

Initial Characterization of the Bluefin Tuna in the Easternmost Mediterranean Sea

Tal Elmaliach

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE MASTER'S DEGREE

University of Haifa

Faculty of Nature Science

Leon H. Charney School of Marine Sciences The

Department of Marine Biology

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Initial Characterization of the Bluefin Tuna in the Easternmost Mediterranean Sea

Abstract

Atlantic Bluefin tuna (ABFT, *Thunnus thynnus*) is an important species due to its high commercial value and its ecological importance across the globe. ABFT are endothermic warm blooded, allowing them to maintain body temperatures higher than that of the surrounding environment. This unique physical trait enable them to exploit a wide range of pelagic environments from the warm spawning grounds in the Gulf of Mexico and the Mediterranean Sea to subpolar waters of the North Atlantic Ocean were it feeds. Over the year, the growing demand for the ABFT meat, especially from the Japanese market, combining with fast improvements in fishing gear and technology have led to the decline of ABFT and today it is defined as endangered species. In order to restore the population there is a need in strict management effort which is taken place by the ICCAT organization since the 1960s. Yet managing a highly migratory fish can be difficult but can be reduced with the understanding of the population structure. With this understanding, ICCAT and the tuna research community is encouraged to provide improved and suitable solutions for management and to keep looking for special differences in the ABFT population. Even though the BFT remains a focal point for much research, there is still lack of information, knowledge and long-term monitoring data in our region of the eastern Mediterranean Sea (EMS). This study aims to provide an initial investigation of the ABFT within the marine space of Israel. During three consecutive years (2016-2018) we sampled 73 ABFT in Israel by our active participants in fishing trips, observations in marinas and port and interviewing collaborating tuna fisherman during the fishing season. In addition 28 fish were sample during the “great tuna race” in Spain. Fish were characterize using five main methods: 1) measuring size and documenting gender. 2) Characterizing the genetic structure of the ABFT population using two mitochondrial genes (COI and mtDNA CR). 3) Measuring trophic levels based on compound-specific nitrogen isotopic ratio. 4) Characterizing feeding habits based on stomach content analysis. 5) Tracking ABFT Routes in the Mediterranean Sea using electronic tags. In addition Israeli tuna fisheries were characterize for their catches, methods, gear and effort. Results pointes on high ratio of small fish (<100kg, n=57) which support the genetic and tagging results implied on the existence of young resident fish that stays in the Mediterranean throughout the year and migrating to feeding grounds in the west and central areas of the Mediterranean sea. Trophic levels results show inconsistence require reanalysis. Tuna fisheries data is already being used for management decision regarding the conservation of the species in Israel. All together my results showing further investigation and long term monitoring is crucial for the sake of population conservation.

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1. Introduction

1.1. Research Topic and Importance of the Resource

Marine ecosystems represent a rich assemblage of species describing complex dynamics. Attempting to model this system as a whole as well as understanding the different modes of interaction between species is almost impossible (Hortal et al., 2015). However, it may be possible to measure some of the critical outputs of the ecosystem that can allow general predictions about the ecosystem as a whole (Camphuysen 2006). In ecological terms, these outputs are represented in either base production of the marine food chain or at the upper limits represented in apex marine predators. Bluefin tunas (BFT), an apex predator, is a key indicator species for understanding the diversity of the marine ecosystem and assessing its health. As numerous marine top-predators species share the coastal environment with humans and consume the same food, they may also serve as effective sentinels for public health problems (Bossart, 2011).

The BFT is the largest member of the Scombridae family, and the common name for three out of the eight species belonging to the genus "*Thunnus*". These are the Pacific BFT (*Thunnus orientalis*), the southern BFT (*Thunnus maccoyii*), and the Atlantic BFT (*Thunnus thynnus*) (Collette 2001). The Atlantic BFT (ABFT, *T.thynnus*) is a large-bodied marine top-predator inhabiting the pelagic zone with a distribution ranging between the North Atlantic Ocean and the Mediterranean Sea. Its maximum length can exceed four meters, and its official maximum weight is 726 kg, however weights of up to 900 kg have been reported in various fisheries (Mather et al., 1995) Earlier studies have found that ABFT reach maturity at around 4 years (approximately 25 kg) in the East Atlantic and Mediterranean Sea, but in the West Atlantic only around 8 years (approximately 145 kg) (Mather et al., 1995). ABFT are endothermic warm blooded, allowing them to maintain body temperatures higher than that of the surrounding environment (Kubo et al., 2008). This unique physical trait of the ABFT enable them to exploit a wide range of pelagic environments from warm tropical to subpolar waters of the North Atlantic Ocean by making deep vertical dives and migrate over large distances (Fromentin and Powers, 2005). In contrast to other top predator, for example dolphins or sharks that inhabit a specific ecological niche, the ABFT is cosmopolitan, cross boundaries predator that travel between different countries and even continents (Block et al., 2001).

The ABFT fish is an important natural resource with significant economic value. Sustainable ABFT fisheries in the Mediterranean have existed since ancient times, with documented archaeological evidence dating back from the 7th millennium BC (Mather et al., 1995). However, the sustainability of this fishery did not last into the 21st century. Since the late 1960's the range and reported catch per unit effort (*CPUE*) of the species has declined (ICCAT, 2003). An increasing and intensive demand for this fishery occurred in the early 1980's when the Japanese sushi market began to incorporate and the ABFT meat becoming a basic and popular dish in every gourmet restaurant (Fromentin and Ravier, 2005). At once the exploitation of ABFT became significantly more profitable than before and connoisseurs were willing to pay hundreds of dollars and even millions of dollars for a single 200 kg fish (Porch, 2005). In addition, during the 1990's, a sharp increase in the efficiency and capacity of the ABFT fisheries occurred due to major improvements in fishing gear and technology (Fromentin and Ravier, 2005). Ultimately, this chain of events led to the decline of ABFT spawning stock biomass by 81% and 52% in the Western and Eastern Atlantic populations, respectively (ICCAT, 2006). Today the ABFT is defined as endangered species (IUCN Red list 2011).

