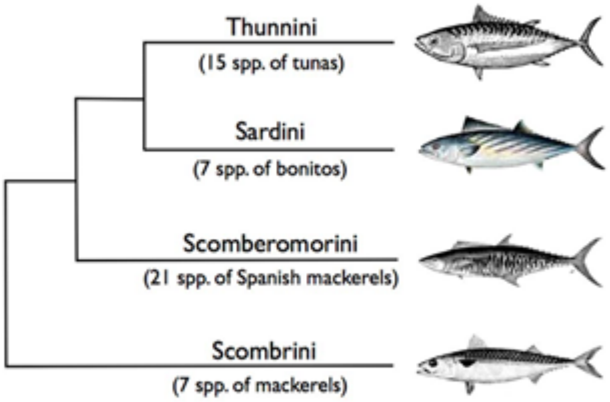

Figure 1 - Morphological phylogeny of the Family Scombridae showing the subfamily Scombrinae composed of four Tribes. From Juan-Jordá et al., 2013.

Among the fifteen species of Thunnini, seven are the principal market Tunas due to their economic importance in the global markets (Juan-Jordá et al., 2013)

The Thunnini tribe comprises 15 species and is a sister group of the Sardini tribe, to which the bonitos belong (Graham & Dickson, 2004).

A constant and continuous swimming, typical of Tuna, has led to changes in the body's shape, alterations in the blood supply, and variations in arteries and venous branches, laying the basis for heat conservation (Graham & Dickson, 2004). Indeed, *Thunnus* and close relatives have evolved a specialized vascular system for heat exchange, and apparently, the four species of subgenus *Thunnus* have more effective heat conservation mechanisms (Graham & Dickson, 2004). Multiple origins and diverse strategies for endothermy exist among these fish to retain metabolic heat that increases their body temperature. Although, this evolution seems to have been selected to expand the thermal niche and not for greater aerobic capacity (Block et al. 1993).

ABT is part of the Thunnini, and it falls within the *Thunnus* genus, which includes eight species. These include both tropical and temperate species. It is the largest Tuna. The maximum-recorded length is 427 cm with a weight of 600 kg (Juan-Jordá et al. a, 2013; Froese & Pauly, 2021) (Fig. 2 A). They are epi–mesopelagic fish with a temperate distribution. They carry out trans–oceanic migrations, spending their life between oceanic and temperate zones (Juan-Jordá et al. a, 2013).

Figure 2 - Adult of ABT (A), Albacore Tuna (B) and Bullet (C), from Froese & Pauly, 2021.

Bullet Tuna belong to the genus *Auxis*, composed of two species. According to a study (Collette & Aadland, 1996), there are two new subspecies, *Auxis thazard brachydorax* and *Auxis rochei eudorax*, in the Pacific waters. They are the smallest members of the Thunnini tribe, with a maximum length of around 48 cm (Fig. 2 C). They are neritic and epipelagic fish, and they prefer inshore waters and those close to islands. They are considered tropical Tuna (Juan-Jordá et al., 2013).

Albacore Tunas, like ABT, are part of the genus *Thunnus* also. They are large Tuna but reach a maximum size of 140cm in length and 60.3 kg (Froese & Pauly, 2021) (Fig. 2 B). They are epi–mesopelagic, considered subtropical Tuna (Juan-Jordá et al., 2013).

1.4 ABT, Bullet, and Albacore distribution, migration and life cycle

The Thunnini tribe has broad geographic distribution throughout tropical and subtropical oceanic and coastal ecosystems, although most prefer open marine waters (Graham & Dickson, 2004; Juan-Jordá et al., 2013). The principal market Tunas have oceanic distributions and are highly migratory species, particularly the three species of bluefin Tuna that migrate and spawn seasonally from cold waters, where they feed, to warm waters to reproduce (Graham & Dickson, 2004). They tolerate significant thermal changes from one area to another (Juan-Jordá et al., 2013). There are species with long life histories that tend to live in temperate climate zones and species with shorter lifetimes typically found in tropical waters (Juan-Jordá et al., 2013). In the Mediterranean Sea, there are five species of Tunas. All are of commercial interest: ABT, Albacore, Bullet Tuna, Atlantic black skipjack (*Euthynnus alletteratus*) and Skipjack Tuna (*Katsuwonus pelamis*) (Alemany et al., 2010).

There are two major behavioral phases in the life of Tuna: a reproductive phase, with marked aggregating characteristics, superficial navigation in favor of the current and a dispersive, erratic step, in search and hunting for prey, in which the animals in small groups move towards the feeding areas. In both cases, loyalty to places is evident (Bombace, 2017).

Tuna migrations for reproductive purposes are concluded in warm waters for spawning (Graham & Dickson, 2004). Depending on whether they are tropical, subtropical or temperate Tuna, the period and the duration of the spawning season suggests an association between spawning duration and environment characteristics (Fig. 3 C) (Juan-Jordá et al., 2013). Temperate species of Tuna spend most of their annual cycle in colder, higher latitudes and perform large-scale migrations to exploit relatively predictable seasonal environmental cycles. This enables reproduction at a particular time, and in well-defined warmer habitats during the springtime (Block et al., 2003).

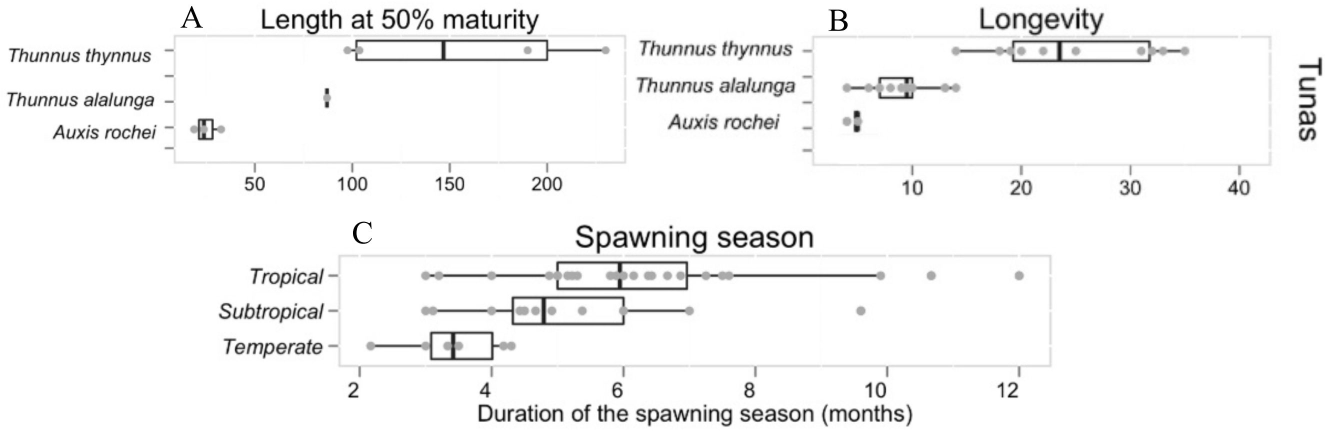

Figure 3 - Length in cm at 50% maturity estimates (A). Longevity in years of the three studied Tuna species (B). Duration of spawning season of scombrid species by type of climate (C). Modified from Juan-Jordá et al., 2013.

The reproductive migration to favorable habitats is a strategy to ensure that offspring have a better chance of survival. Migration within a specific time window and areas allows highly migratory Tunas to minimize the environmental variability to which their larvae will be exposed to; selecting sites with suitable conditions to develop as many individuals as possible (Alemany et al., 2010).

Longevity varies greatly depending on the species, but on average, they are very long-lived fish (Fig. 3 B). Sexual maturity is reached at different times, depending on the size of the species (Fig. 3 A). The smaller species mature at big sizes than their maximum possible size, and the larger species are the opposite (Juan-Jordá et al., 2013).

Tunas are batch spawners with asynchronous oocyte development to have multiple reproductive events within a reproduction window. All Tunas spawn pelagic eggs that hatch into larvae within a few days (~3–4 mm long), grow fast, develop foraging and swimming organs, and then metamorphose into juveniles during the first month of life (Miyashita et al., 2001; Margulies et al., 2007). The three Tuna studied in this thesis share large-scale spawning areas. Still, at a regional level, they have differences, preferring coastal areas or offshore areas. Some seem to be guided more by environmental signals than geographical signals or a mix between the two (Reglero et al., 2012). This happens because spawning adaptations to changing environments and the mechanisms to maximize fitness may differ among Tuna species. Details of the reproductive strategies of each species examined here will be given in the following paragraphs.

Tuna are voracious predators, from the earliest life stages to the adult stage, during which they have enormous energy needs. Their diet varies according to the ontogenetic period, starting by feeding on zooplankton during the earliest larval stages, then becoming piscivorous by feeding on other larvae. At sub-adults' stages, they prey on medium pelagic fishes, shrimps, and cephalopods. As adults, prey on cephalopods and larger fishes (Sarà & Sarà, 2007; Reglero et al., 2011). The larvae are fast-growing, thus remaining vulnerable to predation for shorter periods than other species (Rodríguez et al., 2017). In fact, at early life stages (2.8-8 mm), larvae may grow 0.3 mm/day (Itoh et al., 2000, Miyashita et al., 2000). The transition from endogenous to exogenous feeding is one main bottleneck affecting Tuna larval survival, as observed for other fish species (Hjort, 1914). In most marine fish species, in the first year of life, the survival of the larvae is less than 1% (Houde, 2008), and early arrival at the exogenous feeding stage is successful because it leads to maximizing the number of larvae that will reach the juvenile stage (Hjort, 1914).

ABT

Distribution - It can be found in the North Atlantic, from the cold waters between Labrador and Norway, to the warm waters of the Mediterranean and the Gulf of Mexico (Collette et al., 2021).

Rising temperatures have led ABT to colonies Greenlandic waters (Di Natale, 2012) (Fig. 4). In ancient times, it was present in the Black Sea, and there was an annual migration to Eastern Mediterranean spawning grounds, but, due to the changing environmental conditions of the Black Sea, ABT is now extremely rare, albeit numbers have increased in recent years (Di Natale et al., 2019).

Adults are found in waters from 1 to 29°C and from the surface (where they spend most of their time) to depths as deep as 800 m (Collette & Graves, 2019).

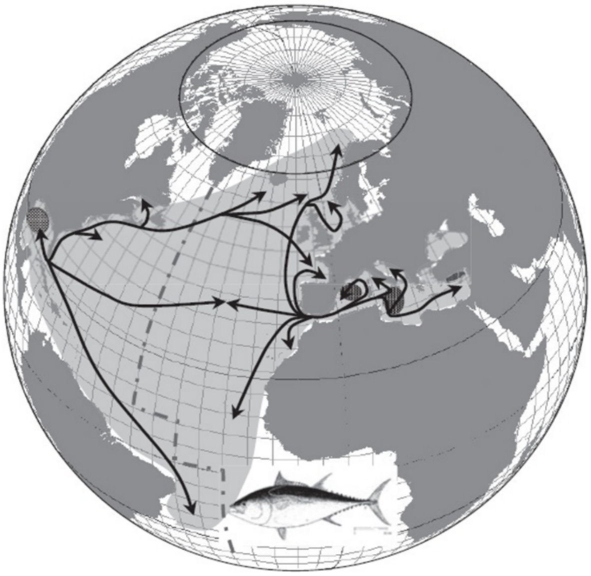

Figure 4 – Spatial distribution of ABT (gray shading) and main migration routes (black arrows). The vertical dashed dotted line depicts the stock delimitation between the two current International Commission for the Conservation of Atlantic Tunas (ICCAT) management units. The main spawning grounds (dark dotted gray areas) being currently assumed in the literature are located in the Mediterranean Sea and the Gulf of Mexico. From Fromentin & Powers, 2005.

Population trend and stock assessment - It is divided into two stocks, the Western Atlantic stock and the Eastern Atlantic and Mediterranean stock. The Western primarily spawn in the Gulf of Mexico and the Eastern in the Mediterranean Sea. The two stocks are genetically distinct (Carlsson et al., 2007; Boustany et al., 2008), but despite this, outside of the spawning grounds, there can be considerable mixing of the two stocks throughout much of the North Atlantic (Block et al., 2005). Since 1966, the International Commission for the Conservation of Atlantic Tunas (ICCAT) recognizes and manages the two stocks at the 45th meridian (Fig. 4). However, they mix during the feeding phases in the North Atlantic. Based on historical catch estimates for each stock, the Eastern Atlantic stock constitutes approximately 78% of the global population of ABT (IUCN, 2021).

From the last International Union for Conservation of Nature and Natural Resources (IUCN) assessment of 15 January 2021, the global ABT population is categorized under ‘Least concern’, a notable change from the previous evaluation that classified the population as ‘Critically endangered’.

Based on spawning stock biomass (SSB) of the Eastern stock and the total biomass of the Western stock, from the most recent 2017 and 2020, there has been approximately no significant decline in the global size of the ABT population (Collette et al., 2021). This trend is a sign that ICCAT conservation measures and management are paying off. Nevertheless, adequate restrictions and a high guard must remain in place to ensure that the global population of ABT remains free from risk of extinction. However, considering the Mediterranean population in isolation, it remains at risk due to over-exploitation, with data defining it as in decline and an IUCN classification of ‘Endangered’ (Di Natale et al., 2011).

Reproduction and migration - The global spawning season occurs between May and July, while in the Mediterranean, it stretches from May to August when the temperatures average 22.5-25.5°C (Rodríguez et al., 2017). Adults mature at 155.2 cm (at 36% of their maximum body size, combining information for both Eastern and Western populations), and they can live up to 35 years (Juan-Jordá et al., 2013 a) (Fig. 3 A-B). The two stocks seem to reach sexual maturity at different sizes. It has been hypothesized that this is due to different resource exploitation (Di Natale et al., 2019). Females produce as many as 10 million eggs per spawning season (Corriero et al., 2005). Egg size is 1.0 mm, and larval length at hatching is 2.8 mm (Fig. 5 A). With a temperature of 24°C, embryo development lasts about 32 hours and larval stages 30 days.

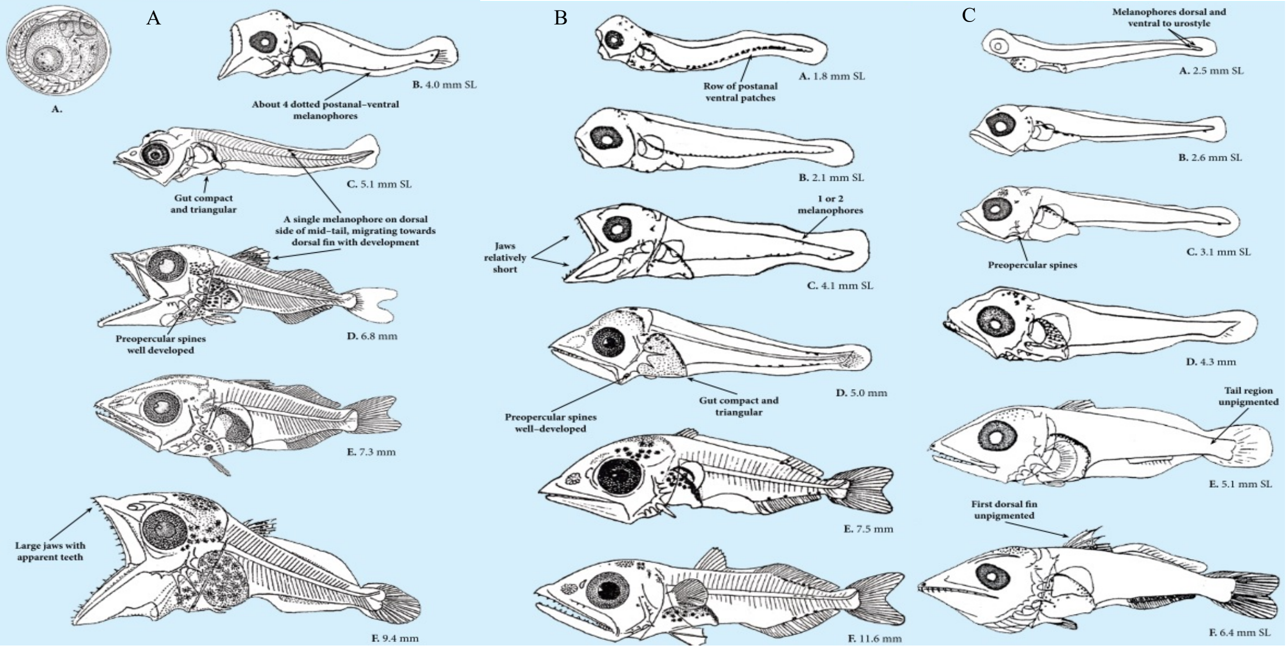

Figure 5 – Morphological and development differences during the larval stage of ABT (A) Bullet Tuna (B) and Albacore Tuna (C). From Rodríguez et al., 2017.

The two stocks have different spawning areas: The Western, whose principal spawning ground is in the Gulf of Mexico (although new evidence suggests spawning also occurs in the Slope Sea), and the Eastern, which spawns in the Mediterranean Sea (Muhling et al., 2017) (Fig. 4). In the Mediterranean, the most studied area is around the Balearic Islands. However, there is also evidence collected from the central region of the Tyrrhenian Sea (around Sicily), the Northeast portion of the Gulf of Sirte, the waters between Malta and Tunisia, and the Eastern area of the Ionian Sea (around the Island of Cyprus) (Karakulak & Yildiz, 2016; Muhling et al., 2017). Large migrant adults enter from the Atlantic Ocean following the Atlantic waters through the Strait of Gibraltar in April-May, and the peak period of spawning is in June-July (Alemany et al., 2010; Reglero et al., 2012; Abascal et al., 2016). It has also been hypothesized that a resident sub-population of ABT within the Mediterranean Sea mixes with Tuna entering through Gibraltar during the spawning period. This sub-population would stay in the Mediterranean Sea during all the year or a part of it, feeding during the winter in different locations than large migrants from Atlantic waters (Cermeno et al., 2015; Livi et al., 2019).

BULLET TUNA

Distribution - It is widespread and abundant, with the highest distribution in the Atlantic, Pacific, Indian oceans, and the Mediterranean (Fig. 6). The Eastern Pacific population is a subspecies *Auxis rochei eudorax* (Collette & Aadland, 1996). There is an intra-Mediterranean migratory species of Bullet Tuna, and a study from the Eastern Mediterranean basin zone observed high larvae concentrations in this area (Oray & Karakulak, 2005).

Figure 6 – Distribution of Bullet Tuna. Modified from (IUCN 2021).

Among all Tuna, the juveniles of the genus *Auxis* are the most abundant and widely distributed in the world, across both tropical and temperate waters. Usually, it forms schools, and it preys on whatever abundant resource is available, with a preference for planktonic crustaceans, small cephalopods, and fish larvae (Mostarda et al., 2007). Bullet Tuna is an essential element of the food web due to their abundance, particularly as prey for other species of commercial interest.

Population trend and stock assessment - The species are listed as ‘Least Concern’ at the global level. The population trend is stable (Collette et al., 2011), but the last global assessment from IUCN is dated 2010. The European evaluation is more recent (Collette & Heessen, 2015). The population is rated as ‘Least Concern’, with an SSB stable trend, but it may be due to no evidence and data.

In the Atlantic, there is a problem with the statistics. It is possible ICCAT included both *Auxis thazard* and *A. rochei* on the catches data, and the proportion between the two species is not known (IUCN, 2021). However, almost all catches in the Mediterranean are thought to be *Auxis rochei* (Collette & Nauen, 1983). This is a common species in fisheries in the Mediterranean, and abundance varies depending on the place and year (Collette & Heessen, 2015). There are no global conservation measures or fishery management plans for this species, despite it being a migratory species and an important fisheries resource for coastal communities. This is due to the lack of accurate data, historical series and analyses. But some data on the catch composition, biology and trends present in the Mediterranean are now becoming available (Collette & Heessen, 2015).

Reproduction - Its spawning season is between May and November (Rodríguez et al., 2017), but in the Mediterranean Sea, spawning occurs from July to September (Allaya et al., 2013). It lives approximately five years and reaches maturity at around two years old, or about 35 cm at fork length (FL) (Fig. 3 A-B), but this can vary by region.

It can spawn multiple times and throughout its range (IOTC, 2007). Its fecundity ranges between 31,000 and 162,800 eggs per spawning (according to the size of the fish) (Collette, 2010). Its eggs measure up to 1.10 mm, and the hatching larvae 2.14 mm SL (Fig. 5 B).

ALBACORE TUNA

Distribution-Albacore Tuna are cosmopolitan in tropical and temperate waters, between the Atlantic, Pacific, Indian Oceans, including the Mediterranean Sea, but they aren't found in surface waters between 10°N and 10°S (Fig. 7). They are abundant in surface waters of 15.6–19.4 °C. The distribution of Albacore juveniles in the Mediterranean is very poorly known.

Figure 7–Distribution of Albacore Tuna. Modified from (IUCN 2021).

Population trend and stock assessment - The stocks are generally largest in the Pacific Ocean and smallest in the Mediterranean Sea. Temperature plays a crucial role in the adult's distribution, setting maximum distribution limits, within which the distribution patches are dictated by food availability (Nikolic et al., 2016). We recognized six stocks: North and South Atlantic, Mediterranean Sea, North and South Pacific, and the Indian Ocean. There is a genetic difference between the six stocks, and they are separately managed (Nikolic et al., 2016). There is no migration or gene flow from the Atlantic to the Mediterranean Albacore population (Nakadate et al., 2005). The Strait of Gibraltar does not act as a barrier, but it seems to be sufficient to differentiate the Mediterranean population of Albacore from the others (Albaina et al., 2013; Laconcha et al., 2015).

There has been an estimated 3–19% decline globally over the past 20–25 years (Collette et al., 2021). The most significant stock decline of up to 40% has occurred in the Mediterranean Sea and is of concern. However, with more than 90% of its global range in areas where overfishing is not happening, the species is not considered overfished, and Albacore Tuna is listed as ‘Least Concern’ but with a decreasing population trend. The Mediterranean stock is estimated to have declined by 45% between 1993 and 2015, it is estimated to be less than 2% of the species global population, and it is considered not overfished (ISSF 2020).

In the North Atlantic, Albacore was overfished during the 2000s but recovered thanks to the ICCAT rebuilding plan of the spawning stock biomass. The stock is now managed with a harvest control rule, including quotas. The first stock assessment was completed in the Mediterranean in 2012, but there is concern that landings have been under-reported. A study by Montes et al. (2012) found two genetic groups of Albacore in the Mediterranean (East and West). The Tyrrhenian and Adriatic Albacore Tuna were found to belong to a separate group from the Balearic samples.

Reproduction-Compared with most other Tuna species, Albacore is relatively slow-growing, late-maturing, and has an extended life span (Nikolic et al., 2016). At maturity, both length and age appear to be smaller in the Mediterranean Sea, and immature Albacore is generally <80 cm.

Its spawning season in the Mediterranean Sea is between June and August, with a peak in mid-July, when sea surface temperatures (SST) exceed 24 °C (Rodríguez et al., 2017). It appears to have a different reproductive strategy in the Mediterranean than in the oceanic spawning grounds. It displays a shorter spawning season and higher relative batch fecundity, and this may be the manifestation of an adaptation to the environmental conditions of the basin (Saber et al., 2015).

Albacore is batch spawners with asynchronous egg development and indeterminate annual fecundity like other Tunas.

Its larvae at hatch size are bigger than the other two Tuna (2.6mm SL) (Fig. 5 C).

The two potential subpopulations are possibly differentiated by their spawning areas, which are: the Tyrrhenian Sea, the South Adriatic Sea, the area around the Strait of Messina, the Aeolian Islands, and the Aegean and the Balearic Islands in the Western Mediterranean (Alemany et al., 2010; Nikolic et al., 2016) (Fig. 8).

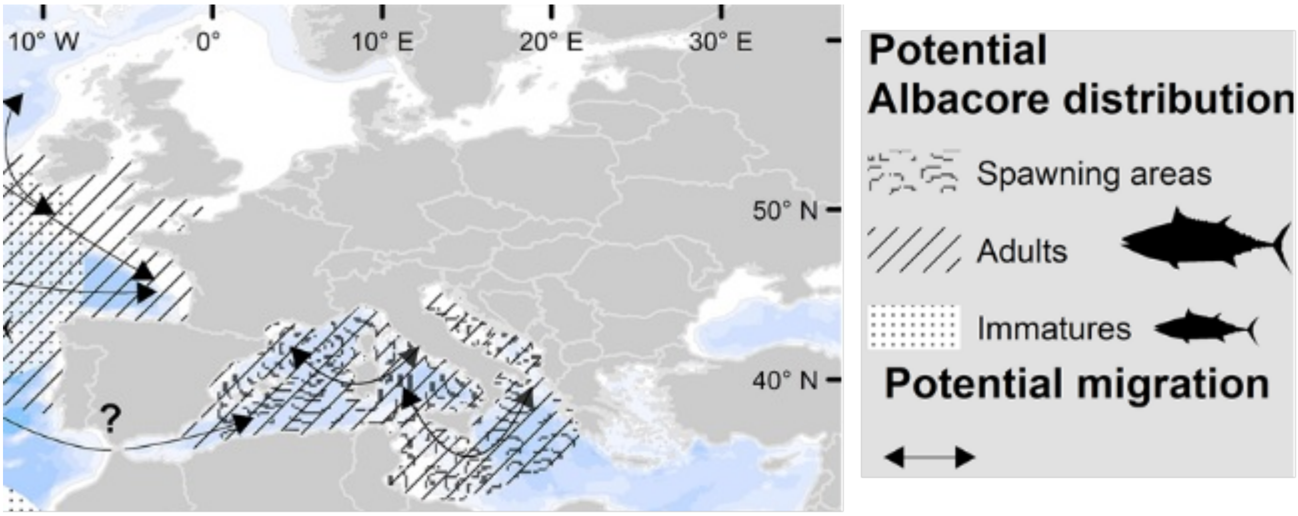

Figure 8 - Schematic distributions and potential (or hypothetical) migrations of adult and immature Albacore Tuna in the Mediterranean. Modified from Nikolic et al., 2016

1.5 Fisheries and Aquaculture

In 1883, during the London International Fisheries Exhibition, Thomas Huxley, biologist and president of the Royal Society asserted, "With existing methods of fishing, it is inconceivable that the great sea fisheries, such as those for cod, herring, and mackerel, could ever be exhausted." (Sims & Southward 2006). However, with the development of large-scale commercial fishing gear, a rapid and repeated negative impact on natural fish populations through overfishing has now been proven. Even large pelagic fishes such as Tuna, overfished for decades and exploited by humans since ancient times, reach one of the highest prices in the international market (Mylonas et al., 2010; Sun et al., 2019).

Scombrids species sustain some of the most important fisheries globally, from large-scale industrial to small-scale artisanal fisheries in developed and developing countries (Juan-Jordá et al., 2013).

There are four methods of fishing for Tuna:

a) Purse seine, used during the morning if shoals or signals are sighted (e.g. ripples on the sea surface, Tuna jumping out of the water, etc.).

b) Long-line, this is a large nylon thread, up to several kilometers long, to which a monofilament is attached to which the bait hook is tied. It is lowered into the water level (generally from 15-20 m to hundreds of meters from the surface), which is presumed to be affected by the school of Tuna.

c) An ancient fishing system, Tuna traps consist of a barrier system of nets placed vertically from the surface to the bottom. The traps reach out around two kilometers into the open sea from the beach. During the spring-summer period, these make it possible to catch Tuna along migratory routes.

d) Sportfishing, with various lines, towed or from a mobile station with a boat, rod, artificial or natural bait.

e) Finally, there are Tuna fattening farms. The adults are caught with purse seines and transported to the farms where they are fed.

ABT- Much of the ABT catch, entering the sashimi market, reaches a very high value (Collette & Graves 2019). Its end value in 2018 was $1.1 million (Pew, 2020). In the 1990s and early 2000s, ranching farms in Croatia and elsewhere in the Mediterranean Sea began operating. With this new practice, reported landings increased in 2007 (IUCN, 2021). The management measures reduced catches (ICCAT, 2020), but the assessment of the two stocks is carried out on adults and on the geographical division that had been decided (45° meridian). This approach leads to problems in stock assessment because, in the Western Atlantic, some studies found increasing percentages of Mediterranean-spawned fish (up to 75% in some areas in some years), indicating that the actual size of the Western Atlantic population is likely even smaller (IUCN, 2021).

Catch quotas for country and a landing declaration with control of the Tuna caught are imposed. Currently, purse seines take 58% of the catch, traps 15%, pelagic longlines 17%, and assorted gear types the remaining 10%. Japan is a major importer of all bluefin Tuna species, and it is estimated that approximately 80% of the Japanese market is supplied by the countries around the Mediterranean Sea (Heffernan 2014, Collette & Graves 2019). Wild-caught individuals became an essential resource for fattening farms, which manage to increase the biomass of Tuna. Once caged, larger individuals can increase up to 25% in size, while smaller ones can increase by 100% or more (IUCN, 2021).

BULLET TUNA- Compared to the other two Tuna, Bullet has the lowest economic value. It supports diverse fisheries and is an essential source of wealth and food security to the fishing communities in many countries (Juan-Jordá et al., 2013).

Small-scale fisheries and commercial fishing in the Mediterranean catch Bullet more as bycatch (Collette & Heessen, 2015).

Pole, purse seine set surface, small drift nets gill nets and line and, accidentally, in artisanal purse seines are the principal method of fishing it.

ALBACORE TUNA- Albacore Tuna is an essential fishery resource around the world, is one of the principal market species of Tuna fisheries worldwide, representing 5% of the total Tuna catch in 2015 (ISSF 2017). It is mainly marketed as canned white meat Tuna. Albacore has been chiefly used for sashimi and sushi in recent years.

The ICCAT manages the Albacore stocks in the Mediterranean Sea. There, the fisheries are predominantly longline fisheries or use troll lines to catch large adult fish, and Juveniles are generally taken with surface gear (Nikolic et al., 2016).

1.6 Methods to study larval ecology

Fish population dynamics are a complex topic. In particular, analysis of the interannual variability or variability in stocks related to the new biomass deriving from the new recruitment. But ichthyoplankton surveys provide a relatively low-cost efficient means to monitor marine fish populations and communities (Koslow & Wright, 2016). The survival of fish larvae may directly influence the future abundance of adult fish stocks (Rodríguez et al., 2017). Once correctly identified, fish eggs and larvae can provide vital information for troubled fisheries concerning population structuring, spawning seasons and locations, as well as SSB or recruitment trends (Puncher et al., 2015). Larval surveys can also help to determine community dynamics or assemblage and favor, with additional data, the development of ecosystem-based management approaches (Zhou et al., 2010).

The principal difficulties are obtaining representative samples of ichthyoplankton, due to the spatial and temporal heterogeneity in distribution and the interaction of fish eggs and larvae with sampling gear. They are not distributed randomly but concentrated in patches that become part of the plankton during specific periods of the year. It depends on spawning adults' strategies, distribution of adult fishes, hydrodynamic processes, and larval behavior. (Rodríguez et al., 2017).

Tuna larvae change morphology throughout their development, making the work of recognition very difficult. Two of the world's former leading Tuna taxonomists warned that ‘‘the young stages of fishes of the family Scombridae are among the most difficult to identify to generic levels and particularly to the species level’’ (Richards & Pothoff, 1974). Moreover, the pigmentation changes depending on the larval stage in which the Tuna is found (Fig. 9). Often, there has been considerable confusion when it comes to recognition at the species level in previous studies, especially where they concern ABT. Puncher et al. (2015) revealed that, very frequently, ABT larvae were misidentified in the Mediterranean Sea. They showed that more than half of larvae submitted by three Mediterranean institutions to an ICCAT funded BFT research project were the wrong species. For that reason, in this study, we relied on the most recent recognition keys recognized as the most suitable for identifying Tuna larvae in the Mediterranean (Rodríguez et al., 2017).

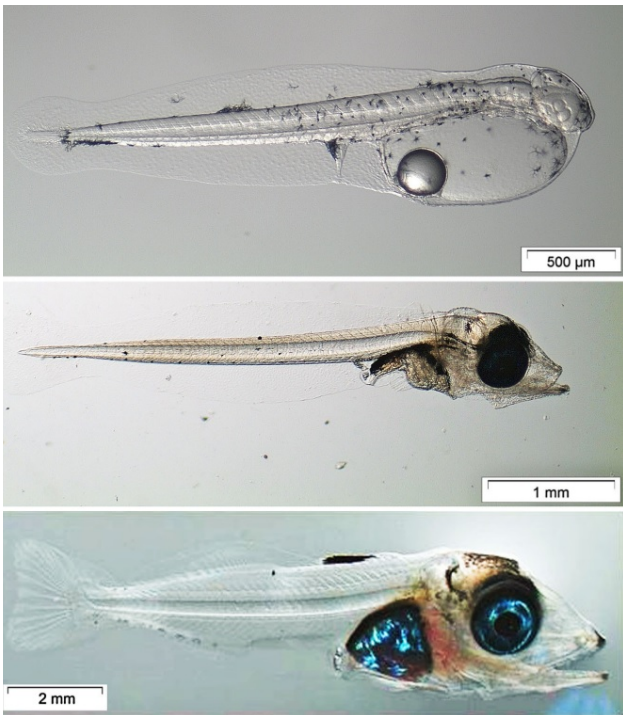

Figure 9- Photos of ABT larvae from Puncher et al., 2015. Larval developmental stages represented are: yolk sac, 8 days post hatching and 14 days post hatching.

1.7 Aims of the study

The Tunas sustain some of the most important fisheries globally from an ecological and economic point of view. Their sustainable management is fundamental, and it depends on a better understanding of their life-history strategies.

This study focused on the interaction between life-history traits and processes and the environment, horizontal distribution, spatial structure and trophic role of three Tuna larvae.

The general objective of the thesis is to verify the influence of the environmental conditions on the distribution pattern of ABT, Bullet and Albacore Tuna spawning grounds and its effects on larval development, and consequently, on their survival probability. Related to this general objective is the analysis of Tuna larval growth and its variability.

Therefore, this study starts from the spatial analysis of larval habitats in the central Mediterranean (Chapter 2) before proceeding to conduct analysis of potential spawning areas through back-calculation and verification of the differences in environmental conditions experienced by the larvae according to their origins (Chapter 3). Finally, the possible effects of environmental change are analyzed (Chapter 4), simulating how these populations can vary within the study area. In this way, the thesis aims to improve knowledge of the growth and survival of the larval stage of ABT, Bullet and Albacore Tuna. This is a novel approach, never attempted in a complex system like the central Mediterranean Sea, where two water masses with opposite characteristics (the Levantine Sea and incoming Atlantic waters) meet.

1.8 General materials and methods

The Central Mediterranean Sea - The study area is located in the Strait of Sicily (Fig. 10). It is the leading fishing site in the central Mediterranean Sea (García Lafuente et al., 2002), a region characterized by a dominant hydrographic feature (the Atlantic Ionian Stream (AIS)) with an evident influence on the spawning strategy and the recruitment success of many fish species (García Lafuente et al., 2002, Cuttitta et al., 2016). It is classified as an oligotrophic area (Malanotte Rizzoli et al., 1997; Van Wambeke et al., 2002), characterized by a very complex sea circulation with possible consequences on the spawning strategy and the recruitment success of many fish species (Cuttitta et al., 2003, 2015; Fortibuoni et al., 2010; Garofalo et al., 2010). AIS seems to advect the larvae towards a retention area at the South-Easternmost corner of Sicily (Capo Passero). In this area, there is a summer stable thermo-haline front (see Chapter 2). It allowed the larvae to maintain their relative position in an area with enhanced trophic conditions, probably providing more favorable conditions for larval fish feeding and growth (Mazzola et al., 2002; García Lafuente et al., 2005).

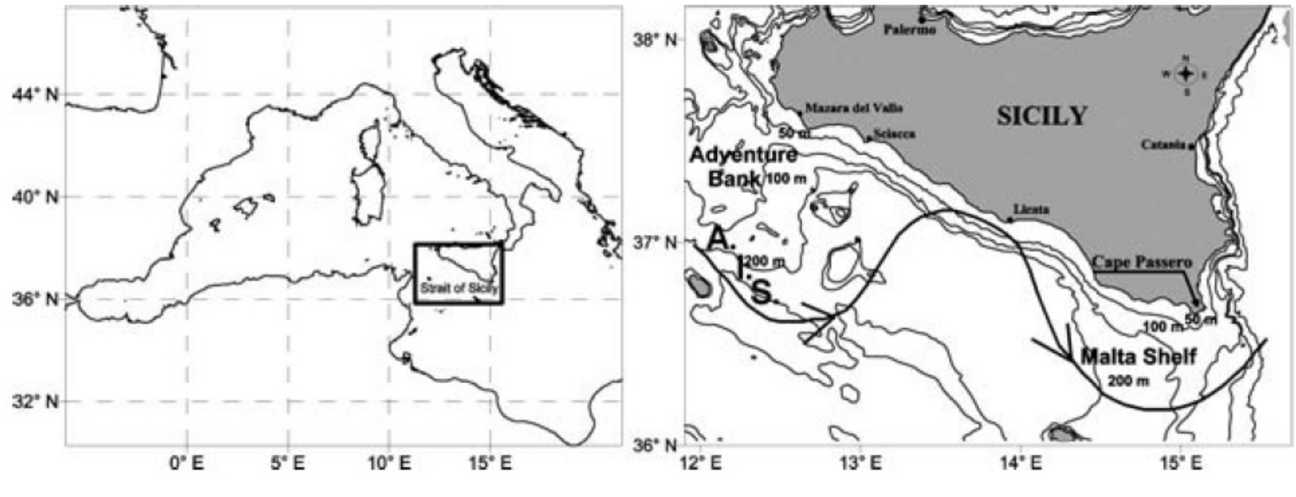

Figure 10- Study area and average path of the AIS (Cuttitta et al., 2004).

General characteristics of oceanographic cruise- Oceanographic data for Chapters 2 and 4 and ichthyoplankton samples for all the chapters were collected during seven oceanographic surveys, derived from the “Bansic” survey carried out by Italian National Research Council (CNR).

The samples occurred from June to August during seven selected years, from 2010 to 2016. The timing and the station number are in Table 1. The sampling was made on a station grid of 1/10°×1/10° along the continental shelf, and 1/5°×1/5° offshore. The stations are located along perpendicular transepts to the Sicilian coast (Fig. 11). Specifically, sampling was denser in the continental shelf area, linked to the oceanography of the Strait of Sicily. In fact, the presence of permanent or semi-permanent structures (gyre, upwelling, etc.) on one side, or some specific water masses, loaded with nutrients (Levantine Intermediate Water LIW) on the other, have modulated the station layout.

YEAR	SAMPLING PERIOD	STATIONS B.40	STATIONS B.90
TOT	26 June/8 August	1167	161
2010	26 June/13 July	185	0
2011	9-22 July	130	0
2012	5-21 July	146	38
2013	27 June/15 July	204	47
2014	23 July/8 August	162	37
2015	17 July/1 August	167	39
2016	1-11 July	173	0

Table 1 - Timing and stations number divided for net type.

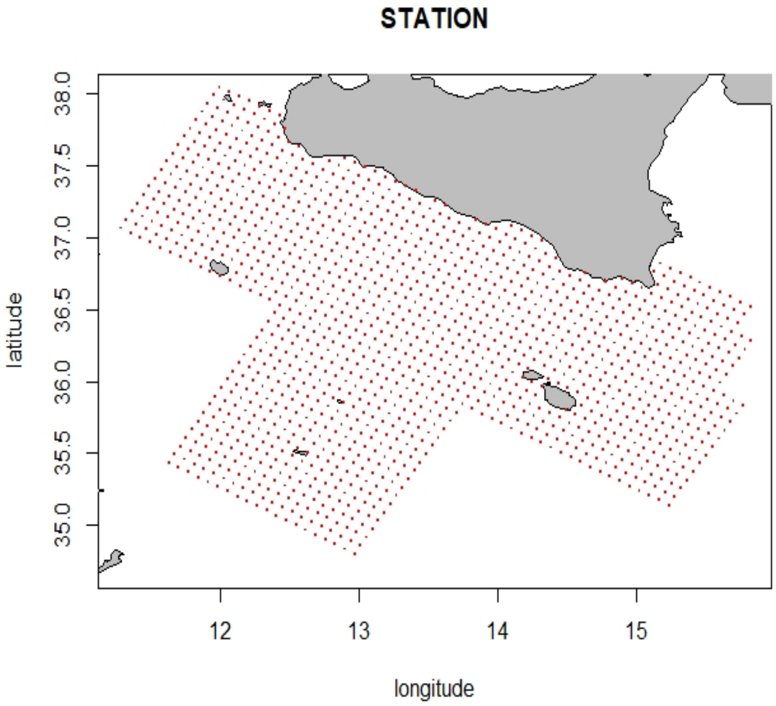

Figure 11- Stations grid and sampling period for each year, showed as day of the year.

In each station, ichthyoplankton samples were collected by a Bongo40 (B.40) net, and in some stations by a Bongo 90 (B.90) net. These are composed of two coupled nets with a mouth diameter of 40 cm with mesh size 200 µm and 90 cm with mesh size 800 µm, respectively. Depending on the study conducted, it was decided to exclude the samples of one of the two nets. The details are reported later in each chapter's specific materials and methods.

The plankton oblique tows were carried with a constant speed of 2 knots and a descending speed of 0.75 m/s, and an ascent to 0.33 m/s, to a depth of 100 m, wherever possible, because this layer characterizes the majority of the fish larvae in the study areas (Olivar et al., 2001, 2014; Sabatés et al., 2008). The larval collecting procedure was conducted day and night because, during the summer, pelagic fish larvae are more concentrated on the surface during the night and more dispersed on the water column during the day (Olivar et al., 2001; Sabatés et al., 2008). The filtered water volume of each mouth was measured by calibrated flow meters (type General Oceanics 2030). The samples were stored on board in ethanol. Fish larvae were sorted from the rest of the plankton and identified to the family taxonomic level on a land based laboratory. Taxonomic identification was based on Bertolini et al. (1956), Costa (1999), Moser & Ahlstrom (1996), and Tortonese (1970), with Rodríguez et al. (2017) being imperative to the specific study of Tuna. The number of fish larvae from each sample was standardized to n/m3 according to Perez Ruzafa et al. (2004). Once finished with the sorting phase, the dried zooplankton weight of the Bongo 40 net was also taken, which refers to the remaining zooplankton weight, another essential data point that gives information on the biotic conditions.

Continuous vertical temperature and salinity profiles were obtained from the surface to the bottom using a multiparameter SEABIRD mod in all the stations. 9/11 plus mounted on a General Oceanics rosette equipped with 24 Niskin Bottles. Before and after the cruise, the probe was calibrated. With the ship stationary, the CTD was lowered at each station. At a depth of 5-10 m, the probe was turned on, and the descent towards the bottom was 5m / s with constant speed. In the acquisition phase, the probe sampled with a frequency of 24 Hz and the raw data were recorded in binary format.

Laboratory works on Tuna- Each identified Tuna species has been photographed and catalogued to obtain morphometric data to study size class distribution and verify the development. Image acquisition was performed with the use of stereo-microscopes with an integrated camera. The management software and image processing © Image-Pro Plus (IPP) was used to acquire the morphometric parameters of the larvae from the photos. The details about the morphometric measure taken are discussed in Chapter 3.

1.9 This thesis

This thesis was carried out in collaboration with the University of Palermo (UniPa) and CNR. A period abroad was carried out at Instituto Español de Oceanografía-Centro Oceanográfico de Baleares (IEO-COB) for the drafting of chapter 2. It was first presented as a poster at the 43rd Annual Larval

Fish Conference, with the title “Characterization of the larval habitat of Tuna species in the central Mediterranean Sea”. This work received a special mention for the job done, graphic presentation, clarity of exposure and short and long-term objectives presented. It was then published in Frontiers in Marine Science. The 3 chapter is in submission with two manuscripts, while the 4 chapter is in the preliminary phase but will soon be sent to be published as well.

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Chapter 2. Unveiling the Relationship between Sea Surface Hydrographic Patterns and Tuna Larval Distribution in the Central Mediterranean Sea

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Unveiling the Relationship Between Sea Surface Hydrographic Patterns and Tuna Larval Distribution in the Central Mediterranean Sea

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Thunnus thynnus (Atlantic bluefin tuna, ABT) and other tuna species reproduce in the Mediterranean Sea during the summer period. Despite the Central Mediterranean Sea, the Strait of Sicily in particular, being a key spawning site for many tuna species, little is known on the effects of oceanographic variability on their larval distribution in this area. The abundance and presence-absence of larval specimens for three tuna species (ABT, bullet tuna and albacore) were modeled in order to examine their relationships with environmental factors, by analysing historical *in situ* information collected during seven annual surveys (2010–2016). The results revealed that most tuna larvae for the three species were found in the easternmost part of the study area, south of Capo Passero. This area is characterized by a stable saline front and warmer nutrient-poor water, and it has different environmental conditions, compared with the surrounding areas. The models used to investigate the presence-absence and abundance of the three species showed that ABT was the most abundant, followed by bullet tuna and albacore. The presence and abundance data collected are comparable with those of other spawning areas in the Mediterranean. Regarding biological and physical parameters, the results suggest that temperature, salinity, and day of the year are the key factors for understanding the ecological mechanisms and geographical distribution of these species in this area. Temperature affects the presence of ABT larvae and salinity, which, with a physical barrier effect, is a key factor for the presence-absence of bullet and albacore and for albacore abundance.

Keywords: ***Thunnus thynnus*, *Auxis rochei*, *Thunnus alalunga*, multivariate statistical analysis, spawning, ichthyoplankton, Mediterranean Sea**

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INTRODUCTION

Atlantic bluefin tuna (*Thunnus thynnus*, Linnaeus, 1758) is one of the most valuable tuna species in the world. Exploited by humans since ancient times, it has long been a valuable resource whose meat can now reach one of the highest prices in the international market (Mylonas et al., 2010; Sun et al., 2019). The International Commission for the Conservation of Atlantic Tunas (ICCAT) manages this species and considers two different stocks: the western stock whose main spawning ground is in the Gulf of Mexico (although according to new evidence spawning also occurs in the Slope Sea), and the eastern stock that spawns in the Mediterranean Sea (Muhling et al., 2017). Within the Mediterranean Sea, three main areas are well recognized, the western area located around the Balearic Islands; the central area in the Tyrrhenian Sea and around Sicily, northeast of the Gulf of Sirte, Malta, and Tunisia; and the Eastern area that comprises the Ionian Sea and the area around Cyprus (Karakulak and Yıldız, 2016; Muhling et al., 2017).

There is substantial discussion about the sub-population structure of Atlantic bluefin tuna (ABT) within the Mediterranean Sea and the occurrence of spatio-temporal mixing during spawning. The major spawning fraction is formed by large migrant adults that enter from the Atlantic Ocean following the Atlantic waters through the Strait of Gibraltar from April to May. Spawning occurs from June to July (Alemany et al., 2010; Reglero et al., 2012; Abascal et al., 2016). Some studies suggest that there is also a resident fraction of adults that remain in the Mediterranean Sea throughout the entire year or at least for a greater part of it (Cermeno et al., 2015; Livi et al., 2019). These individuals feed in different locations during the winter (Cermeno et al., 2015) depending on their ontogenetic stage (Sarà and Sarà, 2007). Although there is no conclusive scientific evidence, this resident fraction is believed to spawn in the Eastern Mediterranean and may also be in the Central Mediterranean (Cermeno et al., 2015). Migrants and residents may mix during reproductive events (Livi et al., 2019), and adult tagging data show that some individuals that remained in the Mediterranean Sea move to the Central Mediterranean during the spawning period where large migrant adults can also be found (Block et al., 2005; Fromentin, 2010). Studies to investigate the lesser-known spawning areas, such as the central Mediterranean Sea, are crucial to increase the understanding of the dynamics of the Mediterranean stock of this species. As such, questions that remain unanswered warrant attention.

The presence of larvae in the Western Mediterranean, one of the best studied spawning grounds, suggests ABT to be an environmental spawner tightly linked to the less saline Atlantic waters that enter through the Strait of Gibraltar and flow northward. There, they encounter the more saline resident water mass around the Balearic Islands, a high dynamic mesoscale area with a marked front (Alemany et al., 2010; Reglero et al., 2012; Alvarez-Berastegui et al., 2014). The Atlantic waters then enter the Strait of Sicily, flowing toward Tunisia and the South of Sicily, from west to east (García Lafuente et al., 2002; Cuttitta et al., 2004; Bonanno et al., 2014). A consecutive sampling of tuna larvae over a 3-year period has shown that the presence of tuna larvae in Tunisia is also related to this water mass (Koched et al., 2012, 2013, 2016; Zarrad et al., 2013). In contrast, little is known about the south of Sicily, an area characterized by high mesoscale variability, vortices, and meanders (García Lafuente et al., 2002) because of incoming Atlantic freshwater and warmer resident water (Robinson et al., 2001). These environmental data have not yet been analyzed with tuna larval data from oceanographic surveys in the study area.

Another common characteristic in the Balearic Islands and Tunisia is that ABT larvae are commonly found together with two other tuna species, bullet tuna (*Auxis rochei*, Risso, 1810) and albacore (*Thunnus alalunga*, Bonnaterre, 1788), suggesting that the three species have overlapping spawning grounds (Alemany et al., 2010; Koched et al., 2013). Evidence from gonad sampling suggests that the timing of spawning occurs around the same time in the Western and in the Central Mediterranean, between June and July (Heinisch et al., 2008), a pattern that is corroborated by temporal changes in the abundance of larvae (Alemany et al., 2010).

Considering these basic knowledge gaps and the apparent need for in-depth analysis, this study aims to better analyze the lesser-known central Mediterranean Sea spawning area. The objectives are to better understand the distribution and abundance of the three species and relate them to the biotic and abiotic factors of the area, thus verifying their main ecological drivers. This can enhance the knowledge of the Central Mediterranean, expanding the ecological knowledge of these species within the basin. We explored the environmental drivers affecting ABT, bullet and albacore tuna at the larval stage by analyzing presence-absence and abundance data, focusing on the early life stage.

METHODS

Field Sampling

The samples are from ichthyoplankton surveys carried out in the Strait of Sicily by the Italian National Research Council (CNR) during the summertime (June–August) from 2010 to 2016. A regular sampling grid was used (1/10° × 1/10° along the continental shelf, and 1/5° × 1/5° offshore). The number of sampled sites investigated in each year, as well as the sampling period, is summarized in **Table 1**. The sites are located along transects perpendicular to the southern Sicilian coast (**Figure 1**).

Ichthyoplankton samples were collected 24 h a day, with oblique tows of a Bongo 40 net with 200 μ mesh size. It was equipped with a flow meter and towed at a ship speed of 2 knots, with a descent speed of 0.75 m/s and an ascent speed of 0.33 m/s. The net was lowered from the seabed to the surface, but sampling depths did not exceed 100 m.

The collected samples were immediately stored onboard in 70% ethanol and later processed in the land-based laboratory for identification of tuna larvae at the highest possible taxonomic level according to Rodríguez et al. (2017). Following larvae sorting, the remaining zooplankton was collected to obtain dried zooplankton weight. Water column temperature

TABLE 1 | Information on sampling strategy and statistics for the three tuna species.

Year	Sampling period	N. Sites	Species	Mean larva (m^3)	SD	Freq. of occurrence
TOT	26 June–8 August	1167	ABT	0.002	0.03	0.05
			Bullet	0.001	0.009	0.05
			Albacore	0.0004	0.003	0.02
2010	26 June–13 July	185	ABT	0.006	0.06	0.038
			Bullet	0.001	0.01	0.043
			Albacore	0	0	0
2011	9–22 July	130	ABT	0.001	0.004	0.031
			Bullet	0.004	0.021	0.1
			Albacore	0.0005	0.003	0.023
2012	5–21 July	146	ABT	0.003	0.01	0.11
			Bullet	0.003	0.01	0.11
			Albacore	0.001	0.01	0.05
2013	27 June–15 July	204	ABT	0.0009	0.005	0.039
			Bullet	0.0008	0.004	0.044
			Albacore	0.0001	0.001	0.005
2014	23 July–8 August	162	ABT	0.001	0.003	0.031
			Bullet	0.001	0.006	0.068
			Albacore	0.0004	0.003	0.031
2015	17 July–1 August	154	ABT	7.9E–05	0.001	0.006
			Bullet	1.1E–03	0.007	0.032
			Albacore	6.9E–04	0.004	0.032
2016	1–11 July	173	ABT	0.005	0.017	0.121
			Bullet	9E–05	0.001	0.006
			Albacore	0	0	0

(°C), and salinity (PSU) measurements were gathered at all sites using the multi-parameter CTD SBE 11 plus probe (underwater unit).

Data Exploration and Statistical Modeling

Mean larval densities at stations with different bottom depths were explored to identify potential spatial preferences regarding bathymetry. The distribution of observed larval presence and absence was explored using density plots. Generalized additive models (GAMs) allowed us to investigate the relationships between the spatial distribution of tuna larval specimens and environmental factors. Presence–absence data, from all the stations, and abundance data, only from positive stations, were used as dependent variables in the models. This helped determine the main covariates that control larval occurrence (calculated as the probability of presence) and the density of tuna larvae per unit area. The binomial distribution family was selected for presence–absence data and the Gamma family for abundance. To transform the catch effort for abundance, we standardized the number of larvae with the following formula:

$$CPUA = (100 * larvae/m^3) * BD$$

where CPUA is the "capture per unit area", larvae/m^3 is the number of larvae divided by the filtered water volume in m^3, and BD is the depth in meters reached by the bongo net during sampling (Alvarez-Berastegui et al., 2018). Considering the volume of filtered water and the maximum depth of the sampling gear, we were able to obtain an unbiased and normalized expression of larval density per m^2.

The first model building phase was dedicated to selecting variables that best describe the system and that were potentially useful for understanding the tuna larvae spatio-temporal distribution. This step also considered information from similar studies performed on other fish species. We started by including all the available and suitable data for the analysis, such as geographic coordinates and dissolved oxygen, but excluding stratification of the surface water, which is a typical condition characterizing the summer season in the southeastern sector of the study area, where most of the tuna larvae were found; and terrestrial runoff, because of limited drainage basin in an island such as Sicily. Finally, tidal excursion was excluded as this is very limited in the waters of the study area.

By Pearson' s correlation, the collinearity among the variables was analyzed. In case of a significant correlation between two variables, we selected the one that fitted best with the dependent variables and excluded the other. For example, longitude was positively and significantly correlated to temperature anomaly, mostly because of the upwelling in the northwestern zone (**Supplementary Material**), which, in some years, was stronger and produced lowest temperature anomaly values.

The last covariates considered in the models were: "Year" as a factor and with random effect, in order to focus the analysis on environmental relationships and not on interannual fluctuations;

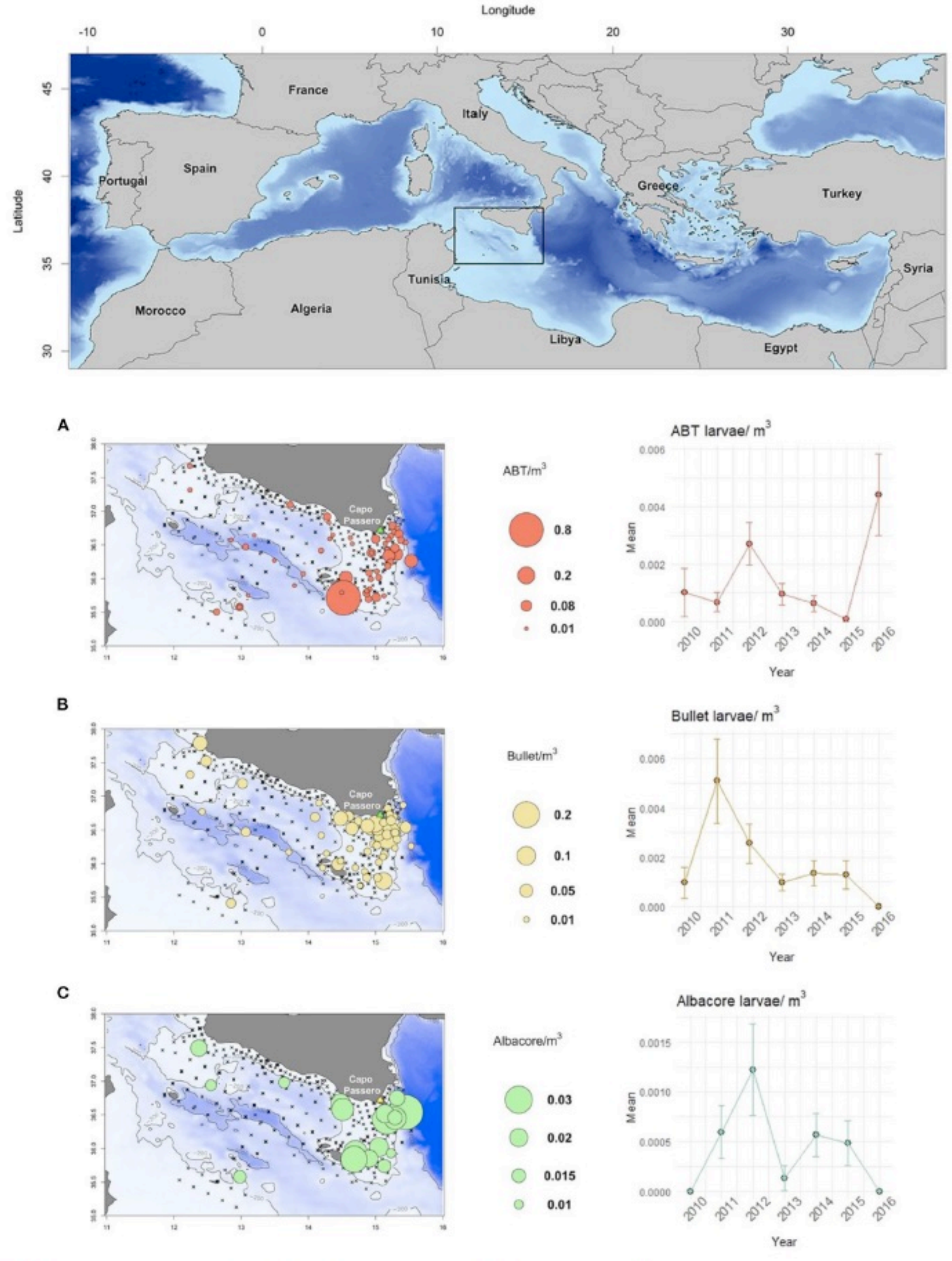

FIGURE 1 | Study area and geographical distribution of **(A)** Atlantic bluefin tuna (ABT), **(B)** bullet tuna, and **(C)** albacore tuna, and interannual changes in nominal larval densities in sites repeated annually.

surface "Salinity" (PSU) as recorded by the CTD probe at 5 m; "Day of the year," to verify the presence-absence and abundance peak, due to the temporal choice for spawning as dictated by the adults; "Zooplankton density" (standardized for the volume of filtered water, dry weight mg/Vol), to understand the role of possible food availability; "Residual of superficial temperature" (°C), calculated as the residual of a GAM, where temperature (at 5m) was fitted to the factor "Day of the year", in order to avoid the correlation between these parameters and allow for the different timing of annual surveys.

The final formulas used for the (i) presence-absence model and (ii) larval abundance model were as follows:

(i) $Probability\ of\ presence \sim Year + Sal. + Yearday + Zoo.\ Den. + Temp.\ residuals + Depth$

(ii) $CPUA \sim Year + Sal. + Yearday + Zoo.\ Den. + Temp.\ residuals + Depth$

A step-wise procedure allowed the progressive removal of non-significant covariates ($p > 0.05$), ensuring that only independent variables were used and non-significant variables were excluded. This had the effect of the most significant variables being easier to understand, removing interferences in the additive model, dictated by less significant variables.

All simulations, statistical analyses, and plotting were performed using the R statistical software (RStudio Inc, 2016). In particular, GAMs were fitted using the mgcv library (Wood, 2011).

RESULTS

Spatio-Temporal Distribution

While the three species under investigation [Atlantic bluefin tuna (ABT), bullet tuna and albacore] are commonly found in the samples (**Table 1**), other tuna and tuna-like species are sporadically found, such as *Sarda sarda*, *Euthynnus alletteratus*, and *Katsuwonus pelamis*. ABT larvae were constantly more abundant in the eastern side of the study area (between 14.5 and 15.5°E longitude) and occurred every year between Capo Passero (the southernmost tip of Sicily) and Malta Island (**Figure 1**). Similar annual abundance was found, but in 2016 there was higher average abundance (**Figure 1A**). Bullet tuna larvae had a geographical distribution similar to that of ABT annually, although they were more numerous closer to the coast and more prevalent east of longitude 15° E (**Figure 1B**). Regarding larval densities, low larval densities were found in the western part of the Strait, while higher densities were found along the eastern end of the Sicilian coast and Malta (**Figure 1B**).

Lastly, albacore, the tuna species found to have the lowest presence probabilities and abundance at positive stations, was found between 2011 and 2015 (**Figure 1C**) and in well-defined areas at the times of samplings (the shelf area between Capo Passero and Malta), with rare presence between longitudes 12 and 14° E (**Figure 1C**).

Hydrographic Conditions

Temperatures were generally warmer in the eastern zone for all the years sampled and 2010, 2013, and 2016 were the years with the coldest temperatures (**Supplementary Material**). The hottest temperature anomalies were detected in 2012, 2015, and 2016 (**Figure 2**). More marked upwellings than usual in the central-western sector were recorded in 2011, 2013, and 2014.

Salinity data showed saltier water masses in the eastern zone for all the years (**Figure 2**). A thermohaline front was found in the same area, every year, beyond 15° E of longitude.

The Zooplankton data showed oligotrophic waters every year, except for a few points with higher biomass in 2010 and general greater abundance in the western sector (**Figure 2**).

Larval Data and Environmental Factors

The mean larval density related to the bottom depth showed that the three species were distributed in areas with different bathymetric profiles (**Figure 3**). ABT was the species distributed more offshore, in areas where the bottom depth is deeper than 100 m. Bullet tuna distribution was more coastal, showing higher probabilities of presence in shallower stations (0–100 m), although individuals were found in the entire bathymetric range. Albacore was present evenly in bathymetric range deeper than 50 m. Regarding temperature, ABT larvae were present at temperatures higher than 21°C. Similarly, bullet tuna was found in waters warmer than 20.9°C, and albacore was found in waters warmer than 20°C (**Figures 4A–C**). With reference to salinity, the presence of the three species showed two peaks at around 37.5 and 38.5 (**Figures 4D–F**). The GAM analysis explored the relationship between larval distribution and putative covariate factors that may drive the presence and abundance of the three species (**Table 2**).

The presence of Atlantic bluefin tuna (ABT) was positively related to "Day of the year," "Temperature residuals," and "Year" ($p < 0.001$ for all the three variables) (**Table 2**, **Figures 5A1,A2**). The presence of ABT increased during the spawning season, reaching a maximum around day 200 (July 19th). The larvae were found in warm waters, and the probability of their presence was higher when temperatures exceeded the average temperature trends. The presence of bullet tuna was positively related to "Day of the year" ($p < 0.001$) with higher probability of finding larvae as the season advances. Regarding "Salinity" ($p < 0.05$), bullet tuna showed a flat trend of up to 38 PSU and increased from that point onward (**Figures 5B1,B2**). As seen for bullet tuna, the presence of albacore increased with the "Day of the year" ($p = 0.01$) and with "Salinity" ($p < 0.001$) (**Figures 5C1,C2**). Regarding the model used to analyze the abundance in positive stations, ABT was found to be negatively related to "Day of the year" ($p < 0.01$) (**Figure 6A**). For the bullet model, even with the stepwise procedure, none of the explanatory variables were significant. The albacore model for abundance in the positive stations showed an increasing linear trend with "Depth," a dome-shaped relation with "Salinity" with a maximum at intermediate levels; and with "Zooplankton abundance," the trend decreased until intermediate values remained constant at higher zooplankton abundances ($p < 0.05$) (**Figures 6B1–B3**). For this species, it should be considered that in the eastern sector where the larvae are more abundant, in the 2 years with greatest zooplankton abundance (2010 and 2016), we did not find

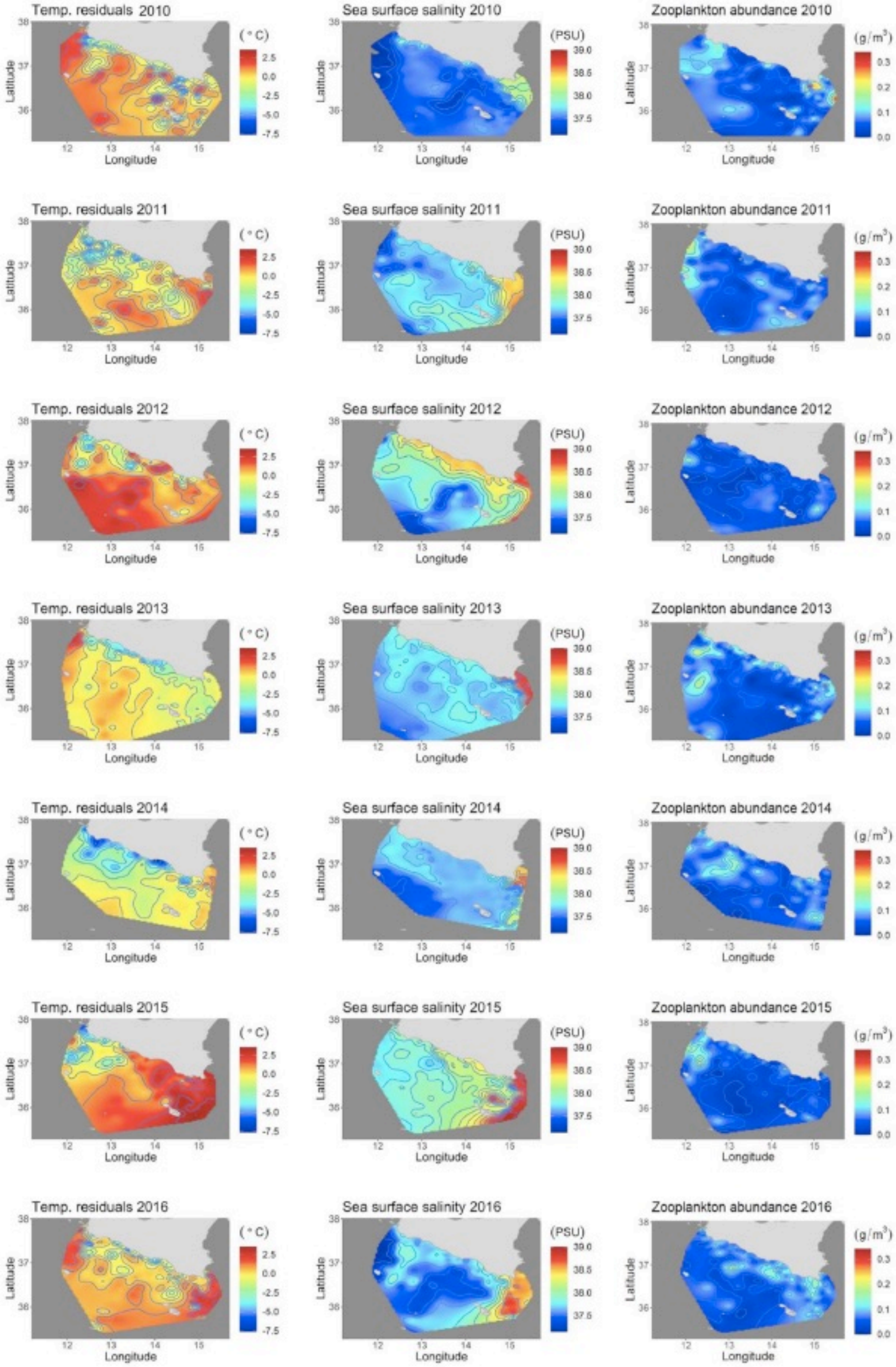

FIGURE 2 | Generalized additive model (GAM) covariates considered in the models. Temperature anomalies in the first column, salinity in the second column, and zooplankton abundance (g/m^3) in the third column.