1.2. Bluefin Tuna Fishing Management

Management of the ABFT is difficult due to its migratory nature, market value, and vulnerability due to late age-at-maturity (Boustany et al., 2008). The continuous decline of the ABFT population has led to the understanding of the importance and the need for international fishing management. Such action took place in 1966 with the establishment of the International Convention for the Conservation of Atlantic Tunas (ICCAT) (Fromentin and Powers, 2005). In the last two decades ICCAT promote ABFT conservation mainly by using four actions; 1) research (GBYP program), 2) annual group meetings within the contracting parties, 3) stock assessments reports, and 4) regulation recommendations includes fishing quotas, defined fishing season, prohibition on any types of unmanned aerial vehicles, and minimum size (ICCAT, 2014). Due to the regulation efficiency, there has been a decrease in overall harvest of both adult and juvenile fish and stocks are showing signs of improvement (García et al., 2013). However, the rate and nature of this recovery is still very much uncertain (Puncher et al., 2015).

The difficulties of managing a highly migratory fish can be reduced with the understanding of their population structure (Boustany et al., 2008). However, the lack of clear barriers in regards to gene flow with such migratory pelagic fishes has complicated the detection of population subdivision (Waples, 1998). since the 1980's, ICAAT has been managing the ABFT population as two separate stocks; an early-maturing eastern stock spawning in the Mediterranean Sea, and a late-maturing western stock spawning in the Gulf of Mexico (Mather et al. 1995). This division was supported by significant genetic variance between the stocks (Boustany et al. 2008; Carlsson et al. 2007).

In addition homing behavior and spawning site fidelity in both sides demonstrated by electronic tagging (Block et al., 2005). However, over the years contradiction evidence documented by electronic tagging suggested a more complex and diverse spawning migration and even mixing between the two management units (Richardson et al. 2016; Galuardi et al. 2010; Rooker et al. 2008; Block et al. 2001; Lutcavage et al. 1999). With this understanding, ICCAT and the tuna research community is encouraged to provide improved and suitable solutions for management and to keep looking for special differences in the ABFT population (ICCAT, 2017).

1.3. Bluefin Tuna in the Mediterranean Sea

The Mediterranean Sea is categorized as temperate with different oceanographic conditions changing sharply from the western to the eastern sections (Robinson 2001). Atlantic surface waters, with relatively low salinity enter the Mediterranean Sea through Gibraltar Strait (Millot, 1999) and move eastward along the North African coast (i.e. the Algerian current) reaching the Eastern Mediterranean, i.e., Levantine basin. The basin is warm, hyper-saline, and highly oligotrophic, as a result of high evaporation rates, very low river runoff and limited vertical mixing (Herut et al., 2000).

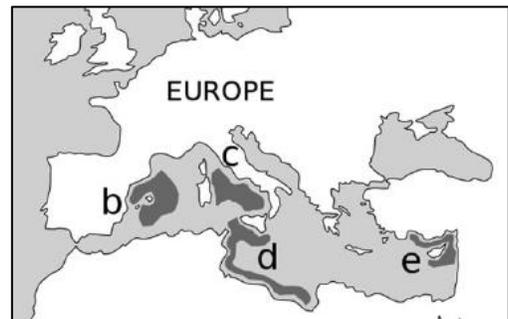


Figure 1: ABFT spawning areas in the Mediterranean Sea. (b) The Balearic. (c) Tyrrhenian Sea. (d) Malta and the Gulf of Sirte (Libya) (e) Levantine Sea (Puncher et al. 2015)

In this complex and changing Mediterranean environment, the eastern stock of the ABFT appear to be using several spawning grounds. Spawning occurs in three specific and restricted

locations; 1) around the Balearic Islands, 2) the Tyrrhenian Sea, and 3) the waters around Malta and the Gulf of Sirte (Libya) (Nishida et al. 1997; Medina et al. 2002; Corriero et al. 2003). Later on the presence of a spawning area in the Levantine Sea has been demonstrated by Karakulak et al. 2004 (Figure 1). Spawning occurs only once a year during the late spring where calm weather conditions allow for the formation of the surface mixed-layer and water temperature reach 24 °C (Schaefer 2001; Druon 2010; Fromentin & Powers 2005). This period is limited to June – early July for the western Mediterranean And around mid–late May–June in the Levantine Sea (Karakulak et al. 2004).

1.4. Problem Statement and Research Question

For the last 30 years a considerable amount of research on the ABFT has been conducted, yet a substantial amount of uncertainty regarding their migration routes, reproduction, feeding behaviors and population structure, still remains (Fromentin, 2003).

Several genetic methods help in demonstrating the possible genetic population structure within the *Thunnus thynnus* species. Still, the present conclusions based on genetic studies on the Eastern ABFT Sea stock structure are rather controversial and not yet conclusive (Viñas et al., 2010). Some studies show significant differences between individuals in different geographic locations in the Mediterranean Sea (Boustany et al., 2008; Carlessen et al., 2004; Carlsson et al., 2007; Reeb, 2010; Riccioni et al., 2013, 2010), while others show no significant spatial genetic divergence and claim a panmictic ABFT population in the Mediterranean Sea (Albaina et al. 2013; Antoniou et al. 2017; Vella et al. 2016; Viñas et al. 2010). In addition, some electronic tagging support the spatial differences hypothesis by detecting residency in several location within the Mediterranean Sea (Cermeño et al., 2015; Metrio et al., 2005, 2004) that can lead to genetically separate population (Rooker et al., 2007).

Focusing in the eastern Mediterranean reveals some evidence of uniqueness of the ABFT population in this basin. ABFT line fishery data confirms the presence of a resident tuna component in the eastern Mediterranean throughout the year as reported in Oray & Karakulak 1997. Microsatellites analysis by Carlessen et al. 2004 suggests the potential for a genetically independent stock of ABFT in the eastern basin of the Mediterranean Sea. In addition, according to Heinisch et al. 2008, the Levantine basin suggested to be function as a habitat for young ABFT,

before gaining a larger body mass and are able to move to the Atlantic Ocean (Heinisch et al. 2008; Sarà 1964). Furthermore, from a map of electronic tag tracks (Figure 2) it is evident that no ABFT, tagged with electronic tags, traveled all the way to the Eastern Mediterranean. This fact, might support the idea of a separate subpopulation in the Eastern Mediterranean and emphasizes the knowledge gap for this area (Natale and Idrissi, 2015).

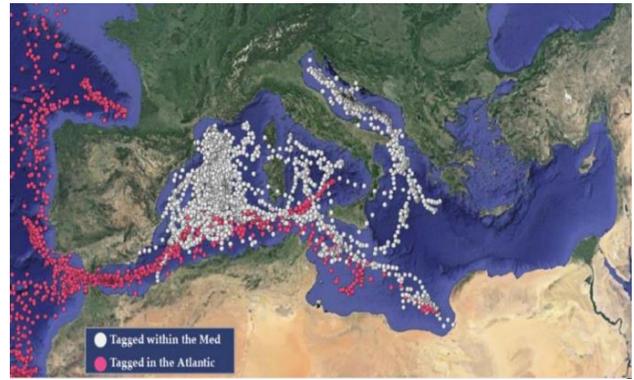


Figure 2: ABFT routes map in the Mediterranean.
The map concludes the accumulated track of tags deployed by GBYP in 2011-2014.

These gaps of knowledge regarding the ABFT population in the east Mediterranean play a role in the decisions made in Israel within the last couple of months. After many years of absence in management and enforcement from the Israeli fishing authorities, a strong pressure from the SPNI (Society for the Protection of Nature in Israel) (<http://mafish.org.il/fishing-reform/the-problem/>) led to legislation of new fisheries management roles. In October 2018 the ministry of environment in Israel declared the ABFT as protected species which means fishing of this kind will be prohibited. This decision was based on the basic definition of ABFT as an endangered species by IUCN and on several papers represent one side of the hypothesis and demonstrate the existence of local subpopulation in the EMS. However, this decision was not supported by any local research or any updated fishing data and was detached from the general effort for conservation of the ABFT in the Mediterranean and without any connectivity to EMS fishing management. It is important to add that Israel is not a member of the ICCAT and therefore is not obligated in reporting on any ABFT fishing operation and no fishing quotas are enforced in its waters. This is in contrast to other countries in the EMS which are members in the committee like Turkey, Syria and Egypt. This decision has led to a strong resistance from the fisherman unions followed by reconsidering this step by the Ministry of Environment in Israel.

The controversial evidence of the population structure and the migration routes in the Mediterranean, the problematic attempt to manage the population locally in Israel, and the lack of substantial information about this species in our area and the EMS calls for a need in creating a base line for the easternmost ABFT population in order to support future population structure studies and fisheries management decisions.

The conclusions above leads to the research question asking:

Does a local- east med ABFT population exist?

In light of the past researches in this field there are three possible hypothesis:

1. The ABFT has a spatial structure in the Mediterranean Sea and a local subpopulation exist in the EMS.
2. The east Atlantic and Mediterranean population present one panmictic population that migrate throw this marine space and do not have a tendency for specific location.
3. Different locations in the Mediterranean might function as a habitat for young ABFT, until they gain a larger body mass and are able to move to the Atlantic Ocean.

2. Objectives and Importance of the Research

This research will provides a preliminary background for the ABFT population that is observed along the marine space of Israel. Three objectives are therefore defined;

1. Creating an Eastern Mediterranean tissue bank for the ABFT which will be accessible for any follow research regarding this population and constitutes as a platform for further long-term monitoring efforts.
2. Characterizes the ABFT along the coast of Israel for their size, gender, genetic structure, trophic levels, feeding habits, and migration routes.
3. Comparing easternmost data with other Mediterranean locations.
4. Create reliable and updated data on ABFT fisheries in Israel methods that could support a local and regional fishery management.

This research will provide information of value for the conservation of the global ABFT population, as well as contribute knowledge to the state of local stocks. In turn, such data may translate into policy measures or other management and planning tools (e.g., fishing quotas, MPAs, etc.). In addition to data collection as part of a long-term monitoring and stock assessment program, the value of this research lies in its ability to inform about the state of the environment with regards to other structural and functional components of the EMS environment.

3. Methods

3.1. Sample Collection

A total of 73 ABFT were sampled during the tuna fishing season (May-June) of 2016, 2017 and 2018 within the marine space of Israel. The first year of the project, 2016, was defined as a pilot and only a few samples were collected. Most of the effort in 2016 focused on building trust with the local fisherman community. In the following years, 2017-2018, Samples were collected with much assistance from fisherman in the region, both commercial and recreational. To characterize the population, the following data and samples were collected; 1) general measurements (length, weight, gender and fishing method), 2) muscular tissues for genetic structure and trophic level analysis, and 3) entire stomachs for stomach content analysis. As not all data groups were collected for each individual,



Figure 3: sampling and measuring BFT on a fisherman boat off the coast of Israel

some fish have only partial data. All muscle samples were kept as duplicates. One set was kept in 70% ethanol and one set in -80 °C. Using both preservation methods means samples will be suitable for most types of future analysis that may be conducted in ongoing research at our lab or in collaboration with other colleagues. All other tissues kept in – 80 °C. Throughout the three fishing seasons, samples were collected either at sea on board by joining the fishing team or at the ports/marinas after fishermen had returned from sea. During the tuna fishing season of 2016-2018 ten different boats were used to supply specimen samples and fishing data, including all of the commercial boats. Collectively, a total of twenty sampling days at sea were conducted by our team.

3.2. West Mediterranean Sea Sample Collection

In June 2017 I attended and participated in “The Great Tuna Race” – a scientific and recreational fishing involving the capture, marking, and release of the Mediterranean caught tuna. The event was organized by the ACPR (ASSOCIACIÓ CATALANA per a una PESCA RESPONSIBLE) and the local fisherman from the Barcelona area. A total of 28 muscle samples were collected from Spain; 11 specimens from the race and an additional 17 specimen were sampled from commercial and local fish markets in Barcelona (Mercabarna Market, Boquería

Market and St. Caterina Market). The samples were collected in order to compare west Mediterranean fish to east Mediterranean fish in the different analyses.

3.3. ABFT Fisheries in Israel

As data relevant to the local Israeli fisheries are absent, fishing data and ABFT fisheries were characterized according to the data collected throughout the seasons of this study. Here data was collected for the amount of active tuna fisherman in Israel, amount of ABFT individuals caught per vessel, total catch weight per season, fishing method, and fishing effort. Fishing effort was calculated as the estimated number of angler fishing trips taken and was calculated as an average effort for both commercial and recreational fisherman separately. Catch rates were calculated as the number of fish caught per 100 hooks and were presented for each one of the commercial boats. Fishing effort and most of the total catch was personally recorded during field sampling while the rest was reported to me by the fisherman both throughout the season as well as follow up interviews conducted after the fishing season was over. In addition, bycatch of several groups of species was documented during the tuna fishing season of 2016-2018 for longline boats in Israel. Data was collected through participant of our crew in several fishing trips as well as by interviews of commercial fisherman