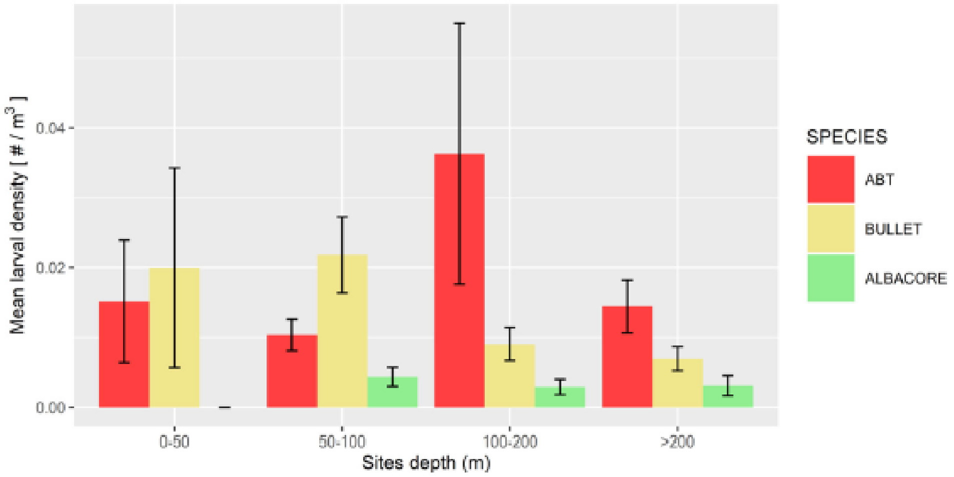

FIGURE 3 | Mean larval density (larvae/m^3) related to site depth, for the three species.

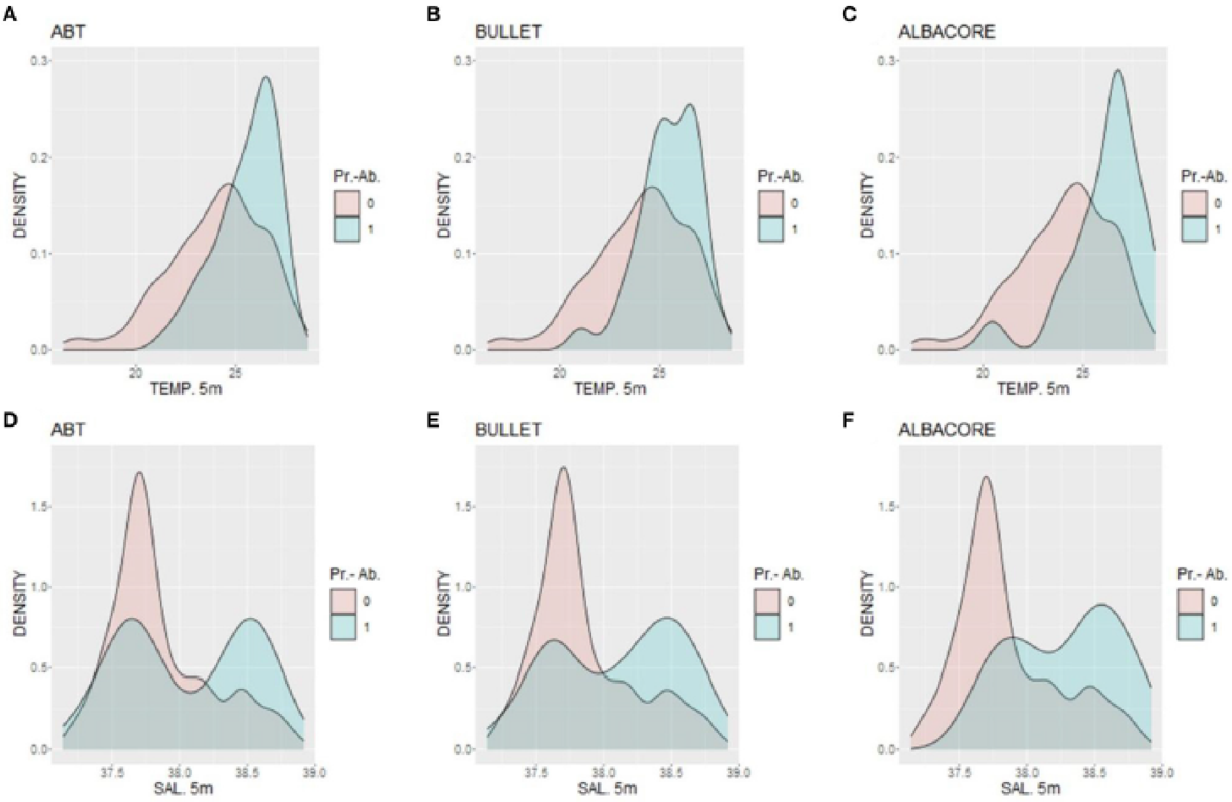

FIGURE 4 | Density of presence-absence data for the three species related to **(A–C)** temperature and **(D–F)** salinity, where 0 is absence and 1 is presence.

albacore larvae. It is also necessary to consider that there are very few events of large zooplankton abundance and that from the low to medium abundance that has been detected, the effect on albacore abundance decreases (**Figure 6B2**).

DISCUSSION

The results indicate that the central Mediterranean Sea, in particular the Strait of Sicily, is a significant larval habitat for

TABLE 2 | GAM output analysis with significance value (0 ‘***’; 0.001 ‘**’; 0.01 ‘*’; 0.05 ‘.’; > 0.05 ‘–’).

Presence absence	Res temp.	Sal.	Zoo.	Year	Depth	Year Day	Deviance explained
ABT	***	–	–	***	–	***	19.3%
BULLET	–	*	–	***	–	***	15.7%
ALBACORE	–	***	–	–	–	*	13.9%
Larval abundance	**Res temp.**	**Sal.**	**Zoo.**	**Year**	**Depth**	**Year Day**	**Deviance explained**
ABT	–	–	–	–	–	**	14%
BULLET	–	–	–	–	–	–	–
ALBACORE	–	*	*	–	*	–	59.7%

the three tuna species considered in this study. ABT and bullet were generally more abundant than albacore, the latter having not been caught in two of the years during the sampling time series. Therefore, there is a temporal-spatial overlap during the early life stages of these species. Temperature, salinity, and day of the year emerged as key variables to better understand the spatio-temporal distribution of larval habitats of these species in the area.

Tuna larvae were found in the eastern side of the Strait, where surface water temperatures were found to be consistently warmer than those of the surrounding areas. The GAM results on the presence-absence of ABT larvae show that the day of the year is highly significant, with a dome-shaped relationship reaching a maximum at around day 200, corresponding to the beginning of the second half of July. Tuna species are found in waters above 22°C, and the timings are consistent with studies carried out in the Western Mediterranean (e.g., Alemany et al., 2010; Reglero et al., 2012) and south of the Central Mediterranean (e.g., Koched et al., 2013; Zarrad et al., 2013). The results reinforce the idea that tuna eggs and larvae are mainly found in oceanic waters and are constrained by temperatures above 20°C, at which they can develop and survive (Reglero et al., 2014). In the study area, we also found some larvae in the August period, suggesting that some adults may have spawned outside the common spawning period, when temperatures are still suitable for egg-hatching.

There was a higher probability of finding larvae at stations where the temperature was higher than the average temperature for that day. Adults can choose where to spawn and may choose to spawn when and where temperatures are favorable for the development of their larvae. ABT have very high fecundity and are multiple batch spawners (Ciannelli et al., 2015). While they are in the spawning grounds, they have a very fast ovarian development and oocyte maturation that is propelled by the presence of increasing warming water, which poses an evolutionary constraint on the spawning locations (Ciannelli et al., 2015). The results suggest that the moment in which adults choose to spawn increases up to mid-July and then decreases. Interestingly, in the study area, we also found some larvae during August. This indicates that some adults have spawned outside the common peak period. The reproductive success of these events that occur later in the year is not known. These smaller ABT that spawned later in the year might be found by larger conspecific larvae that spawned during previous spawning events. If these larger larvae have reached the piscivorous phase, they might feed on their conspecifics and carry out cannibalism (Reglero et al., 2011). As Bongo-40 nets that predominantly catch small larvae were used, statistical analyses on the difference in size distribution across the seasons and/or stations were not possible.

Regarding salinity, the minimum, maximum, and mean values recorded in the stations are typical of the study area, characterized by the confluence of Atlantic waters with Eastern Mediterranean waters in the Ionian Sea. In fact, we have detected a strong variation in salinity values, corresponding to a key oceanographic structure, the saline front in the south of Capo Passero, to the east of the study area. This structure appears to be stable during the summer period, confirming previous observations (Patti et al., 2010; Bonanno et al., 2014; Cuttitta et al., 2016). Across the front the larval occurrence is higher, and this is evident for the three species that show two presence peaks related to salinity. Larvae originating from the spawning activity in the southeastern sector of the study area accumulate with larvae advected by the Atlantic waters from the offshore western area, causing the higher concentrations observed in the retention area for the three species investigated. In the same area, this advection mechanism has also been verified for other species in previous studies (García Lafuente et al., 2002; Cuttitta et al., 2018; Patti et al., 2018). So, definitively the dynamics of this frontal area seems to play a key role for larval retention in the eastern zone. This hypothesis has been confirmed by the GAM models, as bullet and albacore tuna showed a higher probability of presence at higher salinity. This is not dictated by physiological constraints but by a purely physical factor. The different densities of the two water masses where we find higher larvae presence and the presence of the saline front every year suggest that this oceanographic structure works as a physical barrier, concentrating the tuna larvae. Geostrophy and associated surface currents play a key role in determining the fate of the early life stage, and Capo Passero assumes an important function as a retention area for eggs and larvae, not only for tuna but also for other species that gather from the northwest, resulting from along-shore advection (Torri et al., 2018; Patti et al., 2020).

Atlantic bluefin tuna (ABT) larvae were absent in the western sector. This area is characterized by coastal upwelling zones due to mistral winds that induce offshore Ekman transport of surface

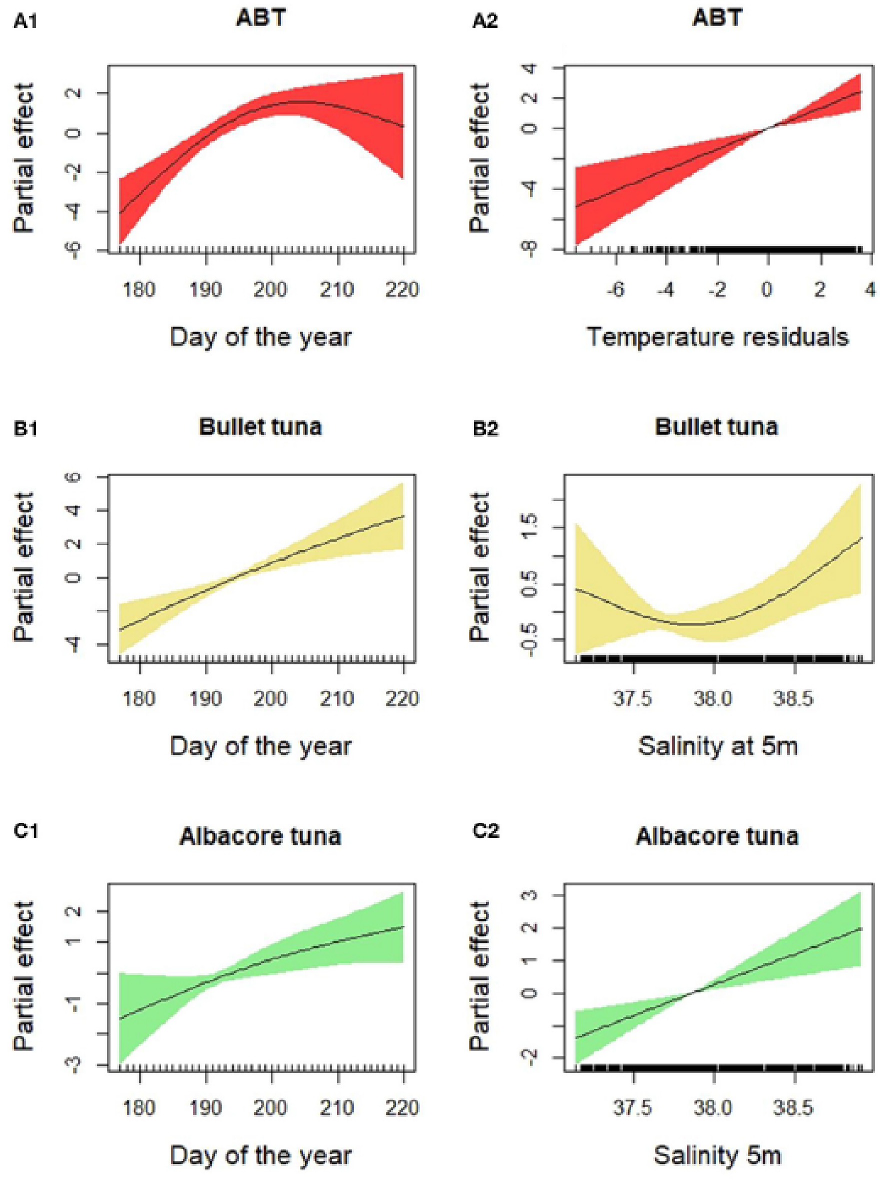

FIGURE 5 | Results of the generalized additive presence-absence models for **(A1,A2)** bluefin tuna, **(B1,B2)** bullet tuna, and **(C1,C2)** albacore tuna.

waters, causing the upwelling of cold and nutrient-rich water masses (Patti et al., 2010; Torri et al., 2018). In general, tuna larvae are found in oligotrophic areas, probably as a strategy to encounter fewer predators (Bakun, 2013). Despite the eastern area being less productive than the western area, local enrichment processes such as cold filaments from the upwelling zone together with the thermohaline front and eddies could concentrate prey, improving feeding opportunities (Cuttitta et al., 2016, 2018; Torri et al., 2018). These oceanographic processes could create patches of higher food abundance that, together with scarcity of predators, could increase the chances of larval survival.

It was not possible to depict the reproductive peak of bullet and albacore tuna in this study, since the data suggest that the spawning window of bullet is longer than the timing of the surveys (Allaya et al., 2013). This is demonstrated by the "Day of the year" results. The bullet tuna reproductive strategy is based on a protracted spawning behavior, compared with ABT. Concerning bullet tuna abundance, the lack of significant variables in the applied model highlights how the larvae of this species are heterogeneously distributed within the study area (**Figure 1B**). We chose to implement a unified model approach for the three species, both for presence-absence and

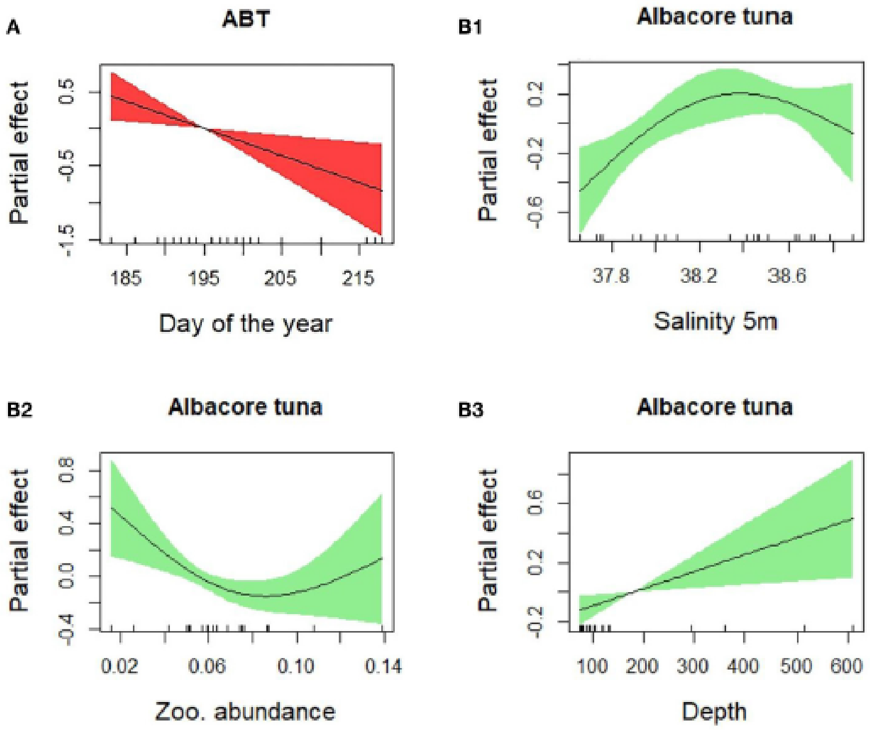

FIGURE 6 | Results of the generalized additive abundance models for **(A)** bluefin tuna and **(B1, B2, B3)** albacore tuna.

abundance, but clearly, each species has its own peculiarities, and the results suggest the need for further species-specific studies. Moreover, because of the complex nature of the ecological data, low values of explained deviance are common, also considering that other environmental variables, not available for this study, could potentially be useful to improve the knowledge of larval distribution.

CONCLUSION

The study area located in the center of the Mediterranean Sea, with direct influence of Atlantic and Levantine waters, creates unique environmental conditions selected as optimal spawning grounds by different tuna species as has been observed for other species (Falcini et al., 2020; Mangano et al., 2020; Patti et al., 2020). In this study, similarities with other well-characterized spawning grounds in the Central and Western Mediterranean were highlighted, where the three selected tuna species also coexist (Alemany et al., 2010; Koched et al., 2013). This study targets the lesser-known central Mediterranean Sea spawning area, revealing that temperature, salinity, and day of the year were the main variables driving tuna larval geographical distribution. However, the lack of a long time series in the region and the different sampling methods in the area compared with the other areas made the detection of significant temporal trends in the observed patterns difficult. As such, this study has given some interesting insights and paves the way for future studies on the impact of environmental variability on the interannual fluctuations in the abundance of these species and in their reproductive success in the Central Mediterranean Sea.

More research in the Central Mediterranean tuna spawning grounds is needed to understand its role compared with other areas in the Mediterranean Sea. It is also possible that there is a spatio-temporal mixing in spawning grounds between the two ABT Mediterranean sub-populations. The crucial issue of the ABT Mediterranean stock structure remains inconclusive. The results could indicate a reproductive event or multiple reproductive events, resulting from Atlantic or resident ABT, or both. We still need to know more about this area, as we still do not know the larval origin and if the larvae come from the resident adult fraction or not. By studying these lesser-known spawning areas, we wanted to take the first step, laying the foundations, to fill critical gaps in the understanding of the unique dynamics of the Mediterranean stock for these species that, to date, have remained unanswered.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SR, AC, and GS contributed to the conception and design of the study. MT, BP, and AC carried out the sampling. SR, AC, and PR taxonomic identification. SR, MT, and BP organized the database. SR, MT, DAB, and PR performed the statistical analysis. SR wrote the first draft of the manuscript. GS and AC secured funding. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.708775/full#supplementary-material

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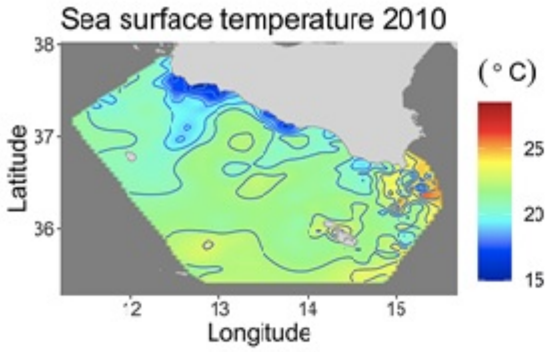
Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2.1 Supplementary material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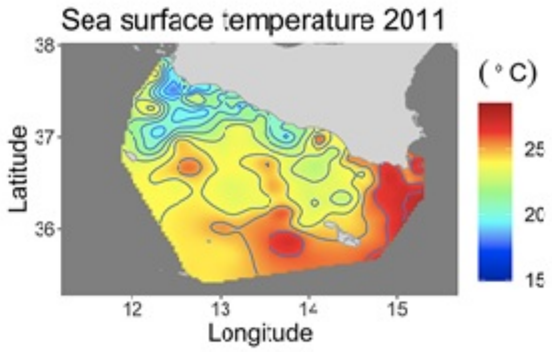
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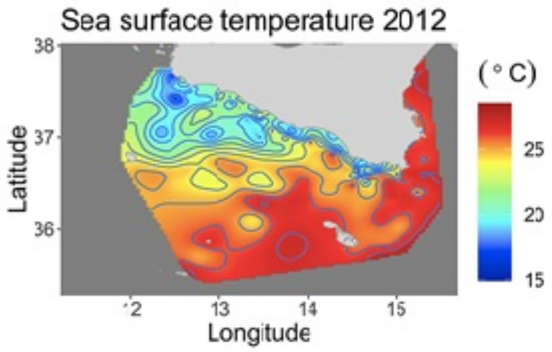
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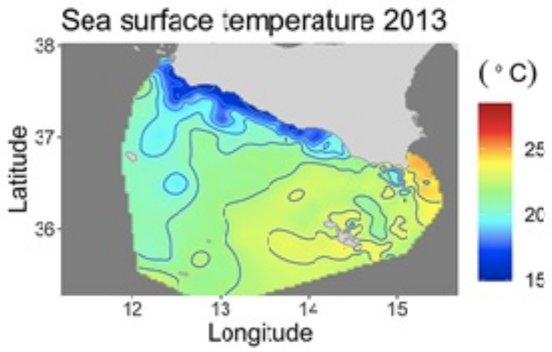
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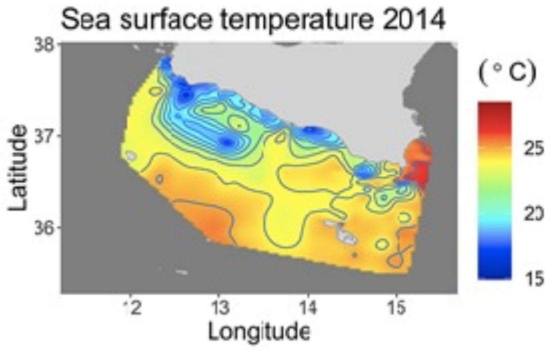
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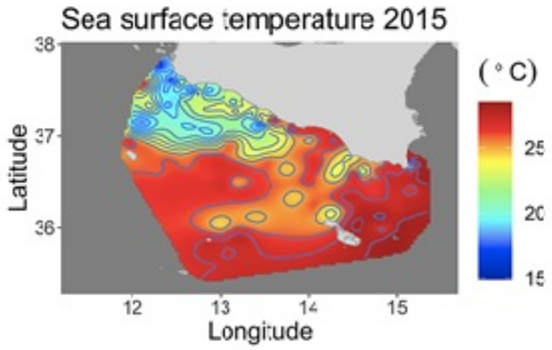
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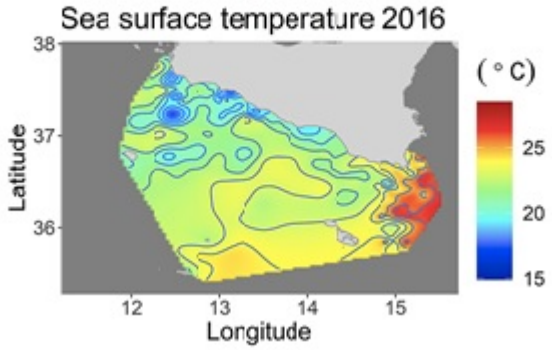
Sea surface temperature 2016

Chapter 3. Can different pathways change the fate of offspring? A back-calculation of Tuna larvae routes in a Mediterranean spawning ground.

Paper extracted from the following chapter

Part of the following chapter has been implemented and published. Below are the manuscript and supplementary materials, followed by the entire chapter.

water

MDPI

Article

Environmental Conditions along Tuna Larval Dispersion: Insights on the Spawning Habitat and Impact on Their Development Stages

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Abstract: Estimated larval backward trajectories of three Tuna species, namely, Atlantic Bluefin Tuna (*Thunnus thynnus*, Linnaeus, 1758), Bullet Tuna (*Auxis Rochei*, Risso, 1801) and Albacore Tuna (*Thunnus alalunga*, Bonnaterre, 1788) in the central Mediterranean Sea, were used to characterize their spawning habitats, and to assess the impact of changes due to the major environmental parameters (i.e., sea surface temperature and chlorophyll-a concentration) on larval development during their advection by surface currents. We assumed that the environmental variability experienced by larvae along their paths may have influenced their development, also affecting their survival. Our results showed that the Tuna larvae underwent an accelerated growth in favorable environmental conditions, impacting on the notochord development. In addition, further updated information on spawning and larval retention habitats of Atlantic Bluefin Tuna, Bullet and Albacore Tunas in the central Mediterranean Sea were delivered.

Keywords: *Thunnus thynnus*; *Auxis rochei*; *Thunnus alalunga*; ichthyoplankton; Mediterranean Sea; backward trajectories; Lagrangian simulations; spawning habitat; larval habitat; tuna

1. Introduction

The biotic and abiotic conditions of the water column, particularly the surface layers, can strongly influence the distribution and the abundance of the fish larval stages, thereby affecting the reproductive success of many fish species [1].

After the spawning events, the fate of eggs and larvae is uncertain. The high mortality rates of these crucial early life phases have a strong impact on recruitment success [2–4]. Marine currents carry and disperse pelagic eggs and larvae through different habitats [5], and consequently affect their fate, which is also driven by variability of environmental forcings and biotic interactions, manifesting deterministic chaos [6]. For instance, predation rather than physical, chemical and trophic properties of water masses are some examples [7]. Any environmental forcing and interactions eggs and larvae experiment along their path can affect the final balance of reproductive success of each individual spawning event. In addition, the influence of environmental factors may vary according to the ontogenetic stage [8–10]. Thus, the pelagic environment is so variable and maximization of the reproductive success is so unpredictable that to increase our understanding of how the resulting stochastic variability can influence the fate of pelagic fishes, such as anchovies, mackerel and tunas, is crucial. This large unpredictability is expected to affect vastly vagrant species, such as adult Tunas, that migrate from feeding habitat to spawning grounds at long distances [11]. Indeed, while adults can select the locations to maximize the reproductive event's success, stochastic events are more effective in addressing the recruitment-related processes. In this, to limit the effects of unpredictability, natal homing behavior is beneficial and is considered a strategy used by many organisms, including Tunas [12,13], to increase the likelihood of reproductive success and maximize the larval survival. Tunas follow specific signals to commence reproductive events together, but this depends on species [14–17]. The spawning behavior of Bluefin Tuna, in particular, can respond to environmental or physical signs, or a mix of both [18]. In any case, the typical strategy is to ensure the survival of as many larvae as possible.

Despite the extent of larval life being relatively short compared with the organism's lifetime, the fate of early life stages is decisive for the future adult stock [19–21]. In fact, larval abundances are currently used as a proxy for assessing spawning stock biomass [22]. During this phase, faster-growing individuals are favored over slower-growing individuals, the latter being exposed for a longer time to a vulnerable status, characterized by higher mortality rates induced by predation and harder feeding conditions [2]. This time interval is also very short for Tuna species compared with other marine fish larvae [19].

During the early life phase, fish larvae can make only small individual, mainly vertical, movements, but they undergo passive travel because they cannot decisively oppose the currents [19,23,24]. Even if limited in extension, these horizontal movements are essential because larvae can be removed from nursery areas and advected towards more, or not more favorable, retention areas [6]. The flexion stage of the notochord is a milestone because larval swimming and feeding abilities improve significantly [8–10]. They become able to hunt better, escape predators more effectively, and make their first active movements by escaping from passive current transport.

Tunas are top predators and targets of fisheries globally. They have an important ecological and economic role, influencing the structure and function of marine communities [25]. Often larval fish habitats are associated with specific oceanic regions with circulation systems generated by particular topographical features [26], and during the early stages, different tuna species overlap in these habitats globally [27]. In the Mediterranean Sea, Tunas' larval habitats seem to be linked to specific temperature conditions, hydrographic characteristics, oceanographic mesoscale structures such as gyres and fronts, and oligotrophic waters that ensure the larvae encounter fewer predators [28–30].

Three Tuna fish species reproducing in the Mediterranean Sea also overlap their spatial ranges during the vulnerable reproductive phase, i.e., Atlantic Bluefin Tuna (ABT) (*Thunnus thynnus*, Linnaeus, 1758), Bullet Tuna (*Auxis rochei*, Risso, 1810) and Albacore Tuna (*Thunnus alalunga*, Bonnaterre, 1788). Their life strategies are complex and different, but they have certain key traits in common, including spawning areas and the larval habitats. Fluctuations in their stock biomass also depend on the planktonic larval ecology, influencing mortality rates and reproductive success. The geographical distribution of Tuna species is closely linked to oceanographic conditions, which also influence their spawning behavior [18]. However, how environmental conditions affect larval survival and development during larval dispersal is still an open question.

Some authors showed that the Strait of Sicily is a spawning area for Tuna species [30–33], whose hydrodynamic complexity, but limited spatial extent, can represent a useful natural laboratory to understand the impact of the oceanographic processes and conditions during the offspring dispersal phase on larval development and survival. Actually, the local surface circulation is dominated by the flow of the Modified Atlantic Waters (also known as Atlantic Ionian Stream (AIS) [34]).

Profiting on the planktonic nature of Tuna early life stages, this study analyzed the backward trajectories of larval specimens of the three Tuna species that overlap spatially during the summer season, sharing their spawning grounds and larval habitats [28,35].

In order to investigate the fate of the larval stages and their relation with environmental conditions, we used a Lagrangian simulation approach, which is able to estimate the larval path [36] and also to identify, by backward calculation, the spawning areas. This technique has already been successfully applied to study larval stages and their relationships with environmental conditions [37,38].

Often larval studies only rely on the environmental conditions observed in the geographical sites where larvae were collected. Instead, in this study it was possible to assess the conditions experienced by sampled larval specimens, from their sampling sites backward to their presumed hatching location (as estimated by Lagrangian simulations).

Here, we verify the hypothesis that the environmental conditions experienced by fish larvae along the phase of passive transport can affect their developmental rates. In addition, the observed changes in environmental factors were related to the notochord development state.

2. Materials and Methods

2.1. Field Sampling

Ichthyoplankton samples were derived from oceanographic surveys carried out in the Strait of Sicily during the summer period (June to August) from 2010 to 2016 (Figure 1).

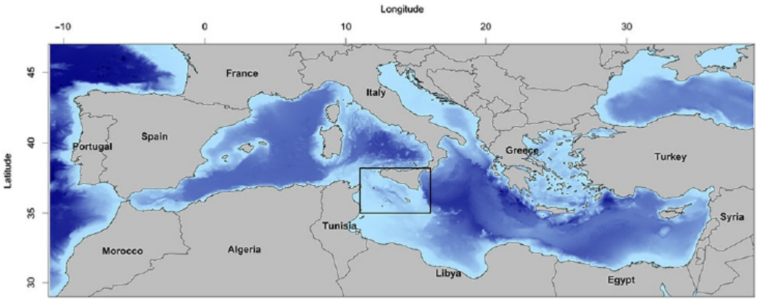


Figure 1. Study area.