3.4. Genetic Population Structure

Several tuna species occupy the marine area of Israel, most of them are easy to identify using only morphology. While *T. thynnus* is the largest species, young and juvenile specimen might be more difficult to classify. The main method for species identification being used today was set by Ward et al. 2005 who suggested the cytochrome c oxidase 1 (COI) region of the mitochondrial DNA as an effective tool in differentiating between species. In this project the COI gene was selected to ensure the samples were not misidentified for their species in case of fault in morphologic identification or in case of sample from unknown source (such as fish market). Over the years, some suggest the COI region to explore the genetic structure within a specie and obtain a better understanding on genetic differentiations between different populations based only on a few individual changes in nucleotides (Bentley et al., 2014; Li Weiwen et al., 2015; Zemplak et al., 2009). The mitochondrial DNA control region (mtDNA CR) was also selected as genetic marker

for the genetic structure in order to compare it to the COI results and perhaps to show a better or different result. The mtDNA CR present greater genetic variability and is more sensitive in differentiated between *Thunnus* species (Vinas and Tudela, 2009), and might be more sensitive in detecting genetic variation among ABFT individuals (Carlesson et al., 2004). These genes were selected against other common genetic markers, like the microsatellites and *SNPs*, due to comparability to other established work as well as time and budget limitations.

Sample Preparation

A total of 41 muscles samples from east Mediterranean (Israel) and 28 samples from west Mediterranean (Spain) were collected and preserved in 70% ethanol. Tissue samples were subdivided into smaller, more easily digested fragments and DNA was extracted using Promega Wizard® Genomic DNA Purification Kit. DNA concentrations and protein levels were analyzed with a NanoDrop 2000 c spectro - photometer (Thermo Scientific) and stored at -20°C until required for use. **COI:** The ~650bp COI segment was amplified by polymerase chain reaction (PCR) using the FISH1 primer as describes in ward 2005:

FishF1- 5' TCAACCAACCACAAAGACATTGGCAC3'

FishR1-5' TAGACTTCTGGGTGGCCAAAGAATCA 3'

With the following PCR protocol: The 50- μL PCR mixes included 25 μL of PCR ready mix gotaq, 18 μL of ultrapure water, 2 μL of each primer (0.1 mM), 2 μL of BSA and 1 μL of DNA template. The thermal regime consisted of an initial step of 45 sec at 95°C followed by 35 cycles of 15 sec denaturation at 95°C , 15 sec annealing at 57°C , and 45 sec extension at 72°C , followed in final extension of 2 min at 72°C and then held at 4.0°C . PCR products were clean using promega Wizard® SV Gel and PCR Clean-Up System kit, measured for concentrations and send for sequencing at Mclab molecular cloning laboratories (CA, USA). **mtDNA CR:** The 868 bp segment of the mtDNA control region as describes in *Carlsson 2004* was amplified by polymerase chain reaction (PCR) using the primers Pro-5'-and 12SAR-3' that set by *Palumbi 1996*: Pro-5' CAC GAC GTT GTA AAA CGA CCT ACC YCY AAC TCC CAA AGC and 12SAR-3' GGA TAA CAA TTT CAC ACA GGG CAT AGT GGG GTA TCT AAT CC. The PCR thermal regime consisted of an initial step of 95°C for 45 sec followed by 35 cycles of 15 sec denaturation at 95°C , 15 sec annealing at 60°C and 1 min extension at 72°C , with a final extension at 72°C for 2 min. PCR products were clean using promega Wizard® SV Gel and PCR Clean-Up System kit, measured for

concentrations and send for sequencing at Macrogen Europe Laboratory (Macrogen Inc., The Netherlands/South Korea). Sequences went through the same procedure as the COI sequences.

Data Analysis

Sequences were converted to FASTA format and were aligned in BioEdit using the ClustalW algorithm and trimmed. For COI sequences, 2 sample from Israel and 9 samples from Spain were eliminated from analysis due to bad quality of sequences. For species identification *T. thynnus* sequences sampled by me compared to representative sequences of other *Thunnus* species available in GenBank the *T. alalunga* (Albacore tuna) and *T. albacares* (Yellowfin tuna).

Tree Analysis

Phylogenetic characterization based on COI and mtDNA CR sequences were inferred from maximum likelihood trees performed with the PhyML v.3.0 program by applying the Kimura 2-parameter model of nucleotide substitution, K80 with four rate categories for COI and HKY85+G+I for mtDNA CR. Robustness of nodes on the phylogeny was assessed by 1000 bootstrap replicates using the ML substitution model defined above. Phylogenetic trees were visualized with the FigTree v1.4.3 software (<http://tree.bio.ed.ac.uk/software/figtree/>). All sample showed identity of other species were eliminated from the next analysis. In addition to phylogenetic trees, sequences from Israel ($n=15$) and Spain ($n=37$) were compared to ABFT sequences from several genetic researches by downloading it through GenBank (NCBI website). Representative sequences from GenBank were chosen only if the location of collection was mentioned. To demonstrate the different haplotypes DnaSP 4.0 software was used to carry out population genetic analyses. The evolutionary relationships and geographic distribution of the haplotypes were visualized using a median-joining haplotype network created with NETWORK v.5.0.0.3 software

3.5. Trophic Level Analysis

3.5.1 GC/IRMS Analysis

Chemical and physical processes change the isotopic composition of organic substances such as carbon, nitrogen, oxygen and hydrogen. For the fish, environmental processes such as

changes in the water composition, temperature, depth and salinity, and the nutritional composition of the food that they feed on during different periods of their lives greatly influence the chemical composition of parts of their bodies, leaving a recognizable "isotopic signature". Main food sources can be characterized by the carbon isotopes ratio which means that the organism contains the same isotopic composition of its food sources. Understanding the trophic level of the specific ABFT individual seen along the Israeli coast using compound-specific nitrogen and isotopic composition of amino acids methods can lead to a wider understanding on the local food chain of the Israeli marine ecosystem and also can provide with information of the main habitats they occupied during the rest of the year. Comparing the trophic level of individuals from different location in the Mediterranean can also imply fidelity to a particular location. The technique is based on the concept that the carbon isotope ratio ($^{13}\text{C}:^{12}\text{C}$) of consumers reflects their food items, while the nitrogen isotope ratio ($^{15}\text{N}:^{14}\text{N}$) exhibits a stepwise enrichment with each trophic level. The isotopic signature can be measured by the gas chromatography combustion isotope ratio mass spectrometry (GC/C/IRMS), a quantitative method based on the direct measurement of essential and non-essential amino acids. Essential amino acids are defined as those produced solely by the primary producers of the system and, therefore, the stable isotope values of nitrogen will remain constant throughout the food web, while the values of ^{15}N (heavy nitrogen) will increase with each trophic level. Using this novel method, it was recommended that a comparison of the $\delta^{15}\text{N}$ values of glutamic acid and phenylalanine is the most useful in obtaining precise estimates of the trophic levels of organisms (Chikaraishi et al., 2009).

Sample Preparation

Muscles samples (38 from Israel and 14 from Spain) were frozen at -20°C immediately after collection and kept at -80°C 1 hour before being freeze dried for 24 hours using a lyophilizer. 1 mg of dried sample was placed inside a 4 ml glass vial with PTFE cap and was acid hydrolyzed in 1ml of 6 nmol HCl at 150°C for 70 min. Samples were cooled to room temperature and then were filtered through 0.22u PTFE filter to remove all undissolved particles. HCl was evaporated in 110°C under a gentle stream of nitrogen. Next samples were prepared for analyze using EZ:faast™ Amino Acid analysis kit with a slight modification of replacing reagent 6 with dichloromethane as a solvent. Final analysis carried out as follows: 1) Helium was used as a carrier gas at constant flow of 1.5ml/min. The amino acids were separated on a Zebron ZB-50 column (25m, 0.25mm and 0.25um) in TRACE™ 1310 Gas Chromatograph (GC). GC condition were set to optimized peak

separation for the desired amino acids as follows: Initial temperature 110°C ramped to 240°C at 8°C per min and then ramped to 320°C at 20°C per min and held for 2.5 min. 2) The separated amino acids were split in to two direction flows, one toward Thermo Scientific™ ISQ™ Series Quadrupole for amino acid identification and the second toward Delta V™ Isotope Ratio Mass Spectrometer advantage for C and N isotope analysis. To define the isotopic ratio of carbon and nitrogen the separated amino acids were combusted in a Thermo scientific GC isolink II at 1000°C c for CO₂ and N₂. Before entering to Delta V for the N₂ analysis the sample went through a liquid nitrogen cold trap to freeze down all other gases. From each sample triplicates injected for nitrogen and carbon.

Data Analysis

To confirm the reproducibility of the nitrogen isotope measurements standard mixtures were used containing eight amino acids (Alanine, Valine, Leucine, Isoleucine, Threonine, Methionine, Glutamic acid and Phenylalanine) with a known isotopic ratio range of -6.69 to +43.25. The standard of amino acids was injected 3 times for carbon and 3 time for nitrogen after oxidation of the reactor and again after 18 samples run. For each fish muscle sample average nitrogen isotopic ratio was calculate for Phenylalanine and Glutamic acid. This result was calibrated using the standard Calibration curve and put into the trophic level formula $TL = (\delta^{15}N_{Glu} - \delta^{15}N_{Phe} + 0.5) / 4.46 + 1$ (Martinez et al. unpublished). Result were not calculate for carbon since all of the samples analyze in the machine before a major calibration was performed, resulting in the analysis results to be irrelevant.

3.5.2. Stomach Content Analysis

Previous investigations on the feeding habits identify North Atlantic ABFT as opportunistic feeder, feeding on a variety of epipelagic and mesopelagic organisms including bony fish, squids, and crustaceans (Crane, 1936). The feeding habits of the ABFT have been well described for the populations in the Atlantic ocean and west Mediterranean with many references (Battaglia et al., 2013; Logan et al., 2011; Medina et al., 2015; Sara and Sara, 2007; Sorell et al., 2017). Yet, so little is known about the feeding habits in the easternmost side of the sea which was only examine by Karakulak et al. 2009 in Turkey. Since oceanographic conditions and non-indigenous species populations shift from the Atlantic ocean, west Mediterranean and east Mediterranean, the composition of the marine species that occupy each area in the Mediterranean is different and

unique to specific location (Coll et al., 2010). The EMS for example, is characterized by many Lessepsian migrant fish species that came from the Red Sea, established large populations and became very common in this area (Golani et al., 2006). An examination of the stomach content from ABFT caught along the Israeli coast can give many clues about this fish path along the Mediterranean and the geographic location he was last feeding at according to the species composition that will be found inside. Stomach content can also be useful as complementary work to the stable isotope analysis of carbon and to give real result about the composition of its food sources. In addition the amount of food inside the stomach can be relate to the reproductive status of the fish (Estrada et al., 2005; Mather et al., 1995).

Sample Preparation

24 full stomach of ABFT were removed from the fish by the fisherman soon after landing at port or at sea right after capture, which they were then kept frozen at -20°C until analysis. Once defrosted, stomachs were opened and large prey items that were easy to spotvisually observable were collected. Such visual prey included cephalopods beaks, lenses, and gladii, fish vertebrates, otoliths, dental bones, eyes and cCrustacean exoskeleton. Occasionally entire fish were observed, as well as cephalopods and crustacean not yet digested. The rest of the stomach contents was flushed out through a 0.5 mm mesh size sieve and assessed for smaller prey items.

Data Analysis

All prey items were classified into large taxonomic categories and preserved in 70% ethanol for future genetic identification and morphologic comparison. Prey taxa were grouped into teleost, cephalopods, and crustaceans (*Supplement Table 1*). Teleost were identified by their vertebrates, otoliths and eyes and sometimes by a full body. As fishermen usually use herring as bait in order to mass the fish in a single area, extracted herring was not evaluated in the stomach analyses. Most cephalopods were largely digested and identified by their remaining beaks. Large and small plastic items were also removed from the stomachs and kept for future analysis.

3.6. Tracking ABFT Routes in the Mediterranean Sea

Pop-up archival tags record several times a day, water temperature, depth, and light intensity that are used to calculate the estimate daily location of the fish. The tag is fixed, through a tether, close by the second dorsal fin of the fish. After a period set by the scientists the tag detaches itself

and transmitting a summary of the recorded data to the closest (Gunn and Block, 2001) However, premature detachment is a common problem with pop-up archival tags that usually cut the time off pop-up to a few months or less (Sibert and Fournier, 2001)

The only ABFT tagging in the east Mediterranean in the last decade presented in GBYP report from 2016 when 30 ABFT were tagged in Turkey. Those tag all ended up popped-off prematurely, providing only partial data on the migration routes of the eastern most ABFT. This needs to be further investigated using satellite tags because at present so little is known about the ABFT in the Levant region of the Mediterranean Sea (Natale et al., 2016).