The sampling was based on a regular grid of stations (1/10° × 1/10° in the continental shelf area, 1/5° × 1/5° in the slope area) distributed along parallel transects perpendicular to the southern coast of Sicily.

Fish larvae were collected by oblique tows of a Bongo 40 plankton net and oblique or horizontal tows of a Bongo 90 plankton net, carried out at a ship speed of 2 knots. The sampling nets were towed from the surface to 100 m depth (Bongo 40) and in the surface mixed layer (Bongo 90).

2.2. Laboratory and Image Processing

After sampling, samples were immediately stored on-board in 70% ethanol, and subsequently processed in the land-based laboratory to identify Tuna larvae at the highest possible taxonomic level, according to Rodríguez et al. [4]. Images of each larva were acquired using stereomicroscopes with integrated cameras. The dedicated Image Pro Plus © (IPP) image management and processing software was used to obtain some morphometric parameters from the photos. The considered measurements derived from Catalán et al. [39].

2.3. Age Estimation

To assess the age (in days) of each individual Tuna larval specimen, we used the empirical relationships estimated in previous studies in the Mediterranean Sea on the daily increment of otolith rings related to standard length (SL) [40,41]. In particular, for Bullet Tuna, we used the relationship estimated by Laiz-Carrión et al. [41] for Mediterranean waters.

2.4. Backward Trajectories Calculation

Once larval ages of collected Tuna larval specimens were calculated, the corresponding larval backward trajectories (in terms of duration of simulation in days) were assessed, in order to infer the location of spawning grounds and the (satellite-based) environmental conditions that larvae experienced along their path, from the sampling sites backward towards the hatching sites.

Specifically, larval trajectories were simulated using General NOAA Oil Modelling Environment (GNOME), a software package designed by the NOAA Hazardous Materials Response Division [42]. Lagrangian elements (particles) movement is simulated within a geospatially mapped environment [43], which offers various opportunities for controlling input data to describe the transport of passive particles (in the present study, Tuna larvae) released at different sites [44,45]. In this study, the daily surface current field, i.e., the main "mover" of the fish larvae, used for the simulation runs, were from altimetry products, as distributed by the Copernicus Marine Service (CMEMS, http://marine.copernicus.eu/, accessed on 20 October 2021). In addition, horizontal diffusion was treated as a random walk process, calculated from a uniform distribution [46]. The GNOME default coefficient of 10^5 cm^2 s^{-1} was applied.

The influence of wind on surface circulation patterns was also taken into account, starting from a value-added 6-hourly gridded analysis of ocean surface winds [47]. Precisely, wind speed and directions were calculated from a zonal and meridional surface (10 m) and wind information was extracted using a 2.5 degrees of latitude × 2.5 degrees of longitude global grid for the geographical area 33°–40° N to 8°–20° E, as available in the link https://psl.noaa.gov/data/gridded/data.ncep.reanalysis.html, accessed on 20 October 2021 Extracted wind time series were included as additional external movers within GNOME backward Lagrangian simulations.

The start of the simulation runs was fixed at the sampling date of each collected larval specimen, for a duration in days corresponding to the estimated age of each larva based on its length, using the empirical relationships reported by García et al. [40] and Laiz-Carrión et al. [41]. In each simulation run, the release of particles was instantaneous from the selected sampling sites, corresponding to the geographical locations where at least one of the three fish species under study was recorded. The simulation consisted of three steps: (1) 1000 particles were positioned in each location of the selected sampling stations in the study area; (2) using GNOME, the direction and speed of the transport trajectory were calculated for the fixed duration of each simulation; and, (3) for each sampling station and each of the three species, the average final positions of released particles at the end of simulation runs, approximating the geographical location of the spawning sites, were evaluated and plotted.

The effect of the wind on the dispersal of particles was related to the expected vertical distribution of fish larvae in the water column. Given that the bulk of the larval abundance was likely to be concentrated from the surface down to the maximum depth of the mixed layer (from the analysis of temperature profiles using CTD probe, it was about 12 m on average over the six summer surveys included in our study), this reference depth layer was adopted for the simulations.

Taking into account the formulation reported in Patti et al. [36], the windage effect in Lagrangian simulations, i.e., the movement of particles induced by the wind, was set in terms of fractions of wind speed in the range 0.93–0.23%, with values corresponding, respectively, to depths of 1 m and 10 m.

Finally, we obtained probability clouds for the estimated backward trajectories of the released particles (see Supplementary Material, Figure S1h). The paths that impinged the coastline were excluded from the analyses. The calculation of centroids of the daily dispersion clouds made it possible to assess the backward larval path, to the hatching sites. The areas of these clouds represented the basis for the subsequent characterization of environmental conditions.

2.5. Remote Sensing Dataset

Satellite-based datasets were used to characterize the environmental conditions occurring in the identified areas. In particular, sea surface temperature (SST) and chlorophyll-a concentration (Chl-a) values were extracted for each point within the areas covered by each daily dispersion cloud. Subsequently, for each daily area, the average SST and Chl-a values were calculated. All satellite information was from the E.U. Copernicus Marine Service. In particular, we used daily data, with a spatial resolution of 0.01° × 0.01° for SST [48], and of 1 km×1 km for Chl-a [49–53]. The reference geographical domain for SST and Chl-a data was, 34.5–39.0° N and 9–16.5° E during the spawning seasons (June-August) of years 2010 to 2016. This way, daily environmental conditions were associated with the paths of larval dispersion.

In order to investigate how larval development stages were affected by the environmental conditions experienced during their dispersion, the average SST and Chl-a values were obtained to include all simulation days in the calculation, i.e., from the catch day, backward to the estimated hatching day. In addition, in order to better characterize the spawning habitats of three Tuna species, bottom depth data extracted from the "Marpap package" [54] (ETOPO1 database) were associated with the final positions of backward larval trajectories.

2.6. Stages Classification

According to the development of the notochord, each larva was classified in preflexion, flexion and post-flexion stage, following the classification based on morphological characteristics of the notochord and caudal fin rays made by Blanco et al. [55].

We also divided each larval developmental stage into normal, early and late development, following the distribution of the SLs for each of the three Tuna species analyzed in this study.

The sub-categories were selected based on the quartiles of the frequency distributions of the three macro-categories (Figure 2, Table 1). Among the larvae evaluated in pre-flexion stage, normal pre-flexion was attributed for SL values below the 75th percentile, and late pre-flexion for SL values greater than or equal to the 75th percentile. For larvae evaluated in flexion stage, early flexion was attributed to larvae with an SL lower or equal to the 25th percentile, normal flexion for SL values between the 25th and the 75th percentile, and late flexion for SL values greater than or equal to the 75th percentile. Lastly, among larvae in post-flexion stage, early post-flexion was attributed for SL values below or equal to the 25th percentile, and normal post-flexion for values greater than the 25th percentile.

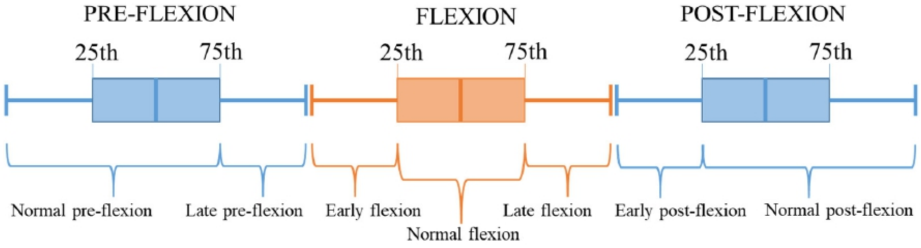


Figure 2. Classification of developmental stages into sub-categories ("Early", "Normal" and "Late").

The comparisons were accomplished, including all available data in the analysis (surveys 2010–2016), and on a year-by-year basis, when the number of observations was large enough.

Table 1. Classification of Tuna larvae in developmental categories and corresponding number of observations by species.

Macro-Category	Sub-Category	ABT	Bullet	Albacore
Pre-flexion	Normal	98	63	21
	Late	33	22	7
Flexion	Early	10	16	10
	Normal	18	31	20
	Late	10	16	10
Post-flexion	Early	9	8	2
	Normal	24	23	5

Non-parametric Kruskal–Wallis test was used to evaluate the significance of the differences in the medians of environmental parameters (SST and Chl-a) among the larval developmental stages. Pairwise comparisons were then carried out using the Wilcoxon rank-sum test in case of significant differences between groups ($p < 0.05$).

3. Results

3.1. Backward Trajectory Calculation

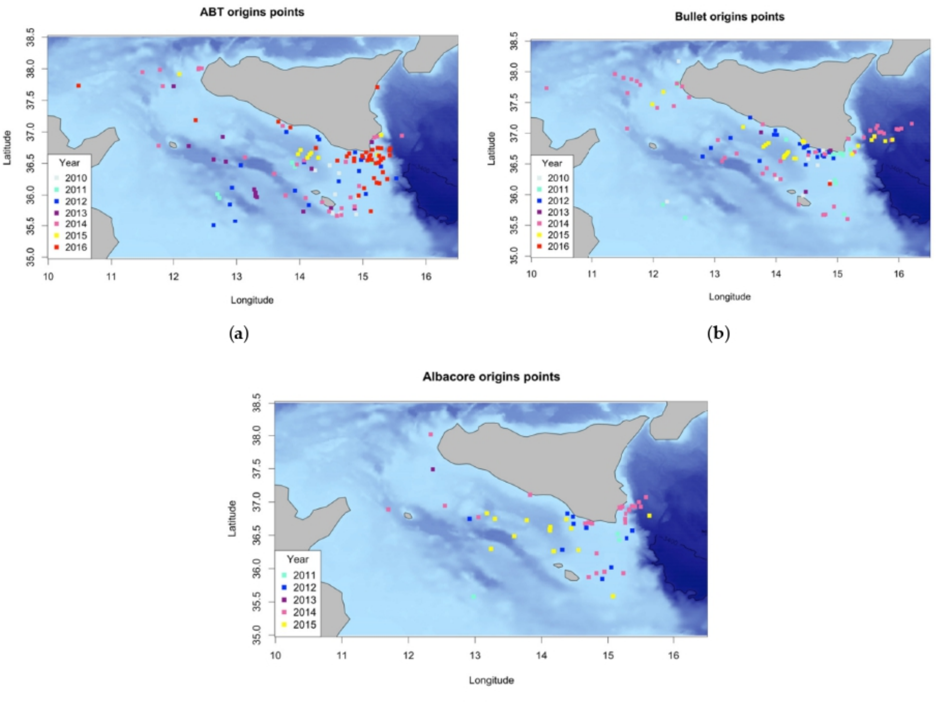
The estimated maximum larval age was 21 days, attributed to an ABT specimen sampled in the 2016 survey. Considering the whole available dataset, the observed average ages of collected larvae were 5 days for ABT, 8 days for Bullet Tuna, and 7 days for Albacore Tuna. The year with the lowest average age for ABT was 2010, due to the large number (60) of 1-day old specimens collected in the same sampling site, whereas the highest mean ages were recorded in surveys in 2014 and 2015. The estimated mean age of Bullet Tuna larvae was relatively stable over the sampling years, except in 2016, which was characterized by a low number of observations. Albacore Tuna larvae showed the lowest estimated average ages in all surveys.

The backward trajectories of some longer-lived larvae, by species and by year are reported in Figures S1–S3. It is worth noting the case of ABT and Bullet in 2014, where the trajectories mimic the average AIS path (Figures S1e and S2e). Pattern distribution of ABT larvae origins evidences their concentration in the southern part of the study area, off the southernmost tip of Sicily (Capo Passero) (Figure 3a). This applies also to many of the larvae whose estimated hatching site is in the western sector (Figure S1). Similar patterns are evident for the other two Tuna species, even though for Bullet Tuna the final positions of backward trajectories appear to be distributed in shallower areas (Figure 3b,c). In general, the paths of larvae of the same age can differ considerably. Surface currents can advect them at relatively long distances, while in some cases they can be trapped by local mesoscale oceanographic structures.

The variability in spawning sites seems to reflect the complex hydrodynamics of the study area. In particular, the Capo Passero area, characterized by a thermohaline front and warmer/saltier surface waters favoring the aggregation of larval stages of different fish species, including Tunas [30,56–58], confirms its features also by the analysis of backward trajectories. Generally, Tuna larvae that hatch in this area are trapped by the thermohaline front and by the local mesoscale circulation, which favor concentration processes also for larvae born elsewhere as long as they are advected there by the average path of surface currents (AIS). Larvae originating from spawning events in the Ionian Sea tend to also converge south of Capo Passero, where they join with larvae from the western sector (Figures S1–S3).

3.2. Characterization of Estimated Hatching Sites

The observed temperatures at origin points of estimated backward trajectories suggest different hatching temperatures for the three species (Table 2, Figure 4a,d,g). Specifically, the minimum temperature value is about 3 degrees higher for Albacore compared with ABT. However, the average temperature values are all in the range of 25–26 °C. In addition, while ABT larvae mainly occurred in waters with a surface temperature around 25–26 °C, Bullet showed a wider thermal range skewed towards warmer waters. The same applies to Albacore, though results for this species is based on few observations.



(**a**) (**b**)

(**c**)

Figure 3. Calculated average origin point by year, for ABT (**a**), Bullet (**b**) and Albacore tuna (**c**).

Figure 4b,e,h shows that sampled larvae of the three species originated in highly oligotrophic waters, with quite low average concentration values (Table 2) especially for ABT.

As far bottom depth distribution is concerned, for ABT, the hatching site is more concentrated below −500 m. The median depth of Bullet (Table 2) also reflects the shallow water habitat of this species. For the same species, greater bottom depth values refer to origin points located in the Ionian Sea, and reflect the local orography, characterized by a narrow shelf area and a very steep continental slope but very close to the coastline.

Table 2. Environmental conditions, SST, Chl-a and bottom depth, in the estimated hatching sites for the three Tuna species.

Environmental Variable		ABT	Bullet	Albacore
SST	Minimum value	20.47	20.71	22.82
	Maximum value	28.67	28.67	28.47
	Median value	25.16	25.98	26.32
Chl-a	Minimum value	0.029	0.033	0.029
	Maximum value	0.174	0.280	0.346
	Median value	0.048	0.055	0.049
Bottom Depth	Minimum value	−1607	−2470	−2484
	Maximum value	−15.7	−9.00	−11.28
	Median value	−283	−209	−507

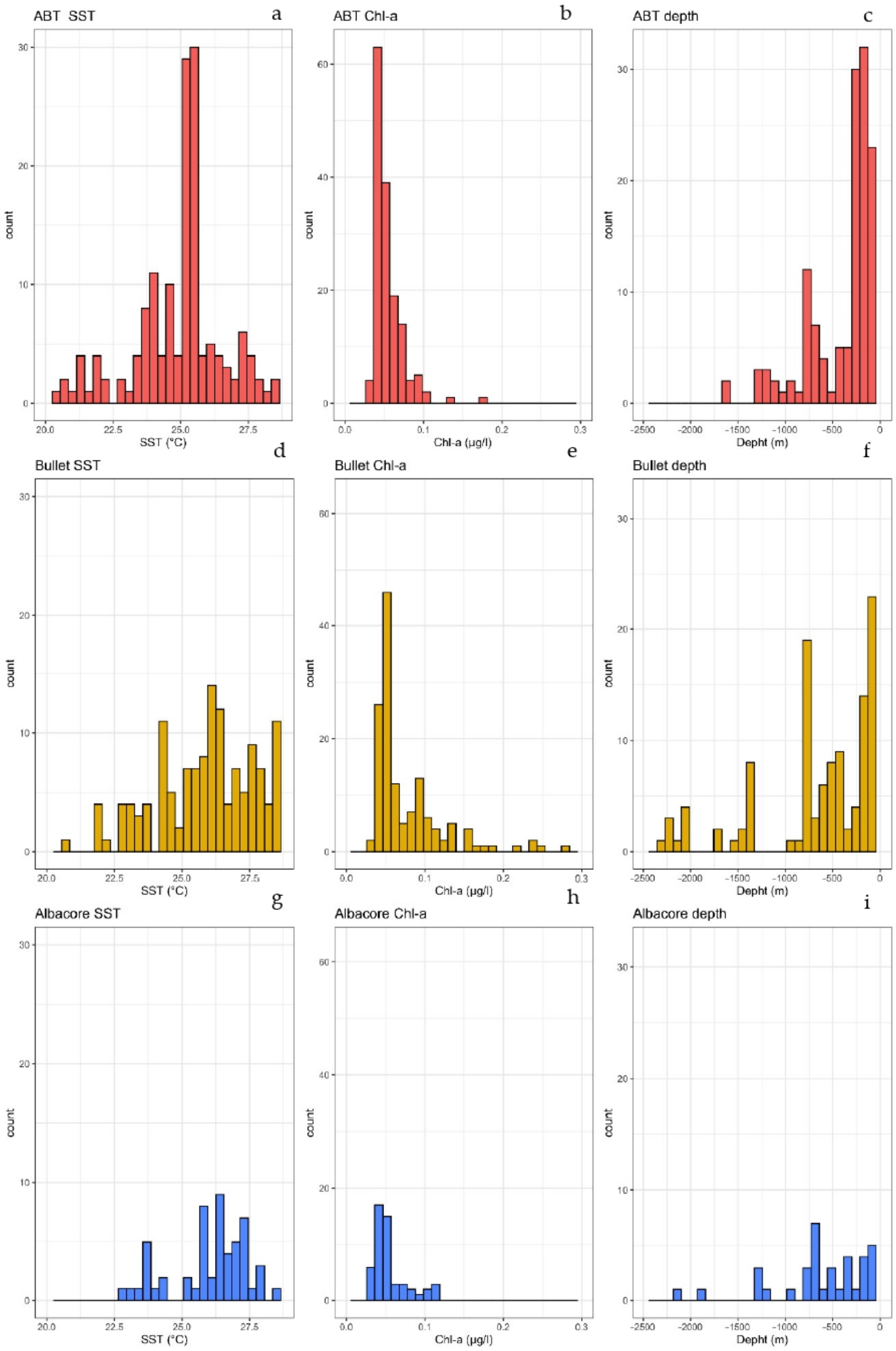


Figure 4. Histograms of environmental conditions, SST (**a**,**d**,**g**), Chl-a (**b**,**e**,**h**) and bottom depth (**c**,**f**,**i**), in the estimated hatching sites for the three Tuna species.

3.3. Relationships among Developmental Larval Traits and Environmental Forcings

Comparing the different developmental stage sub-groups (i.e., "Early", "Late" and "Normal") in relation to the impact of the environmental conditions experienced by larvae along their paths, some differences were found. In the case of ABT at the flexion stage, the Kruskal–Wallis test did not evidence any significant difference. On the contrary, for Chl-a, the difference between normal pre-flexion and late pre-flexion larvae was significant ($p < 0.001$; Figure 5a), with late pre-flexion associated with higher Chl-a concentrations.

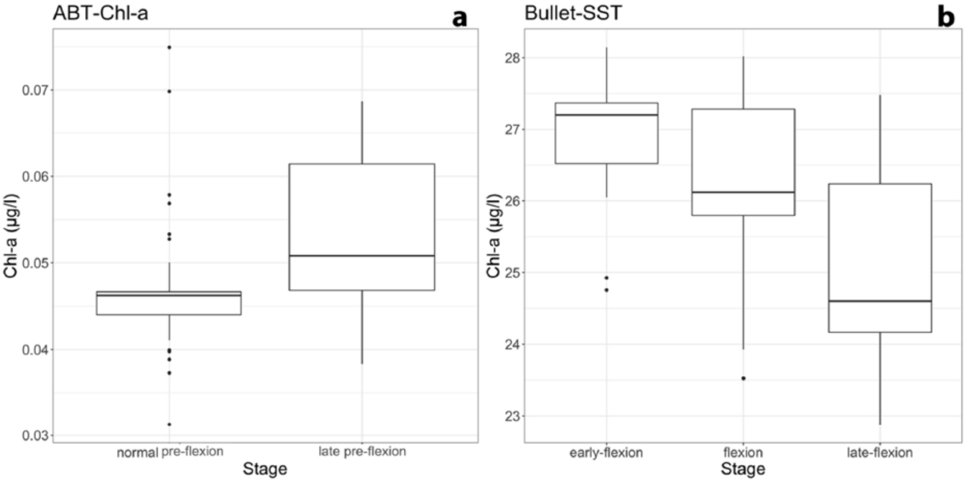


Figure 5. Box plots of developmental stage sub-groups in relation to environmental conditions experienced by Tuna larvae along their path, including information from all surveys. The two subplots show the cases where significant differences between sub-groups were found. (**a**) ABT vs. Chl-a and (**b**) Bullet vs. SST.

For Bullet larval specimens at the flexion stage, the Kruskal–Wallis test evidenced a significant overall difference in SST experienced along the path between sub-groups ($p < 0.01$; Figure 5b). Using pairwise comparisons, a significant difference was found between early and late development (Wilcoxon test, $p < 0.01$). Therefore, the early development larvae were found at higher temperatures and the late development larvae at lower temperatures.

No significant differences were found for Albacore across all years.

Examining ABT data by year, only in 2016 were some significant differences detected when comparing "normal pre-flexion" and "latepre-flexion" larval stages vs. SST ($p < 001$; Figure 6a) and vs. Chl-a ($p < 0.001$; Figure 6b), with late pre-flexion associated with lower SST and higher Chl-a concentrations.

No significant differences were found for Bullet or Albacore Tunas for the year-by-year analysis.

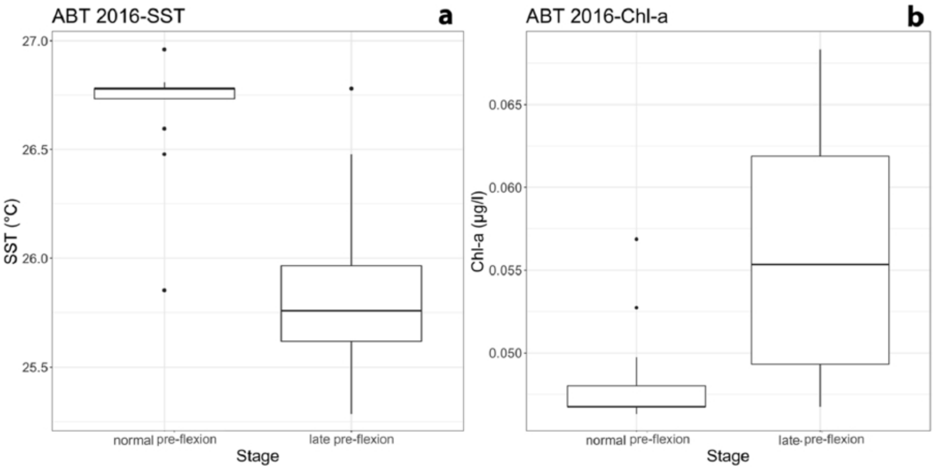


Figure 6. Box plots of developmental stage sub-groups in relation to environmental conditions experienced by Tuna larvae along their path (data from survey in 2016). The two sub-plots show the cases where significant differences between sub-groups were found. (**a**) ABT vs. SST and (**b**) ABT vs. Chl-a.

4. Discussion

The obtained results represent a further step forward the characterization of common spawning areas shared by three important Tuna species in the Strait of Sicily. Larval sampling was conducted during the spawning season, covering an area where larvae experience their development. Spatial distribution analysis of larvae highlighted different patterns among species. In addition, environmental variables seem to be helpful in describing the differences in the larval developmental stages.

The estimated ages allowed us to verify heterogeneity from year to year, indicating multiple spawning events within the studied area at different times. In fact, Tuna fish are multiple batch spawners, and the reproductive events follow one another within their reproductive season [59].

The analysis of backward trajectories showed the paths of each individual larva from the hatching to the catch. In agreement with other studies focused on the same region, AIS was one the main drivers affecting larval advection [36,60,61]. The surface current transported the planktonic fish stages and concentrated them in the southeastern retention area, where other larvae coming from the eastern sector also converge. Through backward Lagrangian simulations runs, the environmental conditions experienced by larvae throughout their lives were evaluated, starting from the origin points of their estimated backward trajectories, which represent the hatching sites. Considering the relatively short hatching time of the Tuna species (e.g., for ABT [62]), this study improved our knowledge about the spawning environment selected by the adults in the study area. The geographical positions of the origin points of backward larval trajectories varied significantly by species and by year. However, in all cases, the larval trajectories reflected the dominant path of the local surface current in the spawning and adjacent areas.

The larvae born at the edge of the thermohaline front tended to be trapped in this area. It was also interesting to note how larvae with similar age, depending on their origins, could experience different paths, covering large or short distances, and could often be found in common retention areas. This, of course, could also impact their development and/or survival. The events that affect larval development, along their path from the spawning sites, are fundamental in assessing reproductive success. In this framework, local oceanographic processes play a crucial role in determining the spatial distribution of the planktonic fish stages, controlling the advection from the spawning areas to more or less suitable retention zones [63,64]. Other important factors affecting larval development and survival are the maternal effect and stochastic events during transport. The choice of the spawning grounds by adult specimens impact on the environmental conditions experienced by their offspring. Even the water masses' origin can affect larval growth, as shown by previous studies on Bullet Tuna [41,65,66].

The analysis of available satellite data (SST and Chl-a) and bathymetry associated with larval trajectories has increased our knowledge about the Tuna spawning habitat in the Strait of Sicily. Some observed patterns, such as the coastal attitude of Bullet or the different distributions depending on water temperature, correspond to what was verified by previous studies on the larval habitat [30]. In particular, backward trajectories analysis highlighted the different origins for larvae from the same sampling area, also delivering further insights into the maternal effect.

We hypothesized that the environmental conditions experienced by larvae along their passive drifting phase would have affected the development of these organisms. In fact, the individual's experience along the paths appears to influence the stage of the notochord, anticipating or delaying its development for ABT and Bullet. We hypothesized that warmer waters could anticipate development, and low Chl-a indicated unfavorable nutritional conditions, slowing the notochord development. Our results confirmed the hypotheses regarding temperatures, as early developing larvae were found in warmer waters, especially in the case of Bullet. For Chl-a, we found the opposite trend to what was hypothesized. This could be due to competition phenomena. We hypothesize that the occurrence of more competitors and predators in richer environments can drive lower feeding rates and energy waste due to escaping and hunting behavior, thereby leading to late notochord development. In this framework, warmer temperatures could lead Bullet, the fastest-growing species among those analyzed here [40], to a more accelerated development than the other Tunas. This, in turn, could lead to resource competition phenomena and/or early juvenile Bullet Tuna preying behavior on other Tuna species, as hypothesized by Bakun and Broad [67].

Condition, growth and survival in marine fish larvae are influenced by food [68], temperature [69], hydrographic patterns [70], and environment in general [71]. However, the parents' genotype could also be an essential factor [72,73]. In this study we did not evaluate any genotypic differences and a parental effect due to purely genetic factors. Embryo and larval characteristics, developmental rate, and metabolism are affected by the parents' genotype [72,73]. Together with the environmental parameters, they can lead to the manifestation of morphological differences and can affect the success of recruitment.

More in-depth studies involving genetic aspects are recommended, especially after recent tagging studies on adult ABT in the same reproductive area have shown different adult migratory behaviors [74]. However, the same study did not clarify whether these migratory patterns are related to two different subpopulations or whether the observed behavior is linked to different spawners' sizes. Therefore, the origin of the ABT population in the area is still an open question.

The early life stage analyzed here represents the most critical period in the life history of fish, affected by the highest mortality rates [75]. Understanding Tuna population dynamics is essential for determining the fundamental features of survival processes in the early life stages [76]. It is also necessary to understand the mechanisms affecting recruitment success for the three Tuna species analyzed here. In fact, understanding the links between ocean patterns, spatial distribution and paths of early life stages, and other environmental parameters is crucial for the sustainable management of fishery resources [77,78].

5. Conclusions

The Lagrangian simulation approach adopted in this study has improved our understanding of Tuna reproductive biology processes and early life history. The origin points calculated varied significantly by species and by year, but the final position where the larvae were found reflect the surface current in the area.

We analyzed organisms that originated from multiple spawning events, finding that, regardless of the spawning area, the larvae released into the area ended up concentrating in a common retention area related to a local frontal mesoscale oceanographic feature. Therefore, the AIS seems to be fundamental in larval advection, and the front plays a key role trapping the larvae in a specific environment. The spawning habitat and the larval habitat have common characteristics, although some larvae have undertaken long transport routes.

We have shown that the environmental conditions experienced by the organisms, in accelerating or slowing down some relevant features of individual fish development (i.e., pre-flexion and flexion stages), could be fundamental for their survival from the first days of life. However, there is still a need for further insights into the physical processes affecting larval fate. Finally, our approach was intended to provide useful information to support Tuna fisheries management. Identifying spawning and retention areas for spawning products could be the baseline for developing fishery-independent recruitment indices. Furthermore, as suggested by Mariani et al. [23], better information on spawning areas and larval habitats can help establish marine protected areas or areas closed to fishing, for a valid protection strategy for these important fish species.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14101568/s1, Figure S1: ABT backward trajectories maps year-by-year, and an example of the cloud points derived from Lagrangian simulations; Figure S2: Bullet backward trajectories maps year-by-year; Figure S3: Albacore backward trajectories maps year-by-year.

Author Contributions: S.R., A.C. and G.S. contributed to the conception and design of the study. M.T., B.P., M.M., T.M., M.V.D.N. and A.C. conducted the sampling. S.R. and A.C. performed taxonomic identification. S.R. carried out the measurement and stages classification. S.R. organized the database. B.P. carried out the backward Lagrangian simulations. S.R. and M.T. performed the statistical analysis. S.R. wrote the first draft of the manuscript. G.S., A.C. and B.P. secured funding for data sampling and analysis. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Conflicts of Interest: The authors declare no conflict of interest.