Tagging in Israel

ABFT were tagged with electronic tags in Israel as part of a collaboration between the Top Predators program in Morris Khan marine station of Haifa University and a team from the Hopkins Marine Station of Stanford University in 2017 and 2018 seasons. In total four electronic tags were used. Tagging was conducted with Pop-up Satellite Archival Transmitting tags (MiniPAT-348 built by Wildlife Computers). The first two tagging in June 2017 were conducted with the help of a recreational fisherman leaving from Ashdod marina on the 12th, 13th and 15th of June. Fishing conducted using rod and reel fishing and trolling lures as bait. This method proved unsuccessful as not even one fish got caught in the first two days. Therefore for the last day a commercial longline vessels provide fish for tagging on the recreational boat deck. ABFT were brought on-board using a lip hook placed in the most rostral position of the lower jaw and pulled from the water to a wet and padded mat. During the time spend on board ABFT had a soft cloth soaked placed over their eyes while a seawater hose oxygenated their gills. A clip of the pectoral fin was kept for future genetic analyses, curved fork length (CFL) was measured. Handling time in total lasted less than 3 minutes. The second tagging in 2018 was conduct with the help of commercial boat using drifting long line and leaving from Ashdod marina on the 11th, 12th, 13th and 14th of June. Tagging protocol was identify to the previous year.

Tagging in Spain

During “The Great Tuna Race” event in Spain 3 ABFT were electronic tagged by the ACPR team, the local fisherman and myself. The fish was caught on a recreational fishing boat using rod and reel fishing and live bait that was caught at sea. The fish handling protocol was the same as

was detail above. A total of 3 Electronic tagging were conducted with Pop-up Satellite Archival Transmitting tags (SeaTag-MOD™ built by desert star) but only one was popped off (the other two tags failed to pop-off) detailed in table 3. Routs maps from both tagging events visualized using google earth.

4. Results

4.1. Sampling and measurements

Out of the 73 ABFT that were sampled between 2016 and 2018, 52 specimen were measured for their weight alone, 39 specimen were measured according to their Curved Fork Length (CFL) and 27 were measured for both parameters (*Supplement Table 1*). Additionally, 35 specimen were reported for their weight in 2018, but no tissue samples were collected. Weight was measured by fisherman after bringing specimen ashore. ABFT weight distribution in the following years is shown that Most of the fish sampled in this study were relatively small fish (<100kg) and the majority were even very young (<50kg, n=35). in *Figure 4*. All weights are represented as gilled-gutted weight and do not include internal organs as well as blood which were cleaned at sea in purpose of maintaining fish freshness. Thirty-one samples were recorded for their gender with 68% (n=21) of sampled fish identified as males and 32% (n=10) female.

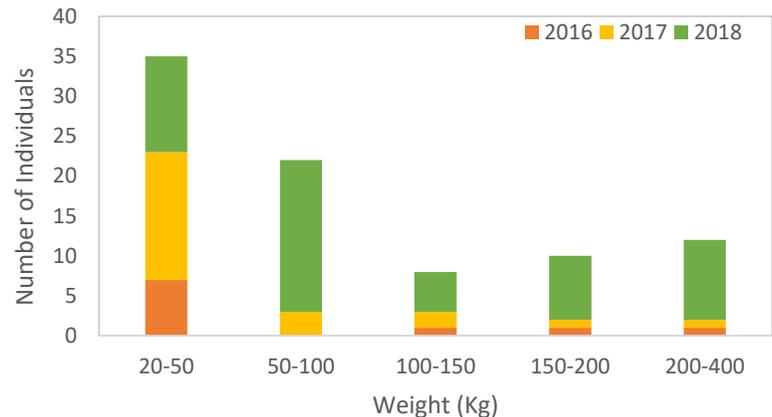


Figure 4: Distribution of ABFT weight. Gilled-gutted weight measurements from 73 specimen fished during season of 2016 (n=10), 2017 (n=23) and 2018 (n=50).

Other tissue sampled from the fish included: 71 muscles, 11 livers, 4 gonads, 4 blood, 8 parasites, 24 stomachs, 3 otoliths. In addition, 28 muscle sample from Spain were collected. All samples are detailed in (*Supplement Table 1*).

4.2. Characterization the Easternmost ABFT Population

4.2.1. Genetic Population Structure

The phylogenetic tree based on COI gene (*Figure 5*) presented total of 58 sequences sampled in Israel (n=37) and Spain (n=19). Out of them 51 sequences presented similarity higher than 99% to *T. thynnus* when compared by blast and were similar to *T. thynnus* COI sequence downloaded from GenBank. Two of the Israeli samples (*ISR_38 and ISR_13*), identified as *T. thynnus* due to morphologic identification by fisherman, presented with more than 99% similarity to *T. alalunga*

(*Albacore tuna*) when compared by blast and were also found similar to *T. alalunga* COI sequence downloaded from GenBank. The *T. alalunga* samples differ by 10 nucleotides from the *T.thynnus* samples. Five of the Spanish samples (*SP_2, SP_4, SP_18, SP_21, SP_26*) collected from the Mercabarna Market in Barcelona and sold as *T.thynnus* presented similarity higher than 99% to *T. albacares* (*Yellowfin tuna*) when compared by blast and were identity to *T. Albacares* COI sequence downloaded from GenBank. The *T. Albacares* samples differ by 3 nucleotides from the *T.thynnus* samples. Six samples (*ISR_2, ISR_12, ISR_21, ISR_36, ISR_46, SP_8*) were outliers from the main *T.thynnus* group and show some genetic variation based on one nucleotide alone.

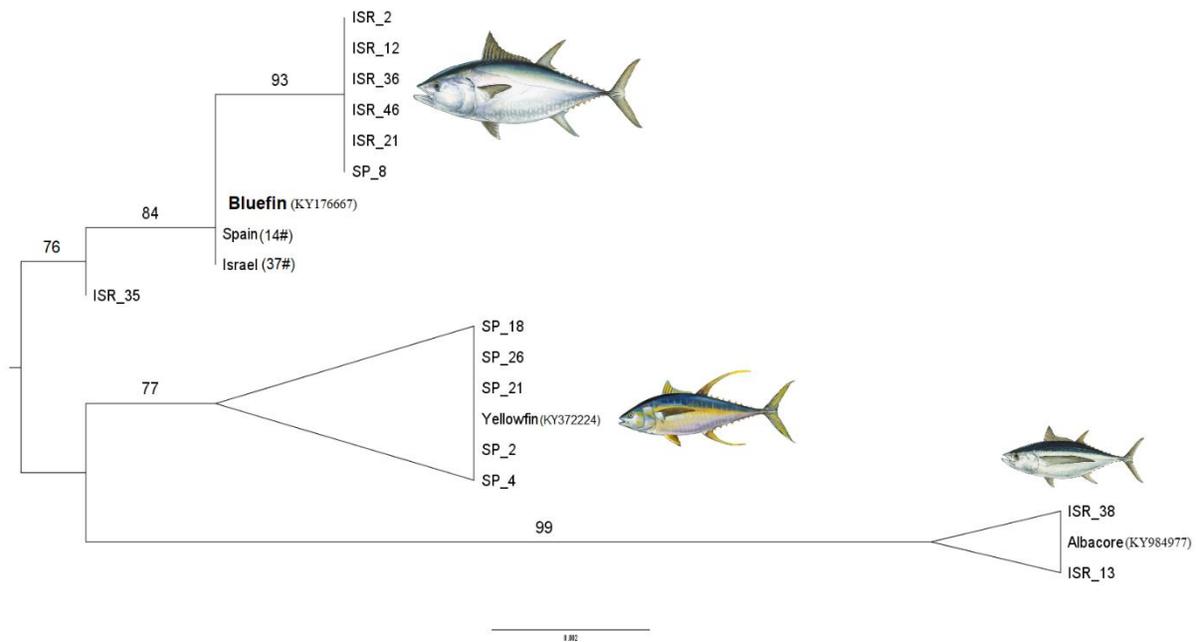


Figure 5: Phylogenetic tree based on COI gene. Maximum Likelihood tree of *T. thynnus* COI sequences. Samples collected in Israel ($n=39$) and Spain ($n=19$). Sequences were compared to representative sequences available in GenBank of Albacore Tuna (*T.alalunga*) and Yellowfin tuna (*T.albacares*) .

A haplotype network was constructed in order to visualize the genetic variation of *T. thynnus* based on the COI gene (Figure 6). Seven haplotypes were obtained from a total of 113 COI sequences of samples collected from Spain ($n=15$), Israel ($n=37$) and additional samples downloaded from GenBank were used (*Italy*=28, *Turkey*=20, *Canada*=8). Haplotypes differed by 1 to 4 nucleotides. Three halotypes were taken from an unpublished data set, and which had not been compared to other genetic data in the Mediterranean Sea. However, these three haplotypes (*H_1, H_2, and H_3*) appeared to be geographically restricted to Turkey. The most abundant haplotype (*H_4*) was found in 70% of sequenced individuals. For the Israeli samples, three different haplotypes were

Genetic variation within the *T.thynnus* population is presented with three groups distinguished from that of the main group. Group I ($n=4$) differed by seven nucleotides from the rest of the samples and group II ($n=3$) differ by six nucleotides but with 4 overlapping nucleotides. Both groups were restricted to Israel. Group III differ by different six nucleotides. All samples that were identify as other *Thunnus* species were eliminated from trophic levels analysis.

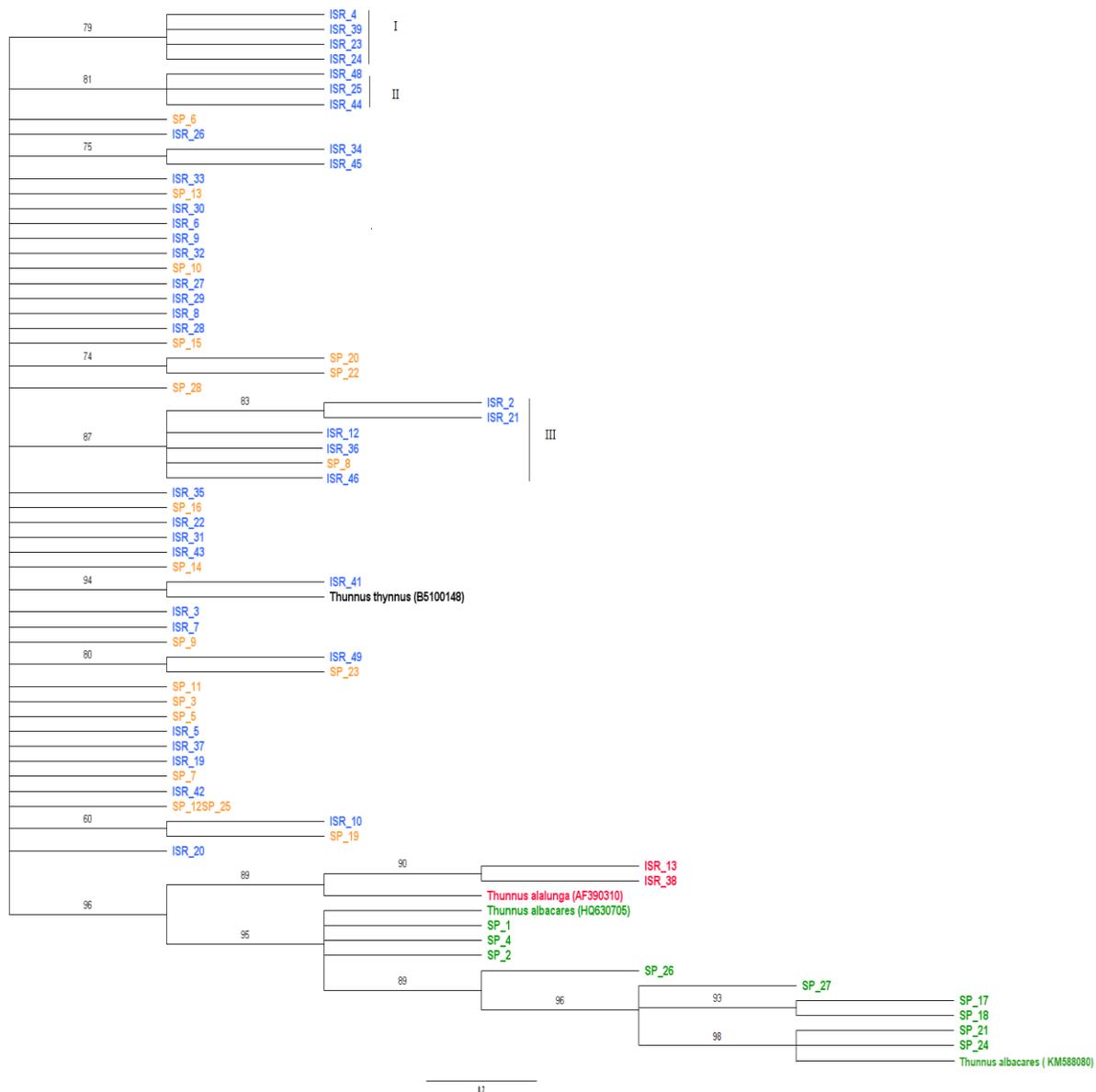


Figure 7: Phylogenetic tree based on mtDNA CR gene. Maximum Likelihood tree of *T.thynnus* mtDNA CR sequences. Samples collected in Israel ($n=41$) and Spain ($n=28$). Sequences were compared to representative sequences available in GenBank of Albacore Tuna (*T. alalunga*) and Yellowfin tuna (*T. albacares*). In addition three genetically distinguished groups (*I*, *II*, *III*) within the *T.thynnus* species.