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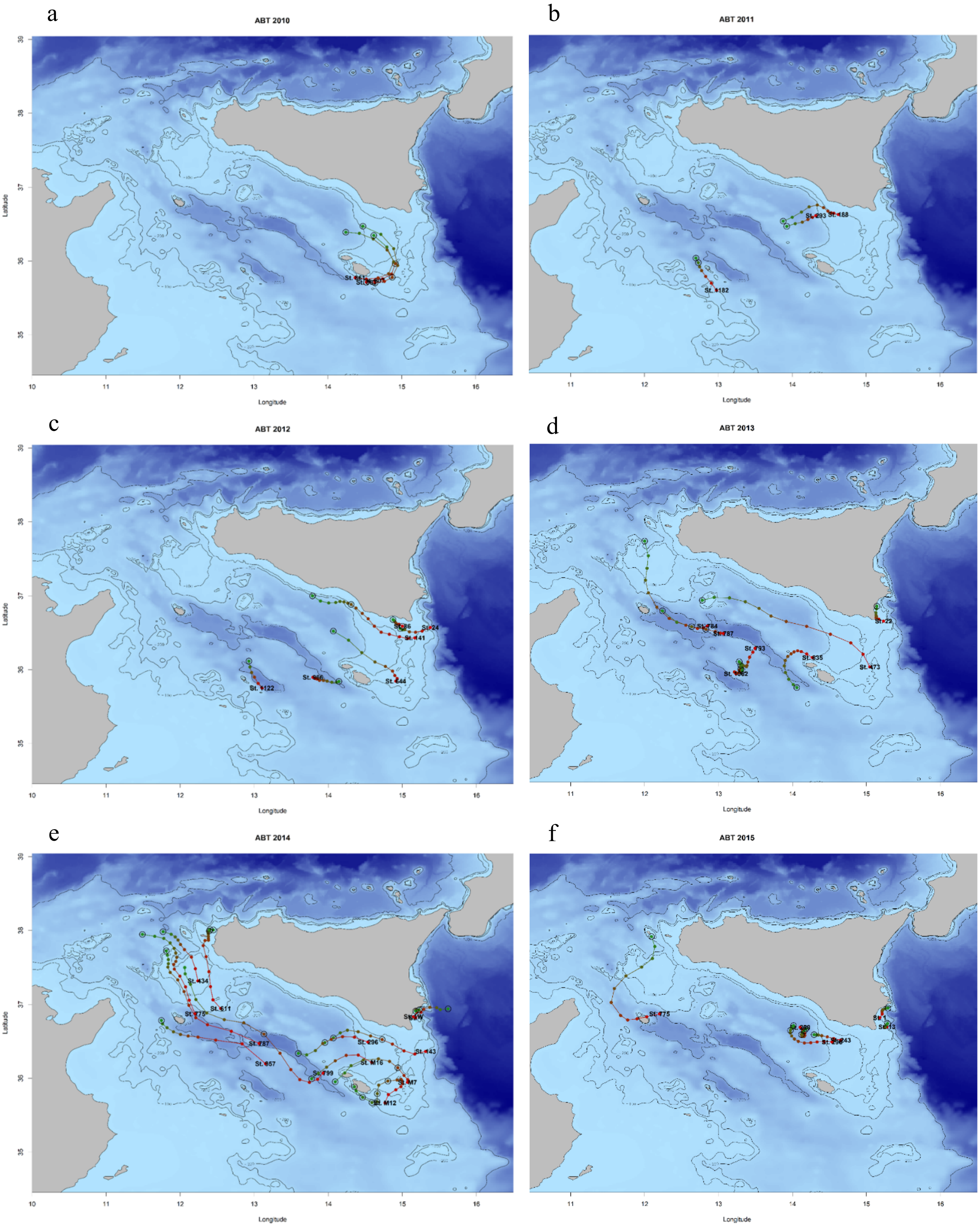
Supplementary Materials:



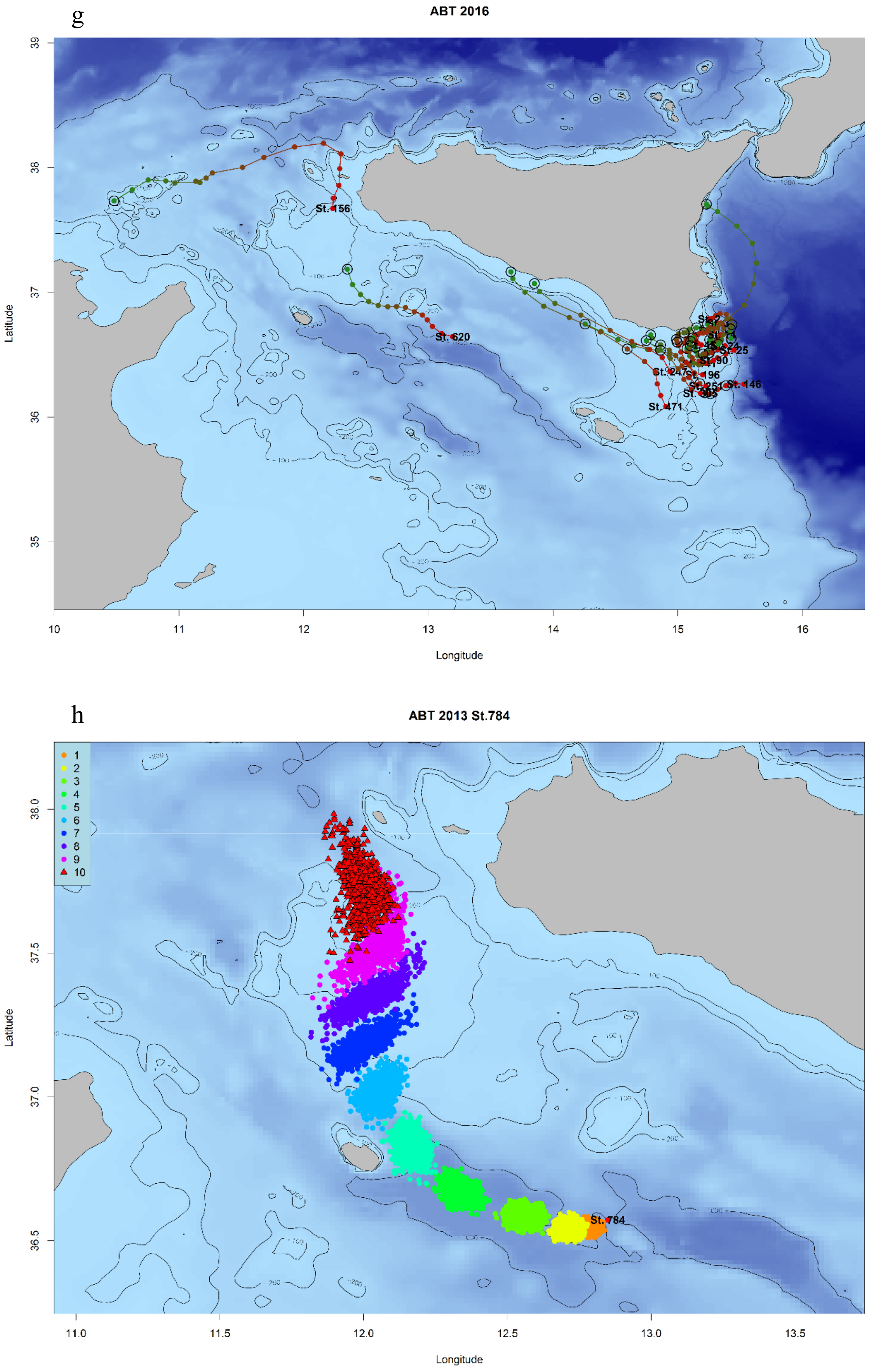


Figure S1. (a-g) Backward trajectories of some longer-lived ABT larvae, by yearly survey. The red points correspond to the sampling stations, the green circles represent the hatching sites as estimated by Lagrangian backwards simulation runs. (h) An example of the calculated daily probability clouds for a 10-day old larva.

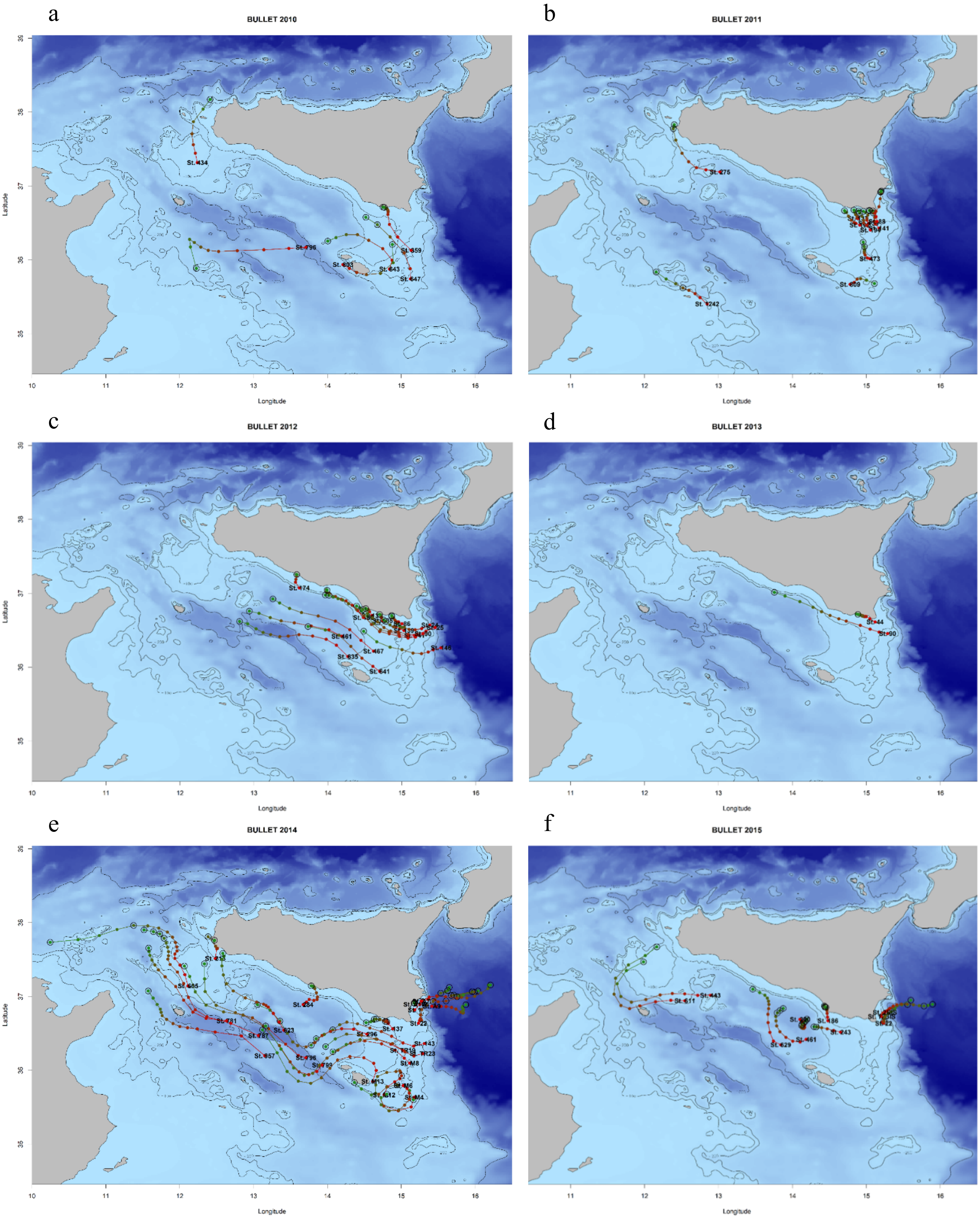


Figure S2. Centroids per year of some longer-lived Bullet Tuna larvae. The red points correspond to the sampling stations highlighted with the identification number, in green circled, the calculated average point of origin (a-f).

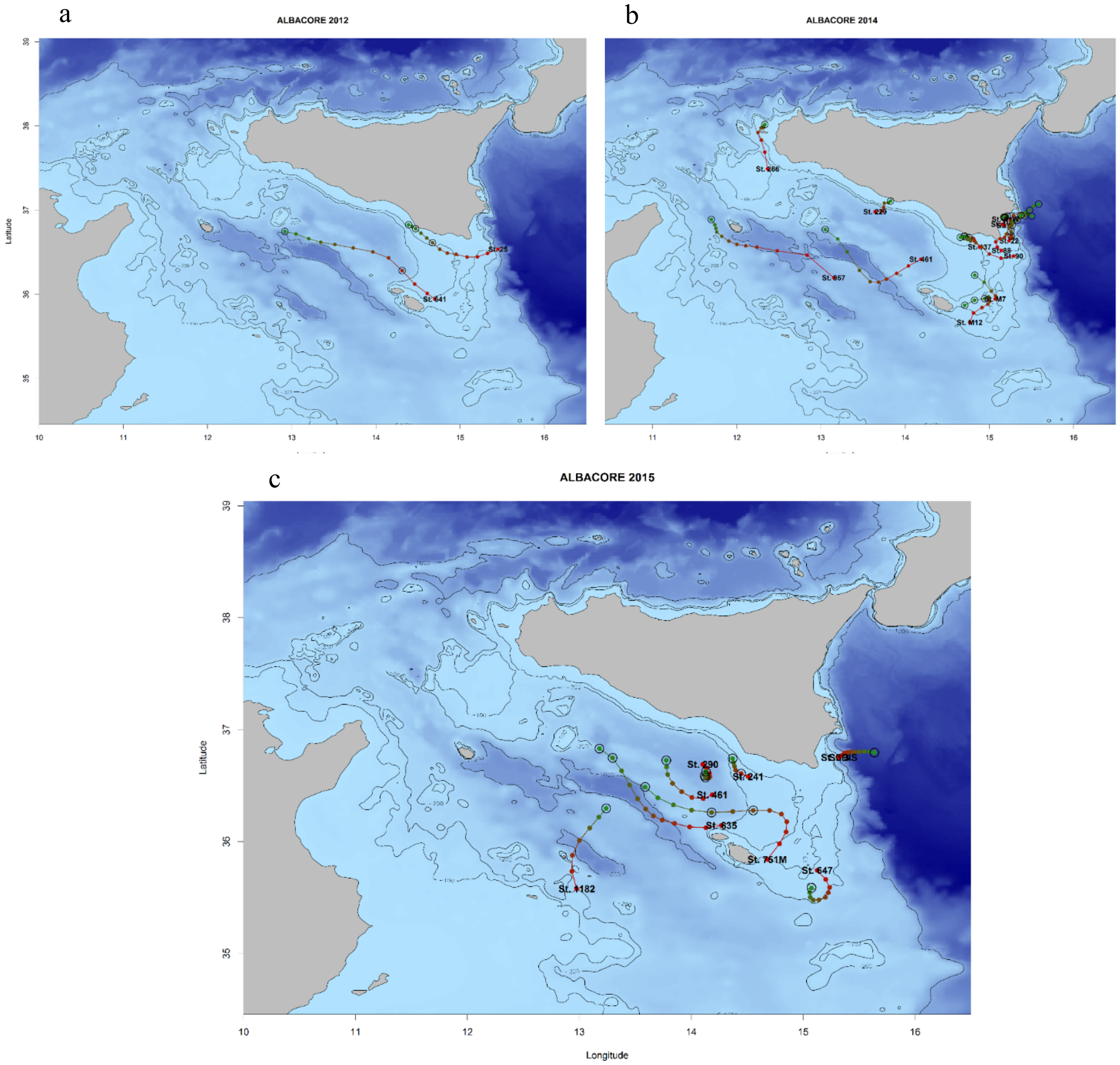


Figure S3. Centroids per year of some longer-lived Albacore Tuna larvae. The red points correspond to the sampling stations highlighted with the identification number, in green circled, the calculated average point of origin (a-c).

Abstract

After large pelagic adults fishes conclude a reproductive event, the fate of their offspring is uncertain. During the first days, the currents passively transport the larvae. What happens to them is dictated by stochastic events and reproductive habitat selection carried out by the adults. Food, temperature, and hydrographic patterns affect the condition and development of fish larvae. The SSTs experimented in the first days of life could significantly influence development before the flexion stage.

In this framework, this study analyses the back trajectories of three Tuna larvae species in the central Mediterranean Sea, Atlantic bluefin Tuna (ABT), Bullet and Albacore Tuna. We check what conditions the adults selected at their origin point and what the larvae experienced in temperature and Chl-a (as a proxy of nutritional conditions) along their path. Finally, we hypothesize that the path taken by each larva may have influenced its early or late development, affecting its chances of survival. Our results help to better characterize the larval and spawning habitats of ABT, Bullet and Albacore Tuna. We have also verified that the larvae undergo an accelerated development in favorable environmental conditions. Finally, we demonstrated that the morphometric measurement we took as “Body depth” can indicate a good larval health state.

3.1 Introduction

The biotic and abiotic conditions of the water column, particularly in surface layers, can strongly influence the distribution, abundance, and reproductive success of many fish species (Cuttitta et al., 2018).

After the spawning events, the fate of eggs and larvae is uncertain. The number that will successfully become an organism, and then an adult capable of restarting the reproductive cycle, is very few (Houde 2009; Rodríguez et al., 2017). Marine currents carry and disperse pelagic eggs and larvae into different habitats (Vasile et al., 2018). It is like a game of probability, within which many factors play a role, manifesting deterministic chaos (Okubo, 1993). Predators, environmental conditions experienced, or food availability are some of these (García et al., 2013). Anything eggs and larvae encounter along their path can affect the final balance of reproductive success for every single spawning event, and the influence of each environmental factor varies, because it may shift according to the ontogenetic stage (Ishihara et al., 2019).

In some cases, adults migrate from a feeding habitat to the spawning area to repeat their life cycle (Mariani et al., 2016). Although an adult can carefully select the reproductive location to give the best possible conditions for the new generations, the process is often dictated by stochastic events. This natal homing behavior can be beneficial and is a strategy used by many organisms, including Tuna species (McMahon et al., 2013; Rooker et al., 2014). Marine organisms can follow specific signals to start the reproductive events, depending on the species (Bromage et al., 2001; Zanuy et al., 2001; Migaud et al., 2010). Bluefin Tuna, in particular, can follow environmental or physical signs or a mix of both (Reglero et al., 2012). In any case, the typical strategy is to ensure the survival of as many larvae as possible.

Larval life stages are relatively short compared with the organism's lifetime, but they are decisive for the future adult stock and spawning stock biomass (Mather et al., 1995). Fish larvae are the smallest autonomous actively feeding vertebrates (Wieser, 1995). During this stage, faster-growing individuals are favored over slower-growing individuals, who are more vulnerable because they are exposed to a delicate life phase for a longer time, being characterized by higher mortality rates induced by predation and harder feeding conditions (Anderson, 1988). This period is very short for Tuna compared with other marine fish larvae (Mather et al., 1995). Larval growth is very fast, and reaching the flexion stage is a milestone because larval swimming and feeding abilities improve significantly (Ishihara et al., 2019). They are able to hunt better, escape predators more effectively and make the first active movements by escaping from the passive currents transport. They quickly pass from a planktivorous to a piscivorous phase, eating other larvae rapidly becoming voracious predators (Blanco et al., 2018). This strategy allows for the larvae to successfully evade predation by becoming predators themselves. In the specific case of Atlantic Bluefin Tuna (ABT), even the hatching time is very fast, 1-2 days, depending on temperature (Gordoa & Carreras, 2014).

However, fish larvae, and Tuna larvae in particular, cannot decisively oppose the currents during the early larval phase (Mather et al., 1995). Therefore, they undergo passive travel, making only small individual movements (Mariani et al., 2010). Passive mesoscale movements are essential, even for a few kilometers, because the larvae can be carried or removed from nursery areas (Bowman et al., 1993; Okubo, 1993). Often larval fish habitats are associated with specific oceanic regions with circulation systems generated by particular topographical features (Wolanski & Hamner, 1988). Tuna larval habitats in the Mediterranean Sea seems to be linked to specific temperature conditions, oceanic conditions such as gyres and fronts, and in general, oligotrophic waters that ensure the larvae encounter fewer predators (Alemany et al., 2010; Russo et al., 2021).

The balance between abiotic and biotic factors experienced by the young larvae is fundamental for future stock. Factors affecting larval development can be essential for their survival (Osse et al., 1997; Cahu et al., 2003).

This study analyses the back trajectories of three Tuna larvae species found in the same area, the central Mediterranean Sea. Three species with different needs but which share larval development and spawning areas (Alemany et al., 2010; Koched et al., 2013), ABT (*Thunnus thynnus*, Linnaeus, 1758), Bullet Tuna (*Auxis rochei*, Risso, 1810) and Albacore Tuna (*Thunnus alalunga*, Bonnaterre, 1788).

We assume that the environmental conditions experienced by these organisms along the passive transport period can affect their development, in the study area where surface circulation is dominated by the flow of the Modified Atlantic Waters, locally named as Atlantic Ionian Stream (AIS) (Robinson et al., 1999). We also verify the hypothesis that larvae came from several areas and experienced different conditions that can anticipate or delay some developmental stages. These events can positively or negatively affect the success of the reproductive event. Reaching the piscivorous stage first can increase survival chances. A larva in the piscivorous phase with a smaller conspecific can carry out cannibalism due to density-dependence processes (Catalan et al., 2007; Uriarte et al., 2019).

Often larvae studies are based only on the points in which they are found. Instead, with this study, we want to look back in time and evaluate the conditions experienced by larvae, starting from their presumed hatching location up to the point in which we found them. This way, the central Mediterranean Sea spawning area is entirely investigated.

3.2 Methods

Field sampling - Ichthyoplankton samples derive from surveys carried out by the Italian National Research Council (CNR) during the summertime (from June to August) from 2010 to 2016 in the Strait of Sicily, a scheme of the sampling period expressed as day of the year is shown in Fig. 1.

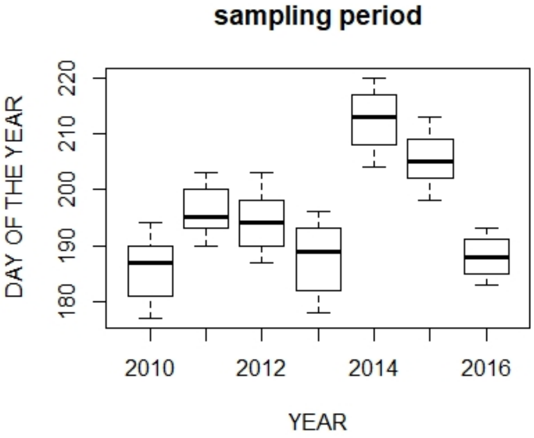


Fig 1 – Sampling period for each year, normalized as day of the year.

The sampling grid was constituted by a regular grid of stations (1/10°×1/10° along the continental shelf and 1/5°×1/5° offshore) located along transects perpendicular to the Southern Sicilian coast.

Ichthyoplankton was collected with oblique tows of a Bongo 40, and oblique or horizontal Bongo 90 nets, with a flowmeter towed at a ship speed of 2 knots, with descent speed 0.75 m/s and an ascent speed of 0.33 m/s. The sampling nets were towed from -100 m to the surface (Bongo 40) and in the surface mixed layer (Bongo 90).

Laboratory and image processing - After sampling, samples were immediately stored on-board in 70% ethanol and subsequently processed in the land-based laboratory to identify Tuna larvae at the highest possible taxonomic level, according to Rodríguez et al. (2017). Images of each larva were acquired using stereomicroscopes with integrated cameras. The dedicated Image Pro Plus © (IPP) image management and processing software was used to obtain from the photos some morphometric parameters. The measurements considered derive from those chosen by Catalán et al. (2011), though other parameters of interest were added to increase the study's accuracy (Fig. 2). Sizes are taken from the larvae of each Tuna species.

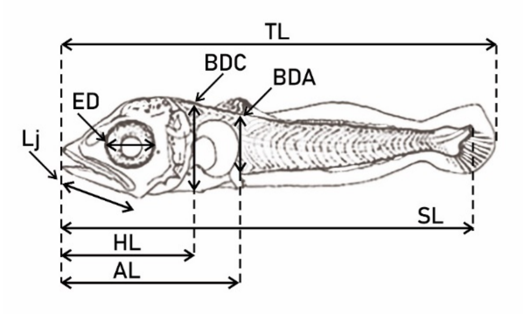


Figure 2 - Morphometric measurements taken for ABT, Bullet and Albacore Tuna larvae: TL=Total Length; SL=Standard Length; AL=Anus Length; HL=Head Length; BDA=Body Depth at the Anus; BDC=Body Depth at the Cleithrum; ED=Eye Diameter; Lj=Lower jaw length.

Age estimation - To allow an approximate calculation of each larva's ages, studies in the Mediterranean Sea on the daily increment of otolith rings related to SL were selected (García et al., 2006, Laiz-Carrión et al., 2013). By applying the relationships estimated in these studies (see Fig. 3), an indicative age was obtained from the SL measurements taken in the laboratory. In particular, the study on Bullet Tuna larvae analyzed two different water masses, and in our study, we selected the relationship estimated for Mediterranean waters (MW on Fig. 3b).

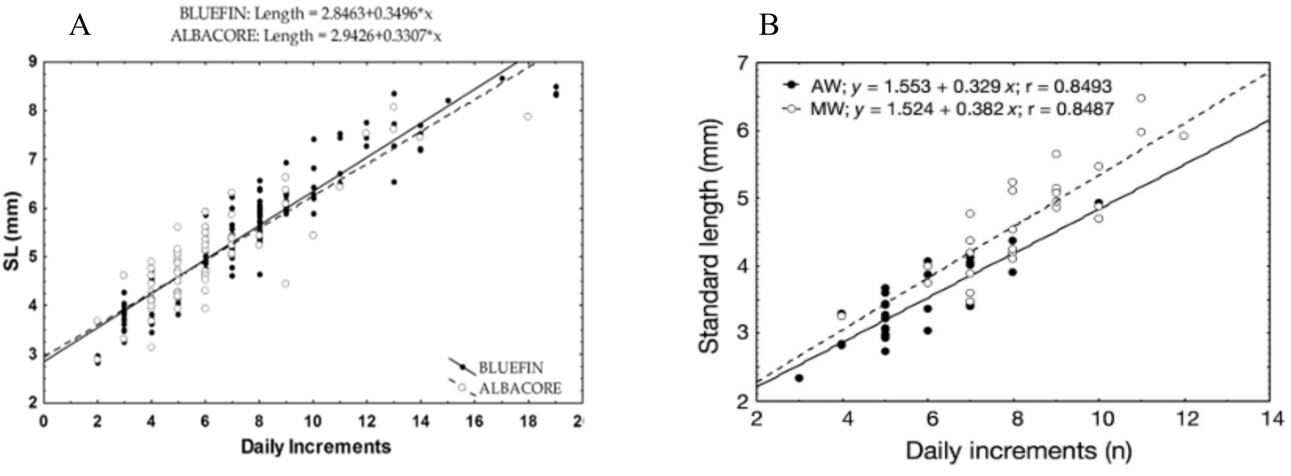


Figure 3- Daily growth model of field-captured larval ABT, Albacore and Bullett Tuna from the Western Mediterranean Sea. From Muhling et al., (2017) modifying Garcìa et al., (2006) (A) and from Laiz-Carrión et al., (2013) (B).

Back trajectories calculation - Once the ages had been calculated, we calculated possible larvae back trajectories to infer their spawning origin and the environmental conditions (satellite-based) that larvae experienced along their route.

Dispersal trajectories of the fish larvae were simulated using the General NOAA Oil Modelling Environment (GNOME), a software package designed by the NOAA Hazardous Materials Response Division (NOAA, 2002). Lagrangian elements (particles) movement is simulated within a geospatially mapped environment (Beegle-Krause & O'Connor, 2005). It offers various opportunities for controlling input data to describe the transport of passive particles (in the present study, Tuna larvae) released at different sites (Engie & Klinger, 2007; Palatella et al., 2014). In this study, the daily surface current fields during the survey periods, as evaluated through the altimetry products, and wind patterns were jointly used and considered as ''movers'' of the fish larvae. In addition, horizontal diffusion was treated as a random walk process, calculated from a uniform distribution (Beegle-Krause, 2001). The GNOME default coefficient of 105 cm2 s-1 was applied.

The influence of wind on surface circulation patterns was evaluated starting from a value-added 6-hourly gridded analysis of ocean surface winds (NASA/GSFC/NOAA, 2009). Precisely, wind speed and directions were calculated from a zonal and meridional surface (10 m) and wind information was extracted using a 2.5 degree of latitude x 2.5 degrees of longitude global grid for the geographical area 33°-40°N–8°-20°E, as available in the link https://psl.noaa.gov/data/gridded/data.ncep.reanalysis.html.

Extracted wind time series were included as external movers within GNOME backward Lagrangian simulations. The start of the simulation runs was fixed at the sampling date of each larval specimen, for a duration in days corresponding to the estimated age of each larva based on its length, using the empirical relationships reported by García et al. (2006) and Laiz-Carrión et al. (2013). In each simulation run, the release of particles was instantaneous from each sampling site, where one of the three fish species under study was recorded. It consisted of three steps: (1) 1,000 non-weathering particles were positioned in each location of the sampling stations in the study area. (2) Using GNOME, the direction and speed of the transport trajectory were calculated for the fixed duration of each simulation and (3) for each sampling station and each of the three species, the average final positions of released particles at the end of simulation runs, approximating the location of spawning, were evaluated and plotted.

The effect of the wind on the dispersal of particles, was also taken into account, relating it to the expected vertical distribution of fish larvae in the water column. The wind is typically included in particle-tracking models assuming that the surface wind-induced current (windage effect) is about 3% of the wind speed (Stolzenbach et al., 1977; Pugh, 1987). The strength of wind-induced current decreases logarithmically to zero at approximately a depth generally assumed to be 20 m (Elliott, 1986). Given that the bulk of the larval abundance is likely to be concentrated from the surface up to the maximum depth of the mixed layer (from the analysis of temperature profiles using CTD probe, it was about 12 m on average over the six summer surveys included in our study), this reference depth layer was adopted for the simulations. The wind-induced current at depth x (in meters) can be estimated using the following equation (Pugh, 1987):

$$u_x = u_0 - \frac{u^*}{k} ln\left(\frac{x}{z_0}\right)$$

Where u0 is the surface wind-induced current, k = 0.4 is the von Karman constant, u* is the friction velocity that can be estimated as 0.0012*W, with W being the wind speed 10 m above the sea surface, and finally, z0 is the sea surface roughness length, fixed at 0.001 m.

Taking into account the above formulation, in GNOME simulations, the windage effect, i.e. the movement of particles induced by the wind, was set in terms of fractions of wind speed, in the range 0.93–0.23%, values corresponding, respectively, to the depths of 1 m and 10 m.

We had finally obtained probability cloud points of the back trajectories of the particle and a cloud of possible origin points (Fig. 5 h). The points that fell on the coast, therefore on land, were excluded from the analyses. We then calculated for each simulation day the average position of the cloud points for all the evaluated back trajectories, thus obtaining midpoints, called centroids, to depict their paths.

Remote sensing dataset - After calculating the daily centroids, we attached them to environmental satellite data, namely sea surface temperature (SST) and chlorophyll-a concentration (Chl-a), extracted from E.U. Copernicus Marine Service Information. In particular, we used daily data, with a spatial resolution of 0.01° × 0.01° for SST (Buongiorno Nardelli et al., 2013), and of 1km × 1km for Chl-a (Lee et al., 2002; Berthon & Zibordi, 2004; Mélin & Vantrepotte, 2015; Volpe et al., 2018, 2019). We considered SST e Chl-a values recorded in the identified spawning area (39-34.5° N of latitude and 9-16.5° E of longitude) for each day of the spawning period (June-August) from 20/06/2010 to 11/06/2016.

In this way, we characterized the origin points of each larva, verified the environmental conditions experienced along its path and related it to the morphometric parameters collected on each specimen.

We also decided to characterize the last two days lived by each larva because these could have had a more significant impact on any differences in fundamental morphometric parameters. In fact, in rapidly developing larvae such as Tuna, the environmental conditions experienced in the short term could interfere with some of the results we have measured on the morphometric characters.

Therefore, we calculated SST and Chl-a at origin (in final day of each backward trajectory simulations), the mean of SST and Chl-a daily values experienced by each larva from birth sites to the sampling points along their whole paths, and the mean SST and Chl-a values experienced in the last two days. In addition, in order to better characterize the spawning habitats of three Tuna species, the bathymetric profiles of hatching points were obtained extracting information from the marpap library (Pante & Simon-Bouhet, 2013).

Environmental parameters and morphometry analysis – First, the morphometric parameters were size-normalized using the procedure presented by Lleonart et al. (2000) to remove the allometric effect of the Standard length (SL). So, all the measurements were divided by the reference SL and comparisons were made on standardized values. This method eliminates the body allometric effect of each larva's growth and normalizes the parameters according to SL. As a reference SL value, we set the average for all the larvae divided by species. Therefore, we could compare the relationships between the morphometric measurements taken as if each larva had equal SL measurement through standardization. This approach allowed us to exclude the effects of spawning events that occurred at different times.

Relationships between environmental (SST and Chl-a) data and morphometric data were assessed by Pearson's linear correlation analysis. A significance level of $\alpha = 0.05$ was considered in all tests. For each species, we first evaluated all the years simultaneously, then individually, excluding the years with few observations.

Stages classification- Each larva was classified according to the state of the notochord in pre-flexion, flexion and post-flexion (see Table. 1).

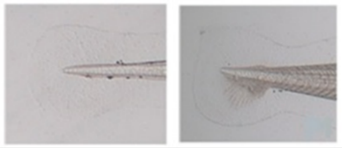
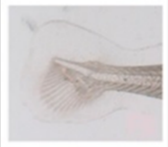
Stage	Description	Example
Pre-flexion	Straight notochord and with some rays in the ventral side	
Flexion	Bending upward of the notochord tip in a very clear angle with an increase in the amount of fin rays	
Post-flexion	The final tip of the notochord disappears. Definition of the hypural plate and caudal fork. The posterior margin of the upper hypural plate is at 90° from the notochord axis	

Table 1 - Description of the three different developmental stages used in this article, based on morphological characteristics of the notochord and caudal fin rays, modified from (Blanco et al., 2019).

We also divided each developmental stage into normal, early and late development, following the distribution of the SLs, thus obtaining sub-categories, reported by number, species and type in Table 2.

Macro-category	**Sub-category**	**ABT**	**Bullet**	**Albacore**
Pre-flexion	Normal pre-flexion	98	63	21
	Late pre-flexion	33	22	7
Flexion	Early flexion	10	16	10
	Normal flexion	18	31	20
	Late flexion	10	16	10
Post-flexion	Early post-flexion	9	8	2
	Normal post-flexion	24	23	5

Table 2 - Classification in categories and relative numbers for each species 2019).

The sub-categories were selected based on the quartiles of the frequency distributions of the three macro-categories (Fig. 4, Tab. 2). Among the larvae evaluated in pre-flexion, normal pre-flexion was considered for SL values below the 75th percentile, and late-preflexion for SL values greater than or equal to the 75th percentile. For larvae evaluated in flexion, early flexion was attributed to larvae with an SL lower or equal the 25th percentile, normal flexion for SL values between the 25th and the

75th percentile, and late flexion for SL values greater than or equal to the 75th percentile. Lastly, among larvae considered as in post-flexion, early post-flexion was attributed for SL values below or equal to the 25th percentile, and normal post-flexion for values greater than the 25th percentile.

This classification was made for all three species analyzed.

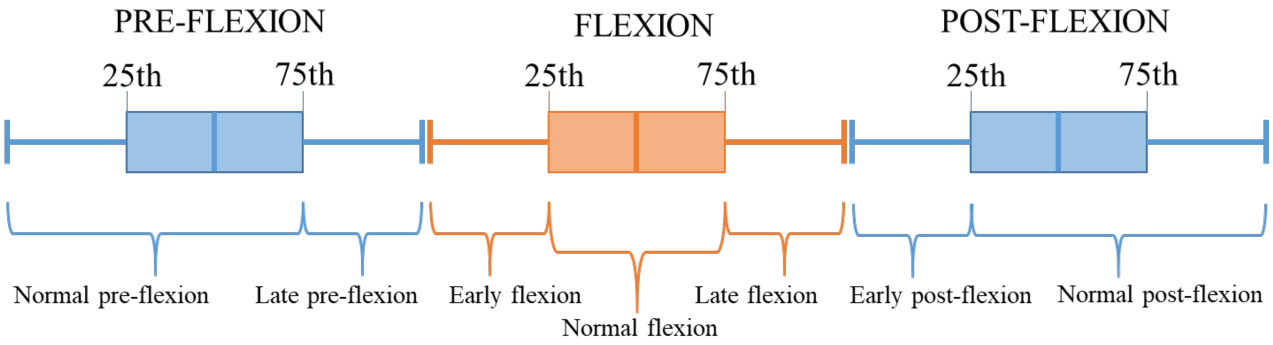


Figure 4 - method of classification of sub categories.

We then used the non-parametric Kruskal-Wallis test to verify the equality of the medians in environmental parameters (SST and Chl-a) among the different group categories. We compared the groups constructed as sub-categories of the developmental stage, with the H0 that the means of the groups are statistically equal. We performed pairwise comparisons using the Wilcoxon rank-sum test in case of significant differences between groups ($p < 0.05$).

This analysis was carried out in two ways, considering each species across all years, and a year-by-year analysis of species, excluding years with few observations.

Body depth and notochord flexion stage analysis - To verify if the larvae that have already reached the flexion developmental stage are in an environment with better nutritional conditions, we carried out the analysis on both BDA and BDC. We also considered the macro-categories pre-flexion, flexion and post-flexion following some recent studies (Cuttitta et al., 2015; Torri et al., 2021), which report that BD is correlated with a good nutritional status of the larvae. This analysis was performed on standardized measures, using the non-parametric Kruskal-Wallis test to verify the equality of the medians of different notochord stages groups, with the H0 that the means of the groups are statistically equal. We performed pairwise comparisons using the Wilcoxon rank-sum test in case of significant differences among groups ($p < 0.05$). Once we verified the differences among the macro-categories, we also analyzed the differences among the sub-categories, delayed, normal and anticipated, to be able to answer the question, "does the body depth vary in early developing larvae?".

All simulations, statistical analyses, and plotting were performed using the R statistical software (RStudio Inc, 2016).

3.3 Results

Age estimation – The calculated maximum larval age is 21 days, derived from an ABT specimen sampled in the 2016 survey. The observed mean age of ABT larvae is 5 days, 8 for Bullet and 7 for Albacore.

As summarized in Tab. 3, the year with the lowest average age for ABT is 2010, due to the large number (60) of 1-day larvae collected in the same sampling site. 2014 and 2015 are the years with the highest mean ages.

The Bullet Tuna appears more constant with its mean ages over the years, except for 2016, where we had few observations. Albacore shows the lowest estimated ages in all surveys.

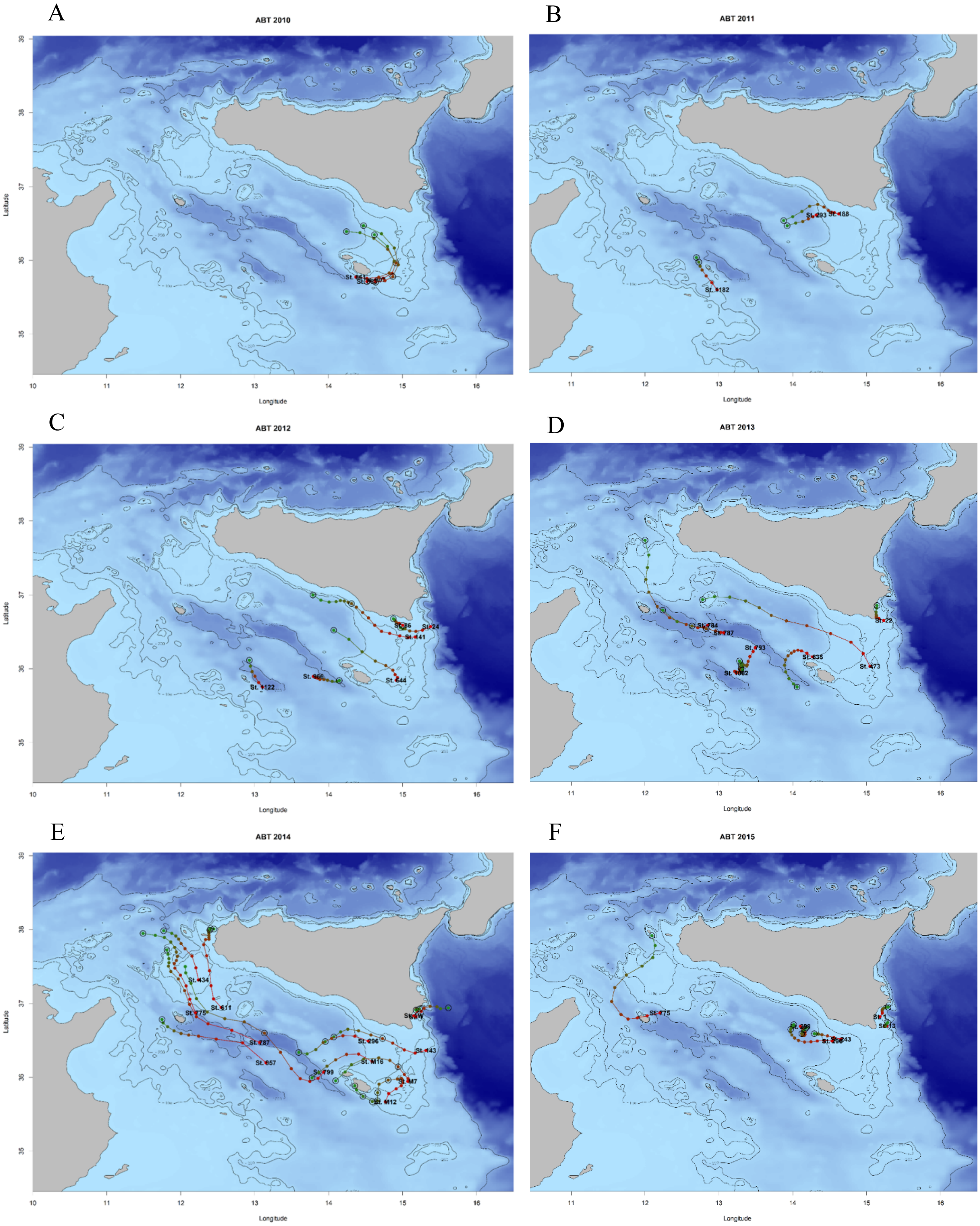
YEAR	SPECIES	MEAN AGE	MAX AGE	N. LARVAE
2010	ABT	1	7	64
	Bullet	6	7	14
	Albacore	-	-	-
2011	ABT	5	9	6
	Bullet	6	11	34
	Albacore	3	4	4
2012	ABT	4	16	25
	Bullet	8	14	22
	Albacore	5	12	12
2013	ABT	7	15	16
	Bullet	9	17	4
	Albacore	2	2	1
2014	ABT	9	17	29
	Bullet	11	16	69
	Albacore	8	12	43
2015	ABT	9	12	13
	Bullet	9	15	37
	Albacore	8	12	15
2016	ABT	7	21	49
	Bullet	2	2	1
	Albacore	-	-	-

Tab. 3 - Mean and maximum calculated age for each species and for each year.

Back trajectory calculation - The longest simulation examined was associated with the 21-day old larva sampled in the 2016 survey. In Figs. 5-6-7, the back trajectories by species and by year of some longer-lived larvae are reported. It is worth noting the case of ABT and Bullet in 2014, where we can see how the trajectories perfectly mimic the AIS path (Fig. 5 e - 6 e). Pattern distribution of ABT larvae collected in 2016 (Fig. 5 g) evidences their concentration in the Southern part of the study area, characterized by a thermohaline front and warmer waters (Russo et al., 2021). The origin points of Albacore are quite widespread (Fig. 7), while for Bullet, final back trajectories positions appear to be distributed in shallower areas (Fig. 6).

The Capo Passero area, which in Chapter 2 was characterized as an aggregation area, confirms this nature with the back trajectories calculation. The larvae that hatch there appear to be trapped by the thermohaline front and local mesoscale circulation. The larvae born elsewhere always tend to end their path in this area, being transported by the surface current (AIS). Those born in the Ionian Sea converge Southwards to Capo Passero, where they aggregate with the larvae coming from the Western sector.

Paths of larvae of the same age can differ considerably. Surface currents are able to advect them at relatively long distances, while in some cases they can be trapped by local mesoscale oceanographic structures, and so remaining in the same sector.


A
ABT 2010
B
ABT 2011
C
ABT 2012
D
ABT 2013
E
ABT 2014
F
ABT 2015
Latitude
Longitude
35
36
37
38
39
10
11
12
13
14
15
16
St. 293
St. 188
St. 24
St. 141
St. 44
St. 122
St. 784
St. 787
St. 793
St. 835
St. 473
St. 22
St. 434
St. 611
St. 775
St. 787
St. 857
St. 296
St. 143
St. M16
St. 799
St. M7
St. M12
St. 775
St. 243
St. 1
St. 13

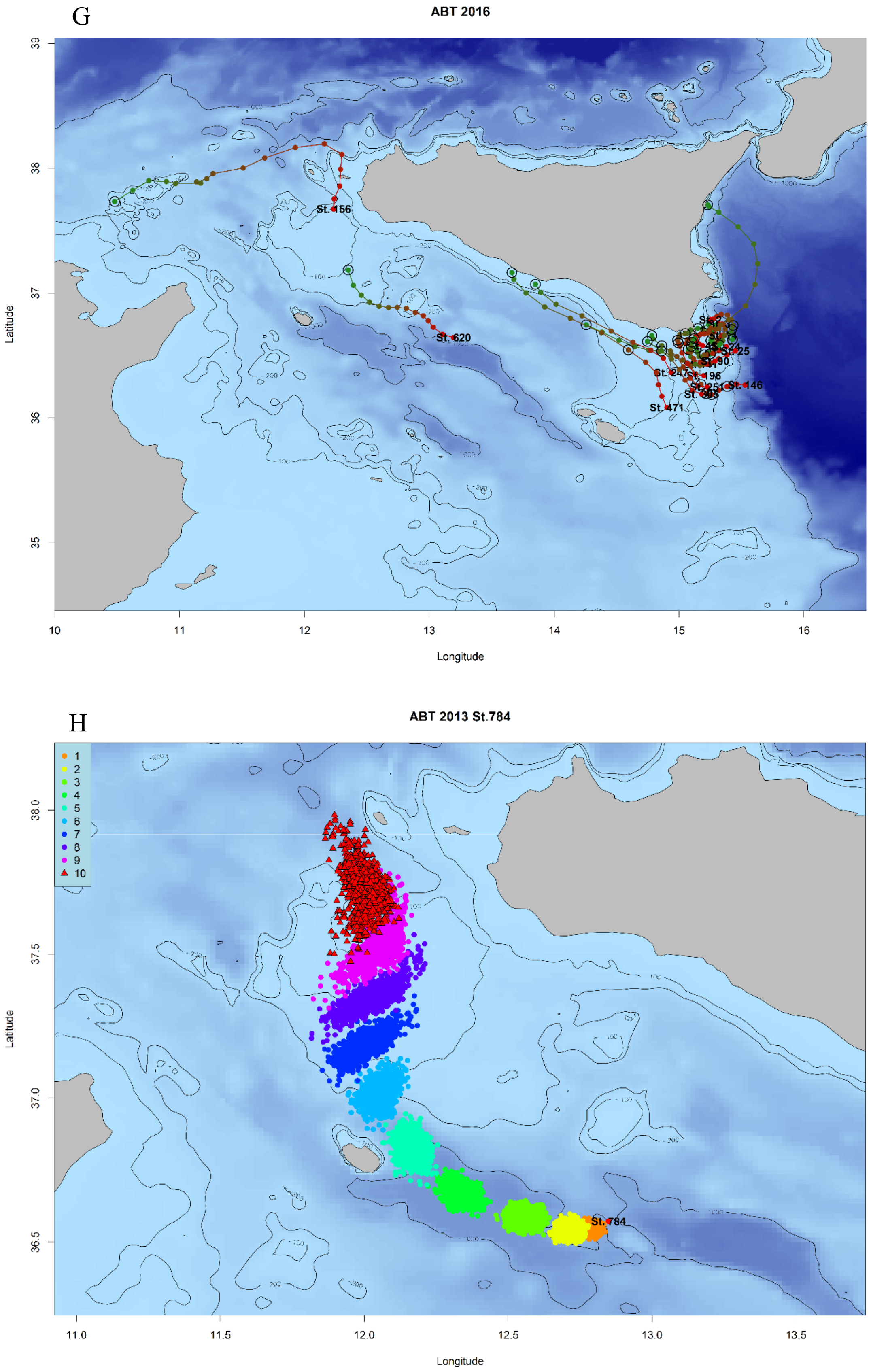


Fig 5 – Centroids per year of some longer-lived ABT larvae. The red points correspond to the sampling stations highlighted with the identification number, in green circled, the calculated average point of origin (A-F). An example of a calculated or probability point cloud of a 10-day larva (H).

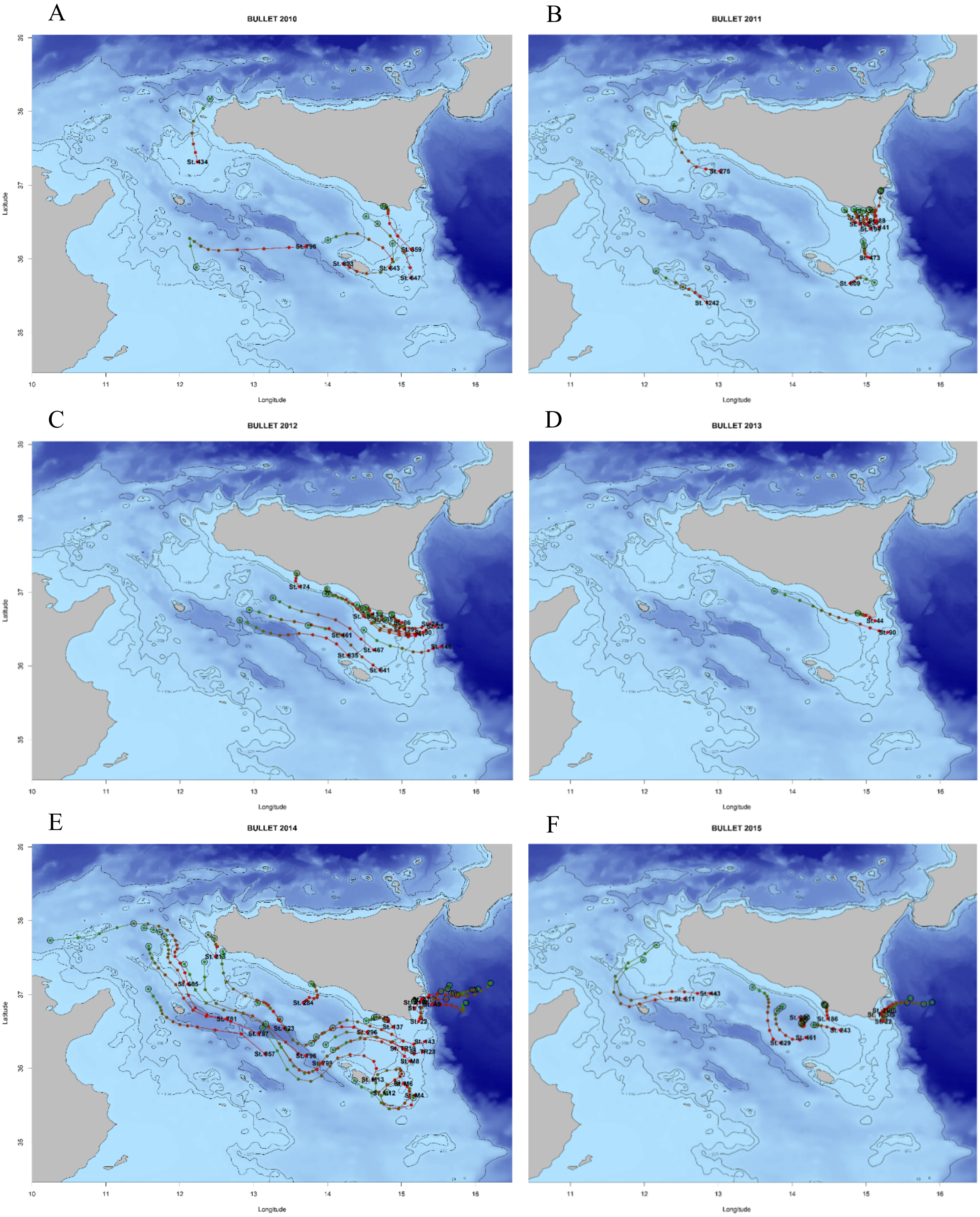


Fig 6 – Centroids per year of some longer-lived Bullet Tuna larvae. The red points correspond to the sampling stations highlighted with the identification number, in green circled, the calculated average point of origin (A-F).

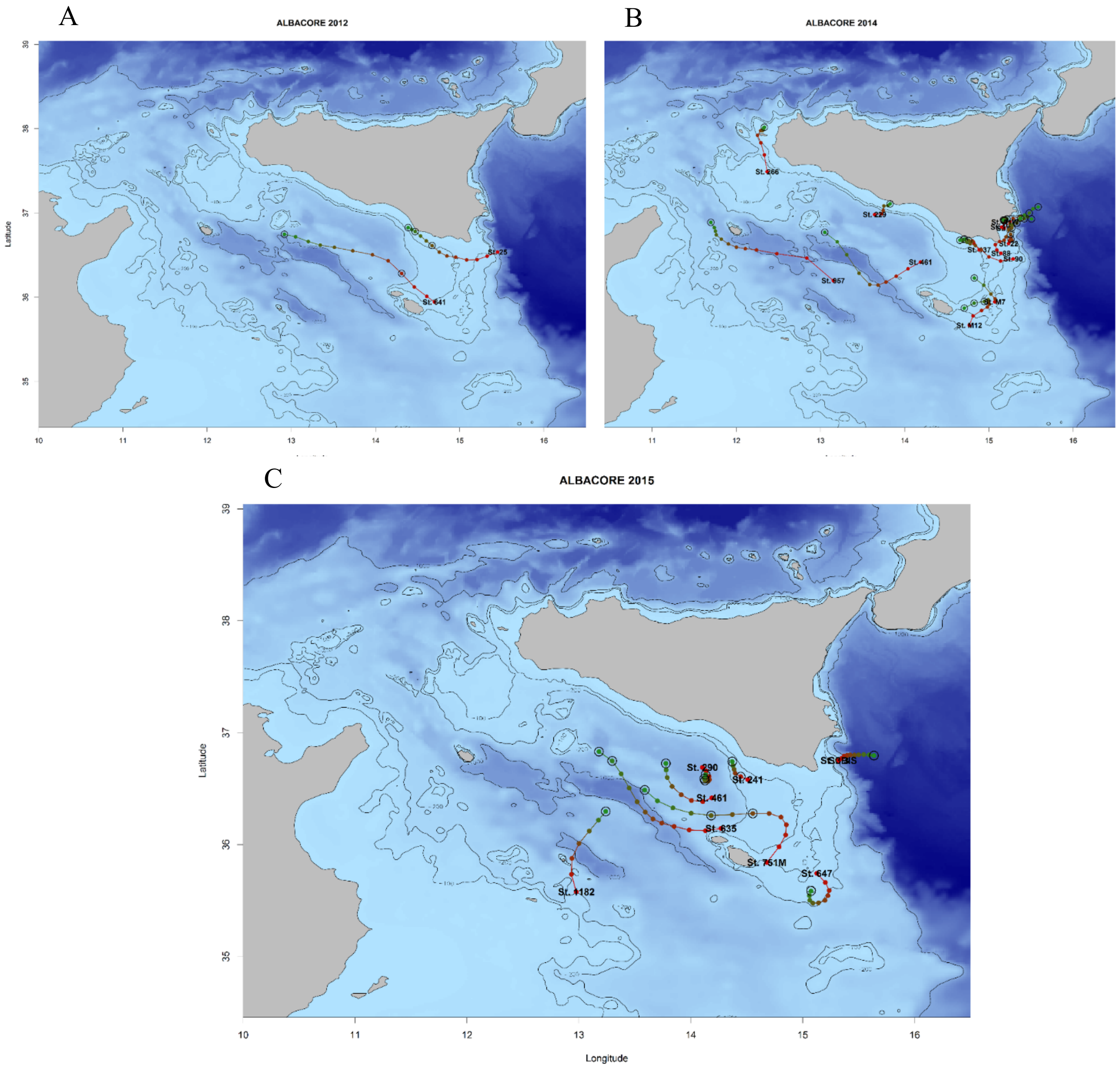


Fig 7 – Centroids per year of some longer-lived Albacore Tuna larvae. The red points correspond to the sampling stations highlighted with the identification number, in green circled, the calculated average point of origin (A-C).

Origin point characterization – The observed temperatures at origin points suggest different hatching temperatures for the three species (Fig. 8). Specifically, the minimum temperature value is about 3 degrees higher for Albacore compared to ABT. However, the average temperature values are all in the range 25-26°C . In addition, while ABT has a higher presence in waters around 25/26°C, Bullet has a wider thermal range but is skewed towards warmer waters. The same applies to Albacore, though the result for this species is based on few observations.

For Chl-a, all three species originate in oligotrophic waters, ABT more than the others (Fig. 8).

As far as depth distribution is concerned, it is broader for ABT compared to the other two species. The median depth of Bullet reflects the shallower habitat of this species, as shown in Fig. 8. For the same species, greater bottom depths of origin points were found in the Ionian Sea in 2014 and 2015, reflecting the local orography, characterized by a narrow shelf area and a very steep continental slope.

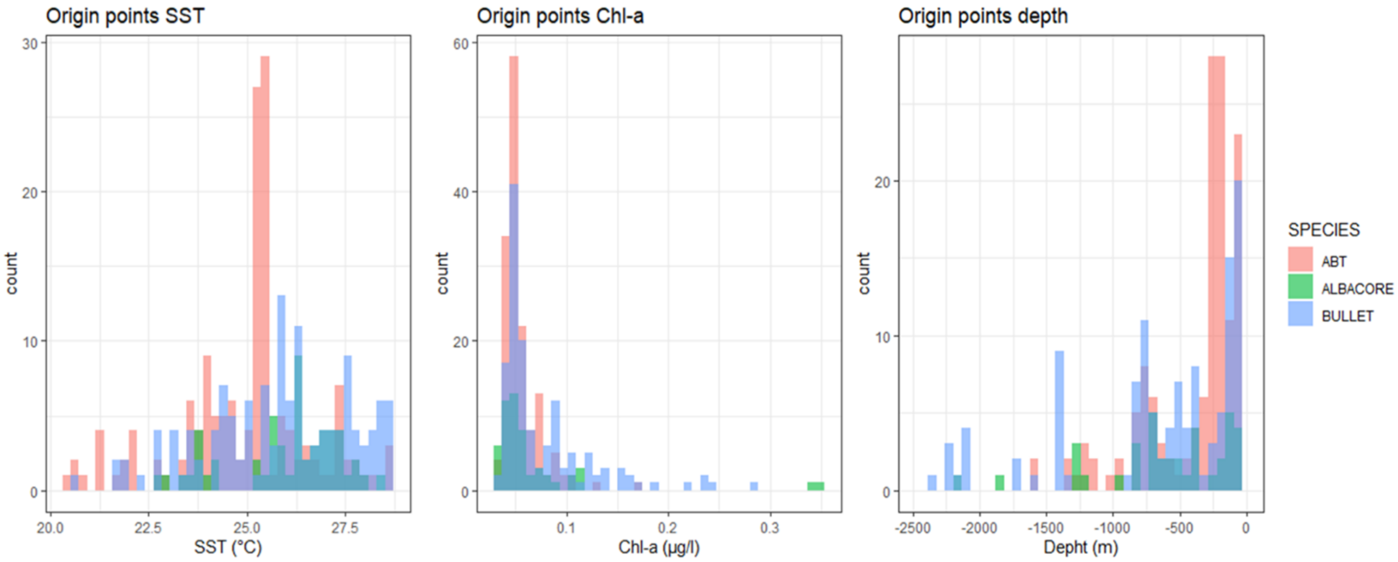


Fig 8 – Characterization of the three species origin points.

Environmental parameters and morphometry analysis - The correlation analysis between mean SST and mean Chl-a along the path of estimated trajectories and the larvae morphometric measurements did not show any meaningful result. Observing the correlations by species and year by year, some significant relationships have been detected. For ABT in 2013, BDC, HL, ED and AL are positively correlated with temperature, and in 2015 BDA and BDC were found negatively correlated with Chl-a. Bullet Tuna from year to year shows both positive or negative correlations with the environmental variables, but without any consistent pattern in the results. The same applies to Albacore, in some of the considered years, also indicating that the two environmental variables considered in this study, SST and Chl-a, have no relationships with the morphometric measurements of Tuna- larval specimens.

When considering just the last two days experienced by the larvae, it is possible to see that Bullet larvae were found in warmer waters, ABT always tended to concentrate at around 26 °C, and finally Albacore at approximately 26.5 °C. As regards Chl-a concentrations, all the three species were found in quiet oligotrophic waters.

Stages classification – Looking at the differences between the groups at developmental stages, Early, Late and Normal, some differences were highlighted. In the case of ABT at the flexion stage, though the Kruskal-Wallis test did not evidence any significant correlation graphically it is possible to note a general trend towards lower temperatures in relation to the development rate of flexion (Fig. 9). On the contrary, for Chl-a, the difference between early-flexion and late-flexion larvae appears to be significant, with a p-value = 9.452E-08 (Fig. 9).

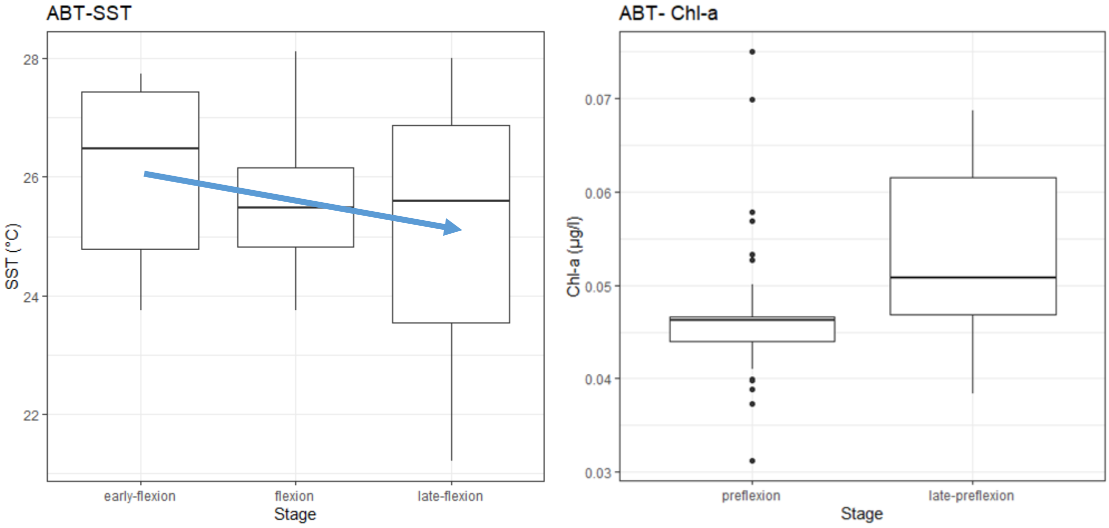
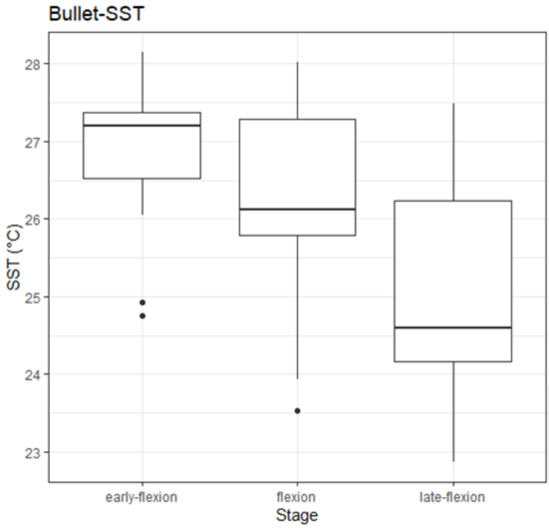
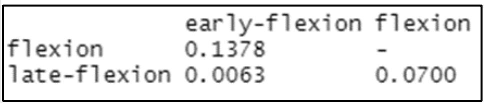


Fig 9 – Significant cases of ABT differences between the groups of developmental stages for SST and Chl-a lived along their path for all the year examined.

For Bullet at the flexion stage, with a p-value = 0.008 the Kruskal-Wallis test evidenced a significant difference between groups for SST experimented along the path (Fig.10). The pairwise comparisons with Wilcoxon rank-sum test shows a difference between early and late development with a p-value = 0.006. Therefore, the Early development larvae are found at higher temperatures and the Late development ones at lower temperatures.





```
             early-flexion flexion
flexion      0.1378        -
late-flexion 0.0063        0.0700
```

Fig 10 – SST a conditions experienced by Bullet Tuna larve along the path of their trajectories in relation to the observed developmental stages groups .

Examining the data from year to year, in 2016 a significant difference is highlighted for the pre-flexion stages with a p-value = 2.686E-05 for the SST and p-value = 0.000356 for Chl-a (Fig. 11).

No significant differences were found for Albacore, so the surface temperatures or the Chl-a concentrations experienced along the paths do not seem to affect the Early or Late larval development of this species.

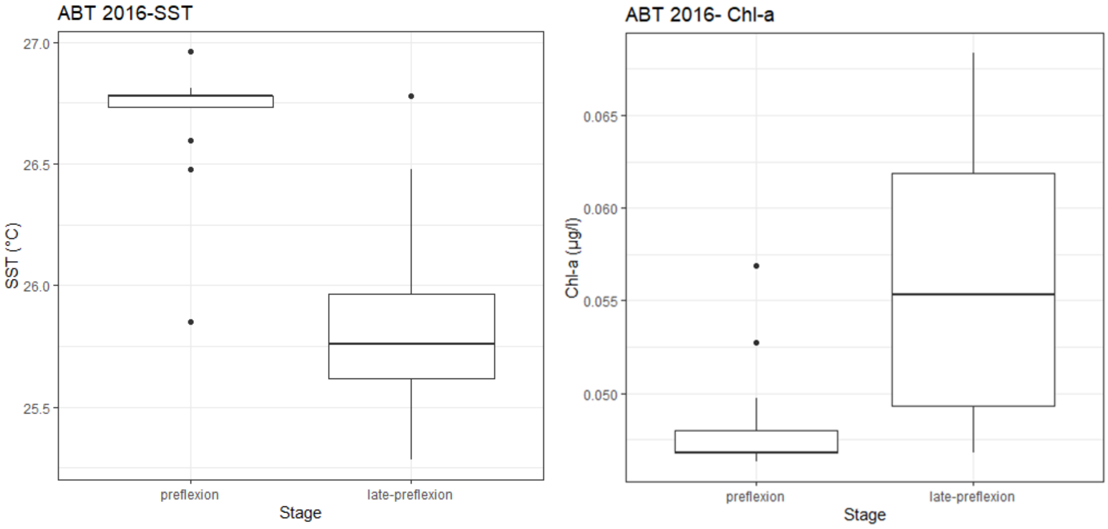


Fig 11 – Significant differences observed year to year in SST and Chl-a experienced along their path by ABT larvae among the groups of developmental stages.

Body depth and notochord flexion stage analysis - The BD analysis with the notochord flexion showed significant differences among the ABT groups with a p-value = 6.288E-10 for BDA and p=1.08E-05 for BDC. For Bullet, the differences are significant between pre-flexion and flexion, as well as for the Albacore BDA.

Closer analysis between early and late-developing larvae showed significant differences among groups for all three species (Fig. 12).

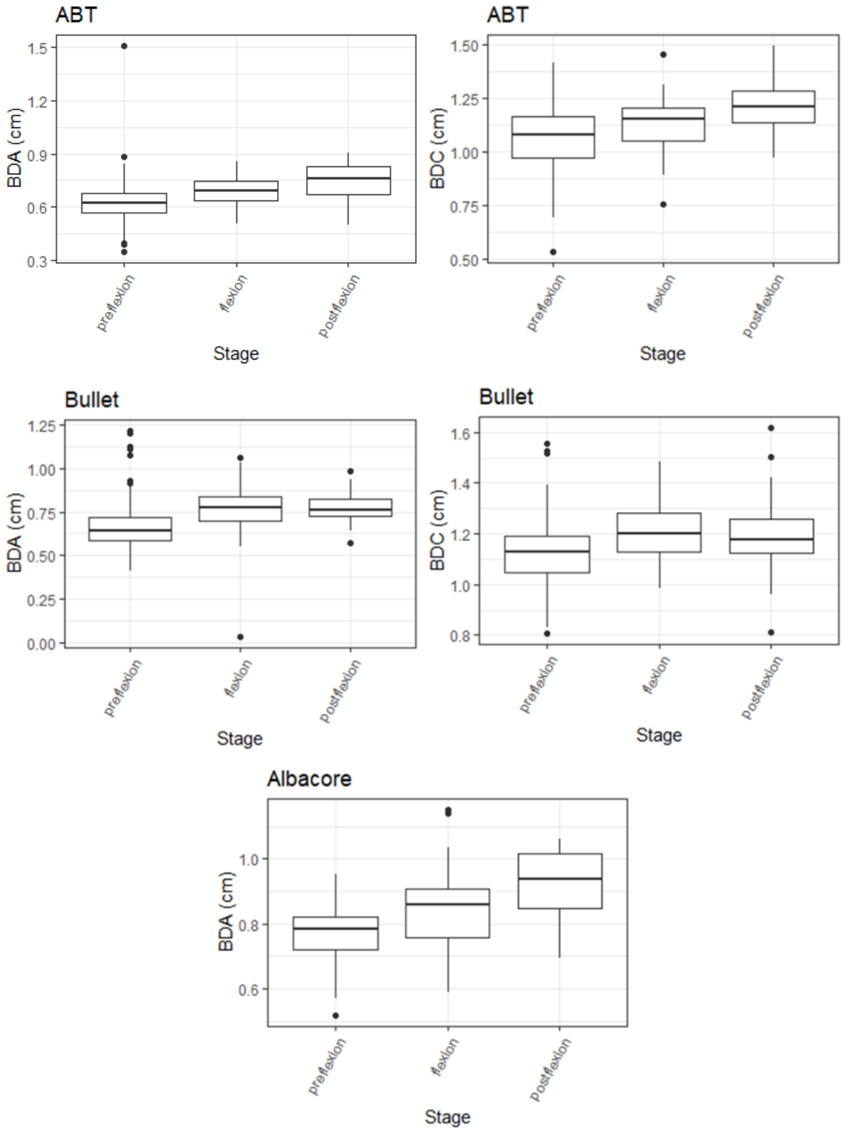


Fig 12 – Significant differences in BDA or BDC among the principal groups of developmental stages for ABT, Bullet and Albacore.

Then, analyzing the differences within the development macro-groups, ABT has significant differences between the Early and Normal post-flexion (p-value = 0.003), Bullet Tuna between the Early and the Late flexion (p-value = 0.02) and the Early and Normal post-flexion (p-value = 0.001), Albacore between all the flexion groups (p-value = 0.03 between early and late flexion and p-value = 0.03 between flexion and late flexion) (Fig. 13). This probably means that early development may have been due to better nutritional conditions manifesting in a larger BD.

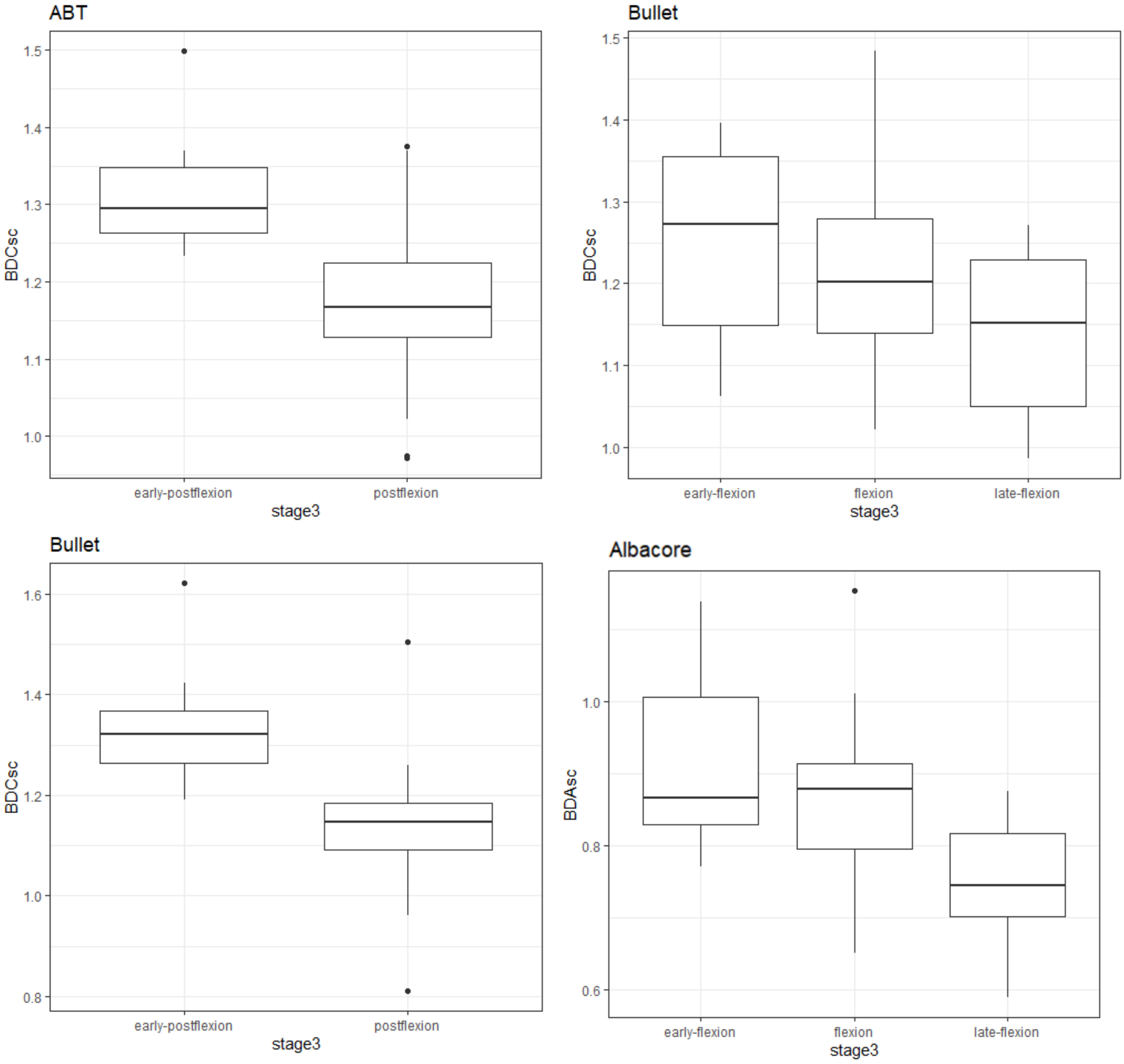


Fig 13 – Significant differences in BDA or BDC among the sub categories of developmental stages for ABT, Bullet and Albacore.

3.4 Discussion

The obtained results represent a further step forward in the characterization of spawning areas common to three important Tuna species. Larval sampling was conducted during the spawning season, covering an area where larvae experience their development. Spatial distribution analysis of larvae in the Strait of Sicily highlighted different patterns among species. Environmental parameters seem to be helpful in describing the observed early or late larval development.

The estimated ages allowed us to verify heterogeneity from year to year, indicating multiple spawning events within the studied area at different times. In fact, Tuna fish are multiple batch spawners, and the reproductive events follow one another within their reproductive season (Ciannelli et al., 2015).

The back trajectories analysis showed the paths taken by each individual larva, and like in other studies focused on different fish species in the same region, the advection was mainly controlled by the flow of AIS (Cuttitta et al., 2004; Torri et al., 2018; Patti et al., 2018, 2020). The surface current can transport the planktonic fish stages and concentrate them in the South-Eastern retention area, where other larvae coming from the east sector also converge. Through running lagrangian simulations (back trajectories estimations), we also evaluated the environmental conditions experienced by larvae throughout their lives, starting from their estimated origin points. This way, we were able to improve our knowledge about the spawning environment selected by the adults in the study area. The geographical positions of the origin points varied significantly by species and by year. However, in all cases the larval trajectories reflect the dominant path of the local surface current in the spawning and adjacent areas. The larvae born at the edge of the thermohaline front tend to be trapped in this area. It is also interesting to note how larvae of a similar age, depending on their origins, can experience different paths, covering large or short distances, and can be often found in common retention areas. This, of course, can also have an impact on their development and/or survival. The events that affect larval development along their individual path from the spawning sites are fundamental in assessing reproductive success. In this framework, oceanographic processes play a crucial role in determining the spatial distribution of the planktonic fish stages, controlling the advection from the spawning areas to more or less suitable retention zones (Pepin et al., 2003; Petitgas et al., 2006). Other important factors affecting larval development and survival are the maternal effect, the area in which they spawn, and stochastic events during transport. Even the water masses origin can affect larval growth, as shown by previous studies on Bullet Tuna (Cortés et al. 2004, Laiz-Carrión et al., 2010, 2013).

The analysis of available satellite data (SST and Chl-a) and bathymetry associated to larval trajectories increased our knowledge about the Tuna spawning habitat in the Strait of Sicily. Some observed patterns, such as the coastal attitude of Bullet or the different distributions depending on the temperatures, were already evident in chapter 1, where the habitat of sampling sites was characterized. However, back trajectories analysis, in evidencing different origins for larvae from the same sampling area, delivered the added value of giving insights into the maternal effect that in the study of the larval habitat was not evident, in these analyses it is revealed.

We had hypothesized that the environmental conditions experienced by larvae along their passive transport phase would affect the development of these organisms. This is not evident by observing the single standardized morphometric parameters. But some correlation is highlighted by evaluating the years individually. However, we do not believe these results sufficiently explain any intraspecific differences between the measurements taken and standardized. The same can be said of evaluating the last two days lived by the larvae, which did not give good correlations between morphometric measurements and environmental parameters. We could hypothesize that temperature and Chl-a alone are not sufficient to explain the intraspecific variability because environmental factors alone explain less than 40% of the difference in larval growth (Caldarone et al., 2003).

However, the individual's experience along the paths appears to influence the flexion stage of the notochord, anticipating or delaying it. We hypothesized that warmer waters could anticipate development, and low Chl-a indicated unfavorable nutritional conditions, slowing the notochord development. Our results confirmed the hypotheses regarding temperatures, finding early developing larvae in warmer waters, especially in the case of Bullet. For Chl-a, we found the opposite trend to what was hypothesized. This could be due to competition phenomena. We hypothesize that in richer environments, there are more competitors and predators, leading to a lower acquisition of energy resources and energy waste for escape and hunting, leading to a late notochord development.

From an ecological point of view, the BD emerged as an appropriate morphometric parameter to detect the larval nutritional condition in these species. This evidence agrees with previous studies on other fish species at the larval stage (Gwak et al., 1999; Diaz et al., 2009; Torri et al., 2021). We hypothesized that BD could be a good indicator for the three Tuna species, and it was confirmed, particularly for ABT. In fact, by evaluating the standardized body depth measurements, we verified that the larvae in flexion stage were larger; experiencing a condition that greatly increases their survival chances (Osse et al., 1997). Also, this trend is confirmed by comparing the body thickness with early and late development. For all three species, the early larvae were thicker than those with normal or late development, further confirming that the body depth is an excellent indicator of the state of Tuna larvae health. Since this is related to early development, it means that the larva has had access to more resources, growing more and initiating the flexion of the notochord earlier. Together with the conditions verified by us, these factors could lead Bullet, the faster growing species among those analyzed here (García et al. 2006), to a further accelerated development than the others. It can lead to resource competition phenomena or early juvenile Bullet Tuna preying on other Tuna species, as hypothesized by Bakun & Broad (2003).

Condition, growth and survival in marine fish larvae are influenced by food (Green & McCormick, 1999), temperature (Green & Fisher, 2004), hydrographic patterns (Utne-Palm & Stiansen, 2002) and environment in general (Pepin & Myers, 1991). However, the genotype of the parents could be an essential factor as well (Chambers et al. 1989; Green & McCormick, 2005). We did not evaluate any genotypic differences and a parental effect due to purely genetic factors in this study. Embryo and larval characteristics, developmental rate, and metabolism are affected by the parents' genotype (Chambers et al. 1989; Green & McCormick, 2005). Together with the environmental parameters, they can lead to the manifestation of morphological differences and can affect the success of recruitment. More in-depth studies that also involve genetic aspects are therefore recommended.

The early life stage analyzed here represents the most critical period in the life history of fish, affected by the highest mortality rates (Hjort, 1914). Understanding Tuna population dynamics is essential to determining the fundamental features of survival processes in the early life stages (Cowen & Sponaugle, 2009). It is also necessary to understand the recruitment of the three Tuna species analyzed here, and this could be essential information for stock assessment evaluations and sustainable management.

3.5 Conclusion

The environmental conditions experienced by the organism are fundamental from the first day of life. In the early life stages, fish larvae move passively, as transported by currents. Therefore, a deep knowledge of the physical processes that affect fate and dispersion of larvae appeared necessary.

Several studies have been done to define the transport dynamics affecting important commercial species' recruitment. Most of them have been carried out on small commercial pelagic fishes (Zarrad et al., 2006; Sabatés et al., 2007, 2013; Torri et al., 2018; Patti et al., 2020). On the other hand, less has been done for other species characterized by fast development rates like Tunas.

Marine fish larval mortality is linked to life-history traits like size and growth, but the processes that influence variability in these traits are poorly understood. Conditions and development in fish larvae are affected by food (Green & McCormick, 1999), temperature (Green & Fisher, 2004), and hydrographic patterns (Utne-Palm & Stiansen, 2002). As hypothesized in a recent study (Ishihara 2019), SST could have a more significant influence before the flexion stage. After this morphological change, the availability of prey influences growth. Therefore, the most influential environmental factor would change according to the larval developmental stage. This hypothesis suggests a focus on future studies on food availability in relation to the developmental stage may be beneficial.

Understanding the link between ocean patterns, spatial distribution and paths of early life stages, and other environmental parameters is also crucial for the sustainable management of fishery resources (Chavez et al., 2003; Pörtner & Knust, 2007). Oceanographic processes and current transport can play a key role in affecting mortality rates, the recruitment and fluctuations of the adult population (Patti et al., 2020). These modelling approaches can improve the understanding of Tuna reproductive biology processes and early life history and eventually contribute to Tuna fisheries management. Identifying spawning areas (e.g., by means of hydrographic models) and retention spawning products could be the baseline for developing fishery-independent recruitment indices. Furthermore, as suggested by Mariani et al. (2010), better information on spawning areas and larval habitats can help establish marine protected areas or areas closed to fishing for a valid protection strategy of these important species.

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Chapter 4. Travelling in time, from past to future. Modelling Tuna larvae distribution and abundance in the Central Mediterranean Sea in a warming scenario.

Abstract

In the study of ecological systems, observations are often brief moments, they describe an instant in an ever-changing system. These moments are informative and helpful to better understand what dynamics affect the studied environment. Still, it is often not taken into account that environmental conditions change rapidly. Global warming is one of the changes that can be the most drastic. Climate models for the future scenario project the ocean temperature values 5 to 7 higher compared to now, by 2100 and due to ocean heating. The response of organisms to this change depends on their plasticity and thermal tolerance. In this study, considering simulated water overheating, we analyze the possible larval habitat changes of three Tuna species that reproduce in the central Mediterranean. We analyze the temperatures they have experienced in the past and then see how an increase can alter their distributions and abundances. Our results show that the potential larval habitat can expand depending on the species, but t there could be a significant loss to Atlantic Bluefin Tuna (ABT) larvae abundance. The ratio between the three species' abundances could vary and lead to new balances. The results obtained here can aid in fisheries management policies for important fish species like Tuna, considering the influence of possible climate changes.

4.1 Introduction

The oceans and seas are the cradles of biodiversity, hosting various ecosystems and organisms of all sizes. Due to climate change, ocean warming affects marine organisms at multiple trophic levels, changing their biogeography, community assemblage and life histories (Pinsky & Fogarty, 2012; Rhein et al., 2013; Bindoff et al., 2019). Climate models for a high-emission future scenario (Representative Concentration Pathway (RCP) 8.5) project the ocean temperature values 5 to 7 higher compared to now, by 2100 and due to ocean heating (Van Vuuren et al., 2011). This phenomenon affects the mixed layer, in which alterations are evident first, and the entire water column, increasing the stratification and impacting all marine life and communities (Bindoff et al., 2019).

The responses to environmental changes depend on species plasticity and, therefore, on their ability to respond to change and the ontogenetic stage that can be mainly affected by these changes (Koehn et al., 2011; Muhling et al., 2015). The most significant effects of a rise in temperatures will be noted where species already exist near the upper limits of their physical tolerances (Pörtner & Peck, 2010).

Although large pelagic fish like Tuna are capable of significant geographical movements, they can be affected at different life-history (LH) traits levels (Kimura et al., 2010). Increasing temperature can affect swimming, physiological function, time and success of egg hatching, larval development and migration patterns (Dizon et al., 1977; Blank et al., 2004; Fromentin et al., 2014; Gordoa & Carreras, 2014). The entire spectrum of life-history can be involved, especially the spawning season (Medina et al., 2002). The planktonic and pelagic phases are delicate. These are crucial for the future stock due to its enormous mortality for many physical, biological, or ecological reasons (Hunter, 1976).

There are temperate, tropical, or subtropical Tuna, and the effect can differ depending on their thermal preference (Juan-Jordá et al., 2013). Temperate ones, in particular, may experience temperature conditions not suitable for their thermal tolerances while travelling to their spawning areas (Reglero et al., 2014). This, however, may be ideal for their larvae, which prefer warm water.

Atlantic Bluefin Tuna (ABT) (*Thunnus thynnus*, Linnaeus, 1758) is a temperate Tuna, its adult behavior with different thermal ranges is well studied. Tagging analysis verified that they escape from warm water masses (Teo et al., 2007). Tropical Tuna can also be affected by the thermal change, but the repercussions are less well known at adult stages (Muhling et al., 2015). The typical Tuna's large movements (horizontal and vertical) make prevision difficult.

For species driven by environmental cues such as the temperature seasonal rise to initiate reproduction (like ABT), an increase in temperatures could anticipate the reproductive peak (Ciannelli et al., 2007; Reglero et al., 2012; Alvarez-Berastegui et al., 2016).

Temperature distribution variation can lead to alteration in larval habitat, with possible loss of actual spawning grounds and use of different areas that are now not common or never used (Ciannelli et al., 2005). Alternatively, they can even lead to the total loss of reproduction of some species in areas where Tuna previously reproduced or fed. Increased sea temperature accelerates metabolic processes of adults and larvae (Duarte, 2007), and larval dispersion process can be altered, changing horizontal transport (O' Connor et al., 2007).

However, the temperature is not the only driver of larval development as for other species. The other conditions must also be maintained (oligotrophy, currents, predators, etc.) since the increasing temperature can alter them. All prerequisites must be met to maintain these areas as larval habitats. In fact, despite the evident importance of water temperature for spawning, actual spawning grounds are much more spatially limited than suggested by water temperature alone (Muhling et al., 2017).

Due to the increasing temperature, economic activities such as fishing are impacted, essential for many coastal countries and the world economy. Together with the effect of climate change, it would add additional complexities. Above all, there could be repercussions at the ecosystem level, as Tuna are top predators in pelagic ocean ecosystems.

The present study analyses the complex Mediterranean Tunas' spawning area, the central Mediterranean Sea. It is a site with particular climatic conditions and water masses circulation, which makes it unique. Chapter 2 has seen the present states from in situ data collections. This work will analyze the larval habitat change in the central Mediterranean for three species ABT, Bullet Tuna (*Auxis rochei*, Risso, 1810) and Albacore (*Thunnus alalunga*, Bonnaterre, 1788). Evaluating changes over time and in possible future scenarios. These three species have slightly different thermal needs at the larval stage, but share space and time during the reproductive and early larval stages in our study area and other spawning sites in the Mediterranean Sea (García et al., 2005). Bullet, tropical Tuna, at the larval stage, has a higher temperature tolerance range than the other two species (Muhling et al., 2017). On the other hand, ABT and Albacore, temperate and subtropical respectively, from previous studies, show a less broad range (Muhling et al., 2011; Reglero et al., 2014).

Past scenario - We modelled the habitat suitability of 8 years of missing larval data to amplify the study time spectrum and compare habitat suitability with actual data from the present. Moreover, getting familiar with the study area extended temperature dataset can improve the base for some assumptions in the manuscript.

Increase simulations - We modelled two different responses with the simulated temperature increase, presence-absence occurrence response (Model 1), and abundance response (Model 2). To obtain the probability of finding larvae in our study area with the temperature increase and how many larvae we could find.

4.2 Methods

Study area - We chose to focus our studies on the Strait of Sicily. It is distinguished by being the meeting point between two different water masses, the Eastern warmest and most saline and the Western one deriving from the Atlantic current entering from Gibraltar, colder and less saline (García

Lafuente et al., 2002). This area has been documented thanks to the historical data deriving from the Italian National Research Council (CNR) “Bansic” oceanographic cruises for ichthyoplanktonic studies. It is a crucial reproduction area for the three Tuna species (García et al., 2005; Puncher et al., 2015; Russo et al., 2021).

Data collection - The Ichthyoplanktonic data for the present scenario and prevision for species responses derives from data collected during the spring/summer of seven years, from 2010 to 2016 included. These surveys were carried out by CNR, with a regular grid of sites (1/10°×1/10° along the continental shelf, and 1/5°×1/5° offshore), collecting ichthyoplankton with two types of nets, Bongo 40 and Bongo 90. The base for temperature modelling comes from CTD data. We analyzed data derived from Bongo 40 and Bongo 90 samples for the presence-absence response. For the abundance, we have selected only data deriving from Bongo 40 because the sampling grid uniformly been adopted as the basis of future forecasts over many years, so we considered it more suitable.

Past scenario - For possible data distributions in past years, from 2002 to 2009, we used sea surface temperatures (SST) deriving from satellite data (MODIS). We extracted the data collected every eight

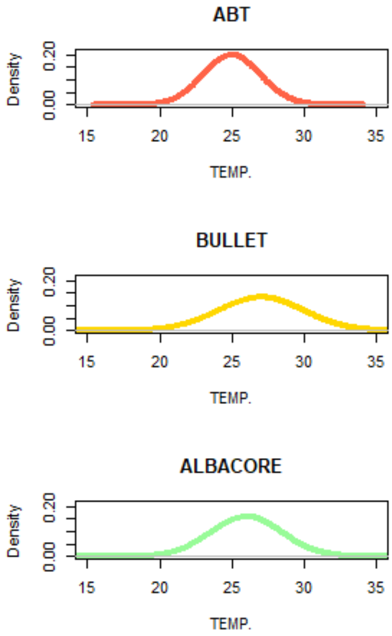


Fig. 1 – Thermal tolerance of ABT, Bullet and Albacore Tuna.

days in the interest area, with a resolution of 4 km, selecting only the months that potentially coincide with the spawning period of the three Tuna. We, therefore, verified what could have been in the past, in the study area, the most suitable periods in terms of temperatures for larval development, starting from the knowledge on larval thermal tolerance. These data for each species at the larval stage was taken from literature in areas comparable with our study area (Muhling et al., 2011, 2017; Reglero et al., 2014). It is reported in Fig. 1, and then used to calculate the probability of the occurrence of each species related to the month.

Increase simulations – Based on in situ data from the CTD, we have elaborated two different Generalized Additive Models (GAMs) for each species, one for presence-absence and one for abundance. At first, we modelled the probabilities of finding the three species in relation to the selected variables and subsequently structured eight matrices, with a simulated temperature increase, to evaluate the possible response of each species to the rise in temperature and how they might vary in presence-absence and abundance. The increment was +0.25 ° C for each simulation, coming to +2°C.

Model 1 - Is a GAM (i) for the presence-absence response, with binomial distributions and surface temperature (◦C), Latitude and Longitude as covariates, to have predictions that also consider the geographical area.

$$(\boldsymbol{i})\ \boldsymbol{Probability\ of\ presence} \sim \boldsymbol{Temperature} + \boldsymbol{Latitude} + \boldsymbol{Longitude}$$

Mean probability of presence in relation to temperature and geography was calculated to show how geographic distribution can vary and how many points of our data sampling grid of the present could be lost or added due to the increase in temperatures or purchased because these have become suitable in areas that previously were not.

Once we calculated the probability of presence for each geographical point, after this referred to as "pixel", we set the probability of finding a larva as a positive chance from 0.05 upwards. The value is not very high, but considering many zero numbers present on our grid and in general, in larval distributions, we have evaluated it as the minimum acceptable probability for the presence.

We also made interpolation maps with the probability to find larvae for each species and for each temperature increase that we calculated.

Model 2 - Is a GAM (ii) for the abundance response, with Poisson distribution and surface temperature (◦C), salinity (PSU), the volume of water filtered (l) as an offset after natural log transformation to account for the effort used in catching the sample, finally Latitude and Longitude as covariates.

$$(\boldsymbol{ii})\ \boldsymbol{Abundances} \sim \boldsymbol{Temperature} + \boldsymbol{Salinity} + \boldsymbol{Latitude} + \boldsymbol{Longitude}\ + \boldsymbol{offset}\ (\boldsymbol{log}(\boldsymbol{Volume}))$$

We also evaluated the salinity for the abundances because the areas that usually show more larvae are close to a saline front, recurrent in the Eastern region (Russo et al., 2021). It creates aggregation areas that affect the abundance values found. The average increase in abundances was calculated for each species and each increasing step to highlight the most significant abundances recorded at what increased degree. The increasing rate between one simulation to succeeding was calculated by subtracting the average value of the expected abundances from that of the previous simulation (e.g. simulation +2 mean – simulation +1.75 mean).

4.3 Results

Past scenario - The SST from 2002 to 2009 show a gradual rise in temperatures with a peak in August (Fig. 2). The thermal tolerance in Fig. 1 shows how Bullet Tuna prefer warmer water and how the three species increase in density from 20° C upwards.

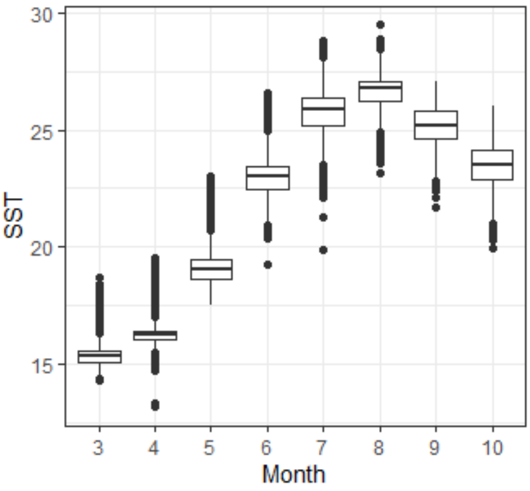


Fig. 2 – SST trend from 2002 to 2009, satellite data.

ABT and Bullet increase the chances of occurrence in May and Albacore in June (Fig. 3). August is the month where the ABT occurrence probabilities decrease due to the too hot waters and then rise again in September, when they drop and go down again in October because they are too cold.

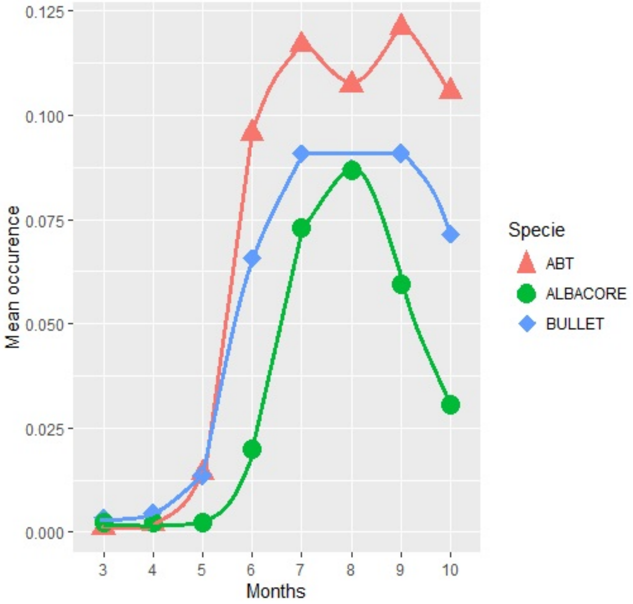


Fig. 3 – Mean occurrence of ABT, Bullet and Albacore Tuna in the past, and related with months.

Looking only at the temperature related to the average species occurrence, Albacore always tends to be the least present species, ABT the most probable and Bullet slightly less present than ABT. These trends are maintained from year to year, but it can be seen in Fig. 4 that in 2005 they were generally lower for all three species. The general trend of temperatures during the years in Fig. 5 shows that 2005 is the coldest year, this explains the decrease in the same year in the species occurrences.

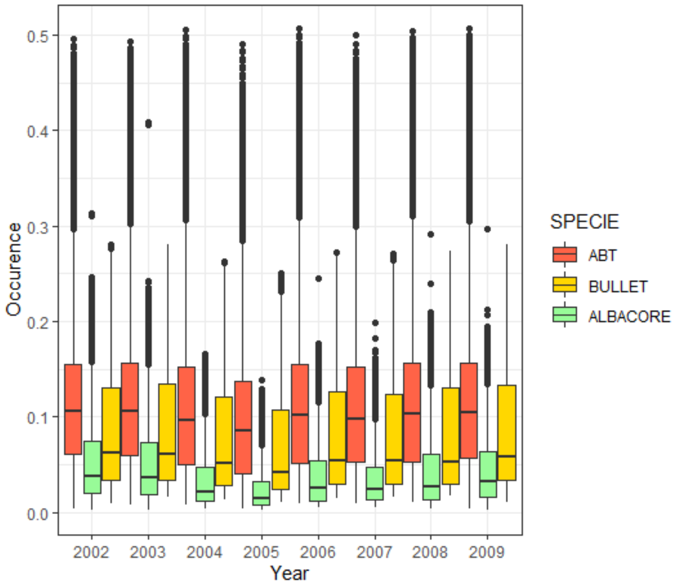


Fig. 4 – Occurrence tend of ABT, Bullet and Albacore Tuna related with past years.

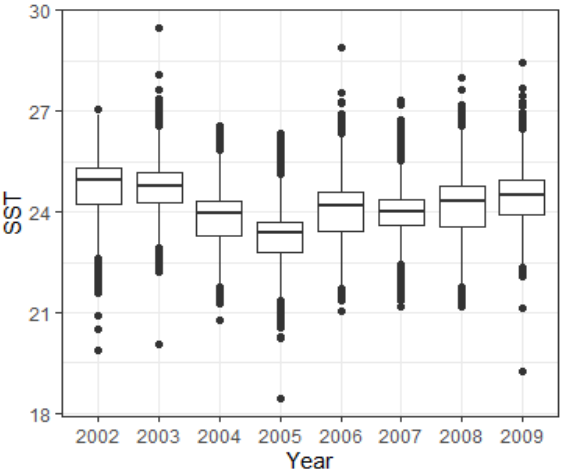
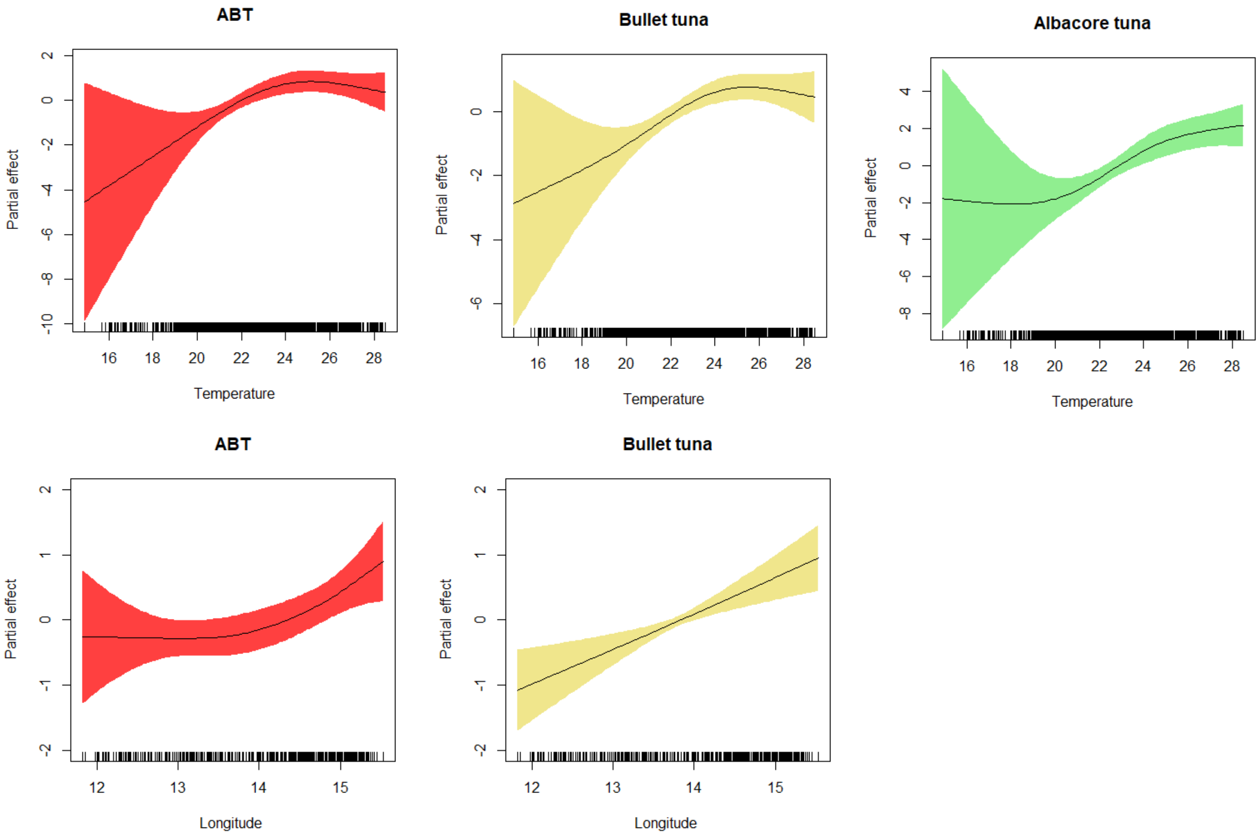


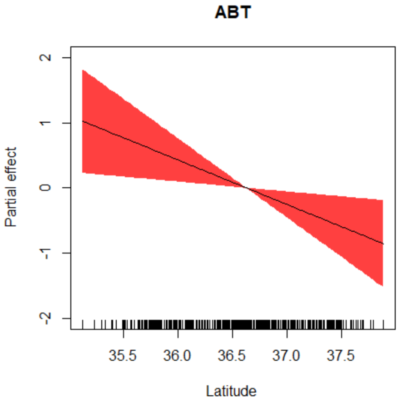
Fig. 5 – General trend of temperatures during the past

Increase simulations – Model 1 - The GAMs analysis explored the relationship between presence-absence and the selected covariates. ABT, Bullet and Albacore presence were positively related to "Temperature". ABT and Bullet with p-value <0.01, Albacore with p-value <0.001 (Fig. 6). There is also a spatial variable's effect on ABT and Bullet with p-value <0.05 for "Latitude" and "Longitude" on ABT and p-value <0.001 for "Longitude" on Bullet.

The prediction's results, applied on the presence-absence GAMs models previously described, gave the probability of finding the three species within the analyzed area, with increasing temperature simulations, summarized in Tab. 1.

The positive chance of finding a larva was set from 0.05 of probability upwards. Albacore showed the widest spatial range for presence data in terms of pixels acquired. ABT does not obtain pixels at +2 but goes negative. All other species have a continuous increase with the rising temperature (Fig.7). Tab. 2 is summarized as mean geographical pixels acquired between the increased temperature simulations and total pixels obtained from the base to the +2°C simulation.





PRESENCE ABSENCE	**TEMP.**	**LAT.**	**LONG.**	**Deviance explained**
ABT	**	*	*	10.9%
BULLET	**	—	***	9.94%
ALBACORE	***	—	—	15.3%

Fig. 6 - GAMs results for presence absence of the tree Tuna and GAMs output analysis with significance value (0 ‘***’; 0.001 ‘**’ ;0.01 ‘*’ ;0.05 ‘.’; > 0.05 ‘—’)

Temp. increment	**MEAN ABT**	**Increment**	**MEAN Bullet**	**Increment**	**MEAN Albacore**	**Increment**
Base	0.06		0.06834		0.027868	
+0.25	0.07	0.002	0.07	0.002	0.03	0.002
+0.50	0.07	0.002	0.07	0.002	0.03	0.003
+0.75	0.07	0.002	0.07	0.002	0.03	0.003
+1	0.07	0.001	0.08	0.002	0.04	0.003
+1.25	0.07	0.001	0.08	0.001	0.04	0.003
+1.50	0.07	0.0007	0.08	0.001	0.04	0.003
+1.75	0.07	0.0002	0.08	0.001	0.05	0.002
+2	0.07	-0.0005	0.08	0.0009	0.05	0.002
TOT		0.008		0.01		0.02

Table. 1– Tuna larvae mean occurrence probability in the study area. Increment = increase in probability between one simulation and another. TOT = total increase from the basic condition to the maximum simulated increment. Base = occurrence probability from real data taken during oceanographic cruises.

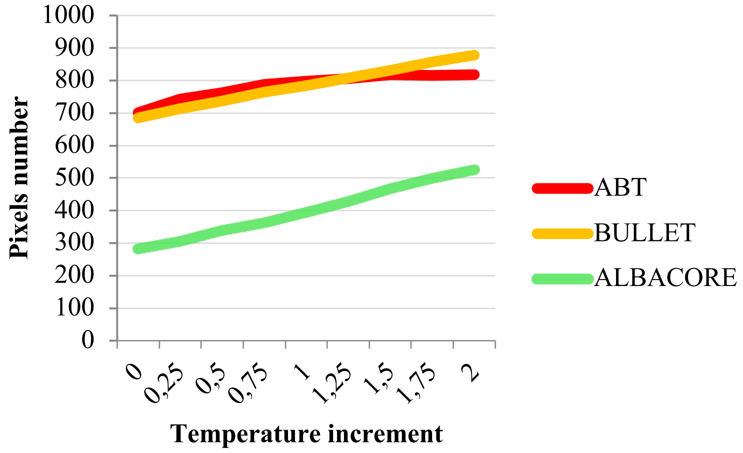


Fig. 7 – Number of pixels for each simulated temperature increase.

SPECIES	**TOTAL PIXELS ACQUIRED**	**MEAN PIXELS ACQUIRED**
ABT	117	14.62
BULLET	193	24.12
ALBACORE	242	30.25

Table. 2 – Geographical pixels acquired with increasing temperature simulation.

The maps can help to appreciate how the species distribution area at the larval stage can expand or shrink in the event of rising temperatures. We assembled loop videos visible at the link below or by scanning the QR code in Fig. 8.

ABT - https://media.giphy.com/media/FnDQTzZ1TezRHgFmB8/giphy.gif

BULLET - https://media.giphy.com/media/oWw28XK6YSskyYSRpz/giphy.gif

ALBACORE - https://media.giphy.com/media/fiP4NqUsxMRCF3nuyz/giphy.gif

a b c

Fig. 8 - Maps showing the occurrence changes for each simulated temperature increase, for ABT (a), Bullet Tuna (b) and Albacore Tuna (c).

Model 2 - The abundances of the three species showed a different response to the simulated temperature increase. The GAMs analysis explored the relationship between abundance and the selected covariates. ABT showed a relation with “Salinity”, “Latitude”, and “Longitude”, with p-value <0.01 for “Salinity” and “Longitude” and <0.05 for “Latitude” (Fig. 9).

Bullet showed a relation with p-value <0.05 for “Temperature”, “Salinity” and “Longitude”, while Albacore only for “Temperature” (Fig. 9).

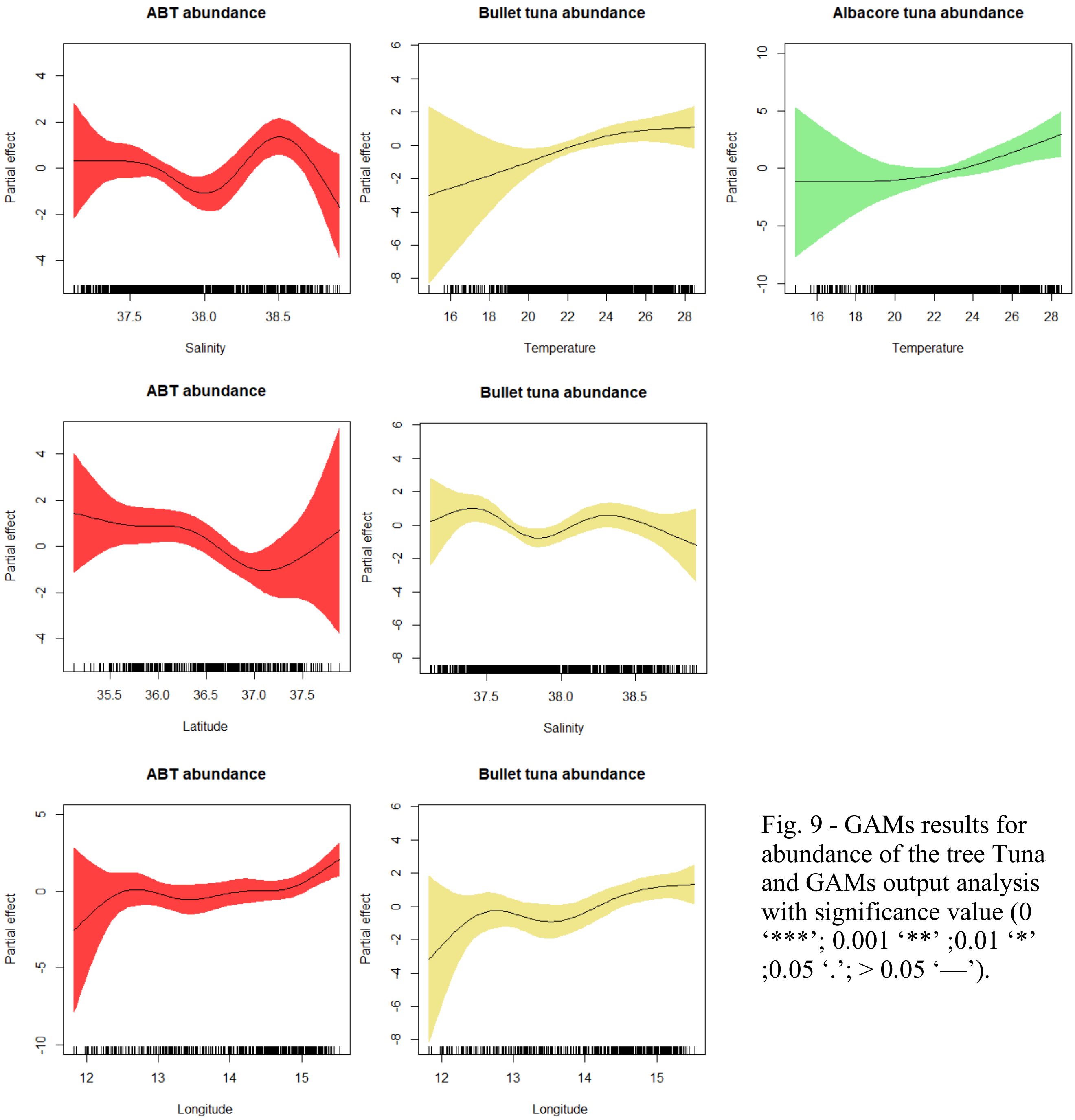


Fig. 9 - GAMs results for abundance of the tree Tuna and GAMs output analysis with significance value (0 ‘***’; 0.001 ‘**’ ;0.01 ‘*’ ;0.05 ‘.’; > 0.05 ‘—’).

ABUNDANCE	**TEMP.**	**SAL.**	**LAT.**	**LONG.**	**Deviance explained**
ABT	—	**	*	**	27.6%
BULLET	*	*	—	*	22.1%
ALBACORE	*	—	—	—	29.2%

The prediction's results, applied on the abundance GAMs models previously described, gave the probability of normalized abundance (log larva/m3) of the three species within the analyzed area, with increasing temperature simulations.

ABT showed a constant decrease in the average abundance as the temperature increased. With a total decrement of -0.04 larvae from the mean of +0.25 °C to +2°C. Bullet Tuna showed a low increase of the mean larva number and a constant increment of the mean rate. Finally, a total increment of 0.02 larvae from the mean of +0.25 °C to +2°C. Albacore Tuna showed an increase in average abundances and a constant increase in the mean rate. With a total increment of 0.12 larvae from the mean of +0.25 °C to +2°C.

Considering the three species together (Fig. 10), what can be seen is that ABT and Bullet have a comparable initial abundance. Still, as the temperature increases, Bullet is favored over ABT. Albacore, a species with minor abundances, assumes values similar to ABT at + 2°C.

A summary of the mean value and mean rate is reported in Tab. 3.

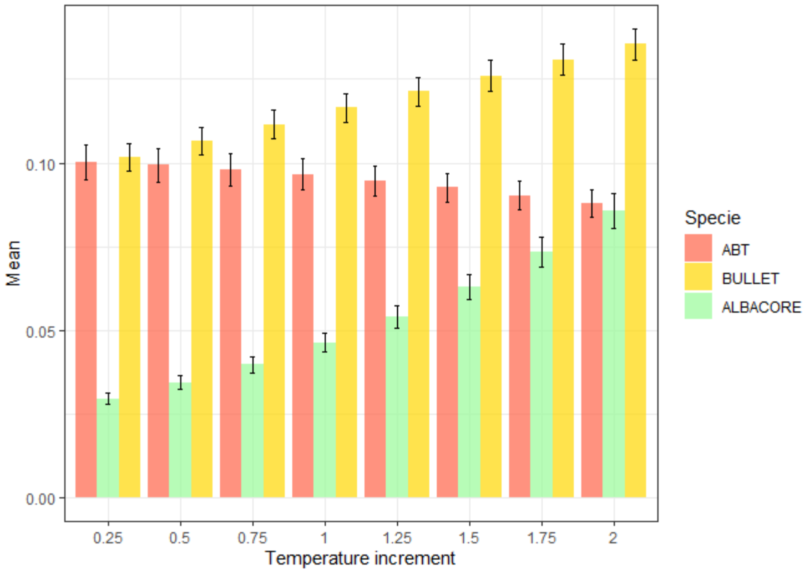


Fig. 10 – Mean larval abundance probability and standard error for each species and for simulated increment.

Temp. increment	**MEAN ABT**	**Increment**	**MEAN Bullet**	**Increment**	**MEAN Albacore**	**Increment**
+0.25	0.1	-0.001	0.1	0.005	0.03	0.005
+0.50	0.1	-0.001	0.11	0.005	0.03	0.006
+0.75	0.1	-0.002	0.11	0.005	0.04	0.006
+1	0.1	-0.002	0.12	0.005	0.05	0.008
+1.25	0.09	-0.002	0.12	0.005	0.05	0.009
+1.50	0.09	-0.002	0.13	0.005	0.06	0.010
+1.75	0.09	-0.003	0.13	0.005	0.07	0.012
+2	0.09		0.14		0.09	
TOT		-0.04		0.02		0.12

Table. 3 – Tuna larvae mean abundance predicted in the study area. Increment = increase in abundance between one simulation and another. TOT = total increase from the +0.25°C simulation to the maximum simulated increment.

4.4 Discussion and conclusion

The results show that, depending on the species, the temperature response in the past, present and future is different. Despite ABT larvae being usually favored by higher temperatures (Russo et al., 2021), we have seen how, in our future simulations, its abundances decrease with increasing temperature. This kind of result seems to be partially valid for Bullet larvae, which in our past simulations of August remain constant rather than grow despite it being a tropical Tuna. Nevertheless, future simulations show an increase in distribution and abundance for rising temperatures. On the other hand, Albacore is the most favored species, with an increased rate higher than the other two in abundance and the possible distribution area for each increased simulation step.

Temperatures in the past were consistent with those observed in the seven years of sampling verified in chapter 2. Therefore, looking only at the simulated occurrence in the past, the proportion between the three Tuna is consistent with studies carried out with data collected in situ (Reglero et al., 2012).

In our results for presence-absence, the amount of available habitat tends to increase for all three species with the increasing temperature. However, this is linked to several factors, in a complex environment such as the one in which the larvae develop, not only the temperature drives the distribution (Alemany et al. 2010; Alvarez-Berastegui et al., 2016). Other components such as food, predators, food for the breeding adults, currents, and many others, also play a fundamental role in the geographic positioning of the larval habitat (Bakun, 1996). In our simulations, Longitude has a role on ABT and Bullet presence, with the tendency for greater presence towards the east. The existence of a saline front in the east and an upwelling area along the Southwest coast of Sicily could be the reason (Patti et al., 2010; Torri et al., 2018). The cold rising waters that constantly form along the Southwest coast drastically reduce the likelihood of finding Thunnini larvae in that area. In fact, they tend to aggregate in warm and oligotrophic waters, probably as a strategy to encounter fewer predators (Bakun, 2013). Latitude also influences the probability of the presence of ABT because there is a spawning zone just around Malta (Druon et al., 2016), thus having an effect on the distributions of this species.

The abundances simulations make the framework more complete, showing how it would seem to increase with rising temperature regardless of the available habitat. The abundance does not necessarily follow the same trend. ABT, for example, although the general range is more extensive at the simulation of +2°C, the abundances decrease. This may mean that the larvae are distributed over a broader range at higher temperatures but in smaller quantities. Salinity also plays a role in abundance. Indeed, the aggregation of more larvae on the saline front, as shown in chapter 2, would explain this relationship, also affecting the predicted geographic distribution.

The proportion between the three species at each point of thermal rise also changes. ABT is initially the most abundant species, followed by Bullet and Albacore. Still, if subjected to a more pronounced temperature effect, then the latter two would increase in abundance, while ABT would decrease. This relationship between the three could have repercussions beyond mere presence, absence, or abundance. There would be fewer factors, such as competition between the three or space could be created for more species that prefer warmer waters, such as the larvae of *Katsuwonus pelamis* (Linnaeus, 1758) (Murua et al., 2017), which is already occasionally found in these areas (Russo et al., 2021).

Our simulation only considers the interaction between the geographical area, temperature, salinity, and species. Still, we must also evaluate that the phenomenon of competition for resources can occur between the three species and change the balance between the abundances of Tuna larvae in our area. For example, an increase in Bullet abundance could further affect the decrease in ABT or attenuate the abundances of Albacore. The species' plasticity determines the responses to each environmental change, biotic or abiotic (Koehn et al., 2011; Muhling et al., 2015), and on occasions such as these, it could prove to be fundamental for the persistence of species in the area.

All three Tuna have the same larval habitat and spawning periods that, more or less, coincide. The increasing temperature could also affect adults' behavior, changing the spawning timing. This can lead to a trickle-down on larvae copresence and further change the competition for resources, particularly in the piscivorous phase.

With the higher temperature, the metabolic demands of an organism are also implemented (Blank et al., 2004) and are not necessarily followed by sufficient food availability. Cross studies between the plankton and the ichthyoplankton trends would resolve the question, seeing the prospects of these species in a single vision.

Even phenomena such as the passage from a planktivorous to a piscivorous stage, stimulated by faster growth due to higher metabolic rates, could be influenced by the temperature conditions, accelerating these passages. Still, it is not sure that the surrounding conditions can go hand in hand with this new possible growth trend.

Finally, another impact of climate change, with repercussions on human communities, can be the increased presence of contaminants like mercury, made more bioavailable due to higher temperatures. Bioaccumulation and few safety regulations around seafood, particularly for large top predators like Tunas, can become another critical impact (Bindoff et al., 2019).

The results presented here show the possibilities of future scenarios, which have some degree of uncertainty, because they attempt to capture only the statistical relationship between the surface water conditions and the abundance or presence of early life stages of fish.

The surface variables considered have been proven to be good descriptors of the spawning habitat and larval environment of all three Tuna larvae in the area (Garcia et al., 2005; Koched et al., 2013).

This kind of study also lays the foundations for re-evaluating how adults can be exploited by fishing in the future. But as suggested by Ingram et al. (2017), before establishing new rules for capturing adults, it is necessary to wait for the species (overexploited for decades) to re-stabilize, reaching a new equilibrium. What could occur is an erroneous assessment because the SSB (spawning stock biomass) would appear to be in continuous growth, and only once a new equilibrium has been reached the actual trends can emerge. This results from the combination of the further exploitation model and future environmental scenarios that affect the success of recruitment and natural mortality.

The conception of a stable environment is the base of the current fisheries management, not evaluating possible future changes that can have enormous repercussions on marine communities (Muhling et al., 2015).

Acknowledging rising temperatures, the new fisheries’ management must include prevision on species presence-absence and abundance trends.

Here we made the basic assumptions that the adults remain in the spawning area for the spawning period despite the higher temperature, that the hatching is successful, and that the larvae can have a favorable development. It is clear that since these are simulations, we wanted to see how the larval habitat could change, but there is no certainty that this will be the trend or that the lost habitat could be exactly what is estimated here. Broadening the knowledge of these systems and larval habitats appears essential to protect the marine environment and the organisms that inhabit it.

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Chapter 5. General Discussion

5.1 Synthesis

This thesis is based on the study of the first life-history (LH) traits of 3 Tuna species in the Central Mediterranean Sea, Atlantic Bluefin Tuna (ABT) (*Thunnus thynnus*, Linnaeus, 1758), Bullet Tuna (*Auxis rochei*, Risso, 1810), and Albacore (*Thunnus alalunga*, Bonnaterre, 1788). We began the cognitive path by characterizing the study area, defining its physical and biological aspects. We then began to understand the larvae's ecological dynamics and why adults select this area year after year.

We have ranged from the past to the present to hypothesize possible future trends, all this to expand the knowledge of these species and to better understand reproductive biology, population structure, and recruitment processes by comparing them with the environmental factors of the Mediterranean, a crucial area for the maintenance of Tuna globally (Boustany et al., 2008).

Contained in these chapters (from Chapters 2 to 4) is a significant advance in the knowledge of the effect of the environmental condition on a broad spectrum of factors involving the three species' LH. The results can be helpful for future stock evaluations or fisheries management of species such as those that are commercially important.

With this work, we have characterized the larval and the spawning habitat from an ecological point of view. Previously, only a few observations confirmed these species present at the larval stage (Rooker et al., 2007) but did not document the abundances or associated ecological characteristics, crucial knowledge for species such as ABT that are environmental spawners (Alemany et al., 2010; Reglero et al., 2012; Alvarez-Berastegui et al., 2014). Furthermore, it is essential to characterize the larval stage, the most delicate and with the highest mortality rates (Hunter, 1976), to understand the factors that could further increase these rates and evaluate the possible future stock state in advance. There are other spawning areas in the Mediterranean, but each site has its unique characters selected by the organisms for reproduction (Karakulak & Yıldız, 2016; Muhling et al., 2017). Getting to know this area in depth was critical. There is also the issue of the possible presence of two Mediterranean

ABT subpopulations hitherto hypothesized and never confirmed (Cermeno et al., 2015; Livi et al., 2019). With this study and the chapters 2 and 3 results, we add pieces to this complex puzzle. Through chapter two, we have seen the areas of greatest abundance, with the third chapter the different origins. We could hypothesize that the Capo Passero area is a meeting and aggregation point for two possible separate spawning stocks, one from the East one from the West. In the future, it would be informative to study and sample the origin areas of the Ionian and add genetic analyses that can better identify the two possible groups.

Finally, the assessments carried out in Chapter 4 allowed us to verify how the three species could respond to predicted increases in water temperatures in the future. This response could lead to new equilibrium and imbalances in the delicate ecological system where these larvae develop and even lead to the disappearance of reproductive habitats. To fully understand the possible environmental effects of an increase in temperatures on the adult populations of these three species.

To understand the demographic processes at the population level, the physiological responses of fish larvae to environmental variability are fundamental. The results of this thesis demonstrate the importance of understanding the complexity of the processes that regulate growth and spatial distributions during the larval stage of these species, which to date were not well known. Field surveys provide essential information to understand the relationship between the abundance and distribution of fish larvae and habitat variables. Still, possible laboratory analyses and simulations in the mesocosm could further improve the responses we observed through the models exposed here in the thesis.

For the three species, this thesis provides knowledge on ecology that can be used to know the effects of environmental variables on specific LH traits of ABT, Bullet, and Albacore Tuna.

Still, there is a long road ahead.

5.2 The difficulty of working with these species

Tunas have a very narrow environmental window for spawning (Alemany et al., 2010), reducing the days to collect these species. Every day for 24h, sampling was carried out during the spawning season. Weather conditions sometimes prevent larvae collection due to too rough seas. Once the plankton specimens had been collected, the sorting and recognition work was very long. In addition, Tunas are among the most complex species to recognize at the larval stage (Puncher et al., 2015).

5.3 Future perspectives

Chapter 2 - Expanding the study area into the Ionian would be interesting. Studying the area's environmental characteristics and adding them to the presently collected data. It would also be helpful and informative to cross-reference data from the central, Western and Eastern Mediterranean. Starting a monitoring program of the entire basin would lead to a complete knowledge of the state of the Mediterranean ABT stock.

Chapter 3 - The study could be integrated with genetic analyses to discriminate possible subpopulations. The obtained information from the chapter can also be the basis for analyzing the piscivorous stage and associated ichthyoplankton community.

Chapter 4 - It would be interesting to integrate it with mesocosm experiments and add studies on the species' metabolic rates to re-evaluate possible future stocks trends related to global warming with greater accuracy.

5.4 Concluding remarks

1. The central Mediterranean Sea, the Strait of Sicily in particular, is a significant larval habitat for the three Tuna species considered in this study.

2. ABT and Bullet were generally more abundant than Albacore.

3. There is a temporal-spatial overlap during the early life stages of these species.

4. Temperature, salinity, and day of the year are key variables to better understanding the larval habitats’ Spatio-temporal distribution of these species in the area.

5. The area dynamics seem to play a crucial role in larval retention in the Eastern zone together with a thermohaline front.

6. Tuna larvae, in general, are found in oligotrophic areas.

7. The back trajectories analysis showed different derivation points for ABT. Some from the Eastern others from the Western sector, we speculate that there may be two ABT subpopulations.

8. For Bullet Tuna, the back trajectories have confirmed the adults' coastal attitude for spawning and the larvae in their spatial distribution.

9. The environmental parameters from the hatching to the sampling time seem to be helpful to describe an early or late development.

10. BD emerged as an appropriate morphometric parameter to detect the larval nutritional condition in these species.

11. In the case of increasing temperature, ABT abundances decrease. This trend is a fundamental factor for evaluating future fisheries’ policies for this species.

12. Albacore Tuna is favored by increasing simulation temperature in abundance and the possible distribution area.

13. In case of temperature increase, the potential habitat of all three species could expand, but not necessarily be followed by the rise in abundances.

5.5 Acknowledgments

I thank my mentor Angela Cuttitta for the given support, patience, and valuable teachings. My foreign tutors, Patricia Reglero and Diego Alvarez, welcomed me to the IEO COB and followed me with dedication during my second year as a PhD candidate. I thank the CNR for allowing me to access the plankton samples banks from the analyzed years and for letting me carry out the oceanographic cruises. Marco Torri for his collaboration on the entire thesis, especially on the statistical front. Lorenzo Ciannelli for the advice given with Walter Ingram during the 43rd larval fish conference. Francisco Alemany for introducing me to the Balearic working group. For support during the morphometric data collection, all CNR technicians, Grazia Maria Armeri, in particular. Dino Patti, for all the help on the oceanographic cruise data and the work done together. For the work-planning phase and the valuable advice during the data analysis, my university tutor Gianluca Sarà. Mr. Emanuele Gentile, Master of the R/V "Urania" and R/V "Minerva Uno " and his crew, are thanked for their work supporting the plankton sampling during the oceanographic cruises.

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Attachments

Characterization of the larval habitat of tuna species in the central Mediterranean Sea

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Atlantic bluefin tuna and other tuna species reproduce in the Mediterranean Sea every summer. Despite the Central Mediterranean Sea, in particular the Strait of Sicily, is one important spawning site for many tuna species, little is known on the relationship between the environmental variables and their larval spatial distribution. Using historical larval collections from seven years (2010-2016) and in-situ sampled environmental variables (Fig.2) we applied a Generalized Additive Model (GAM) approach for modelling the relationships between the presence-absence and the abundance of tuna species and the oceanographic scenario in the Central Med, focusing on Atlantic Bluefin tuna (ABT), Bullet tuna and Albacore (Fig.3). Previously, we conducted a comparison between the two plankton nets used for sampling, Bongo 40 and Bongo 90 (Fig.4) to compare their catchability in relation to the targeted species. The results suggest most tuna larvae were located in the easternmost area in the south of Capo Passero (Fig. 5), an area characterized by specific environmental conditions compared to the surrounding areas.

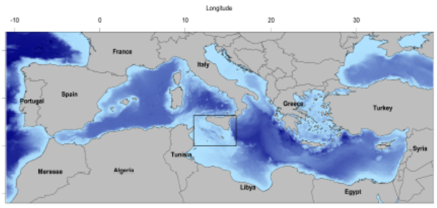

Fig.1. Mediterranean Sea

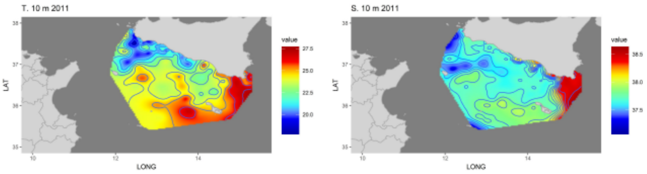

Fig.2. One-year extract showing the geographical tendency of the temperature and salinity variables, common to all the years of sampling

Fig.3. Atlantic Bluefin tuna (a), Bullet tuna (b), Albacore (c)

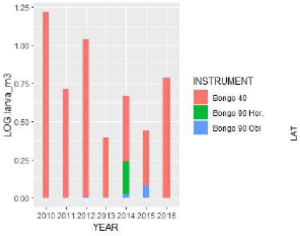

Fig.4. Tuna larva log per m³ per nets type and per year

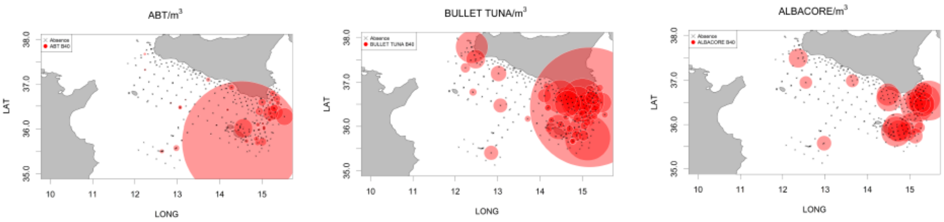

Fig.5. Larva m³ of the three species studied, for all seven years

a

PRESENCE ABSENCE	Temp	Sal	Y.Day	Zoo
ABT	***	--	--	--
BULLET T.	--	*	***	--
ALBACORE	*	--	*	--

b

ABUNDANCE	Temp	Sal	Y.Day	Zoo
ABT	***	***	***	***
BULLET T.	--	**	***	
ALBACORE	--	*	*	--

Tab.1. GAM results for presence-absence (a) and abundance (b) with approximate significance level

GAMs were used in order to explore the relation between environmental parameters and presence-absence or abundance of the species. The results show a probability of ABT occurrence, positively related to temperature (Fig.6 a) the abundance too, but is also related to salinity, zooplankton weight and day of the year, showing that we studied the reproduction peak period (Tab.1, Fig.7). For presence-absence and abundance of bullet tuna, both seem to be influenced by salinity (Fig.6 b, Fig.6 a). It also emerged that the reproduction peak was not studied (Fig.6 c). Similar results emerged for Albacore, with the temperature affecting the presence-absence (Fig.6 d), salinity related to abundance and day of the year that affected the presence absence and abundance (Fig.8 b,c).

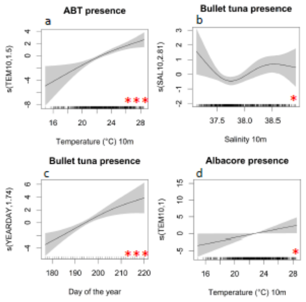

Fig. 6. Environmental drivers for presence-absence of ABT (a), Bullet tuna (b,c) and Albacore (d)

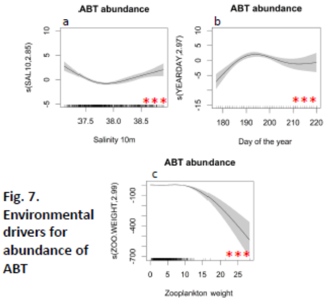

Fig. 7. Environmental drivers for abundance of ABT

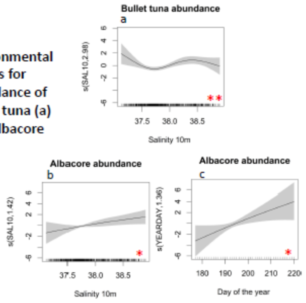

Fig. 8. Environmental drivers for abundance of Bullet tuna (a) and Albacore (b,c)

This study suggests more research in the Central Mediterranean tuna spawning grounds is needed to understand its role compared to other areas in the Mediterranean Sea.

Talk abstract

ICES PICES Early Career Scientist Conference (ECSC4), which will be held on July 17 - 21, 2022. Abstract accepted.

ECSC 2022/1A

Tuna fish life-history traits in the Central Mediterranean Sea and ecological effects on early life stage

Authors: Stefania Russo, Marco Torri, Bernardo Patti, Gianluca Sarà, Angela Cuttitta

Abstract

Thunnus thynnus, Thunnus alalunga and *Auxis Rochei* reproduce in the Mediterranean Sea in the summer period. They sustain some of the most important fisheries on a global scale. Their sustainable management is fundamental and depends on a better understanding of the fish ecology, especially in terms of early life-history traits. Despite the Strait of Sicily (Central Mediterranean Sea) being one important spawning site for Tuna species, little is known on the relationship between the environmental variables and their larval spatial distribution in this area. Using historical larval collections from seven yearly surveys, in-situ environmental measurements and satellite data, we modelled the interaction between ecological conditions and Tuna life-history traits, horizontal distribution, spatial structure and trophic role of the three Tuna species at the larval stage. A multidisciplinary approach involving lagrangian simulations, species distribution modelling, and morphometric analysis has been used to verify the influence of environmental conditions on the distribution pattern of Tuna spawning and its effects on larval development, and consequently, on their survival probability. Finally, the potential impacts of the identified main drivers have been investigated through forecasting models to determine the deposition patterns of Tuna species under possible future climate change scenarios. This work aims to improve the knowledge on the growth and survival of Tuna larval stages, making it under a new light and in a complex system like the Central Mediterranean Sea, where upwelling, gyres and fronts play a key role and where observations could indicate a meta-population structure in Tuna populations.

Keywords:

Thunnus thynnus, *Auxis Rochei*, *Thunnus alalunga*, spawning, ichthyoplankton, recruitment, Mediterranean Sea.