4.2.2. GC/C/IRMS Analysis

Muscle tissues of *T. thynnus* sampled from Israel ($n=23$) and Spain ($n=6$) were measured for their TL. The nitrogen isotopic ratio of glutamic acid and phenylalanine was measured and TL values were calculated based on compound-specific nitrogen isotopic composition of those amino acids (Table 1). Israeli samples of TL values range from 2.26 to 4.52 (average of 3.740 ± 0.480) while the Spanish samples values range from 3.3 to 4.38 (average of 3.592 ± 0.586) (Figure 8). Four of the Israeli samples (73, 41, 4, 5) presented TL values beneath 3 and did not resemble that of any documented TL values of ABFT. Seven samples presented TL values of over 4.0 (39, 31, 21, 22, 26, S20, S23). Twenty samples out of the Israeli samples were measured for their weight allowing for the comparison between TL to fish size as shown in Figure 9. Small size ABFT (0-50kg) presented with a wide range of TL values (2.26 to 4.52). Two of the large size ABFT measured for their size present TL values of over 4.0. Nine sample were measured for all tree parameters: TL, weight and stomach content. None of the parameters showed correlation to each other.

Table 1: TL and Nitrogen isotopic ratio values. *T.thynnus* samples from Israel ($n=23$) and Spain ($n=6$) described for: Sampling location, average nitrogen isotopic ratio and trophic level of.

ID	Location	$\delta^{15}N_{Glu}$	$\delta^{15}N_{Phe}$	TL
22	Israel	26.556	11.338	4.52
39	Israel	24.81	9.649	4.51
21	Israel	24.86	9.915	4.46
26	Israel	24.141	10.786	4.11
31	Israel	19.635	6.342	4.09
25	Israel	24.069	11.598	3.91
33	Israel	23.181	10.901	3.87
27	Israel	18.779	6.873	3.78
37	Israel	23.573	10.39	3.77
7	Israel	22.3	10.861	3.68
19	Israel	23.573	12.666	3.56
24	Israel	23.775	13.967	3.31
32	Israel	18.973	9.211	3.3
20	Israel	17.266	7.569	3.29
36	Israel	18.345	8.667	3.28
3	Israel	23.88	14.479	3.22
29	Israel	24.409	15.307	3.15
23	Israel	18.036	9.215	3.09
30	Israel	21.113	12.495	3.04
41	Israel	20.024	12.236	2.86
73	Israel	21.056	13.7	2.76
4	Israel	20.391	15.228	2.27
5	Israel	19.492	14.377	2.26
S23	Spain	24.965	10.39	4.38
S20	Spain	25.706	11.84	4.22
S22	Spain	25.193	14.143	3.59
S5	Spain	22.972	12.213	3.52
S1	Spain	26.216	15.728	3.46
S3	Spain	22.275	12.393	3.33

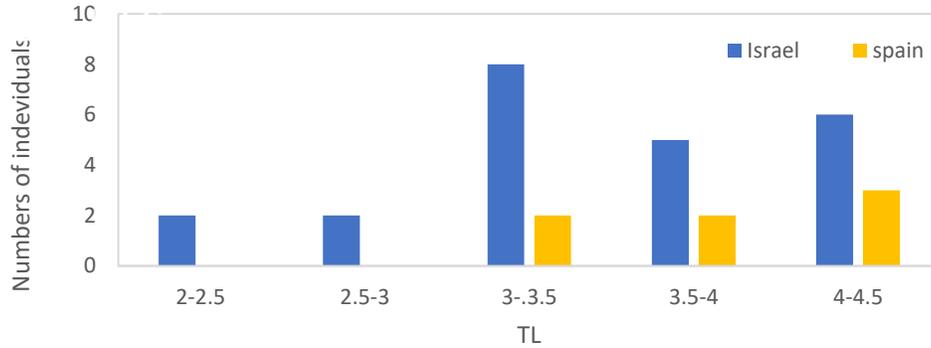


Figure 8: TL value distribution. Numbers of individuals for each TL category from Israel ($n=23$) and Spain ($n=6$).

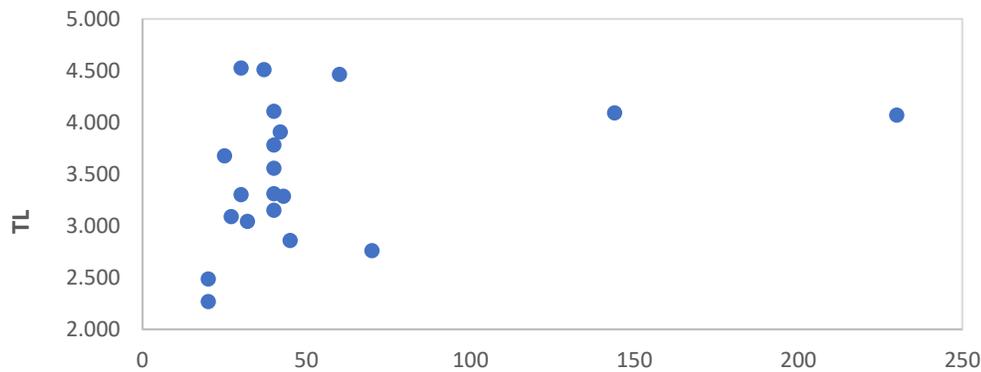


Figure 9: Comparison between TL to fish size. Relationship between body weight (kg) and trophic level for 20 individuals sampled in Israel.

4.2.3. Stomach Content Analysis

24 ABFT stomachs were examined, and content items summed in *Table 2* and *Supplements Table 4*. Size of *T. thynnus* examined ranged from 20 to 231 kg. Of the 24 stomachs examined 14.3% were empty. Of samples containing food 80.9% of the stomach contained teleost remaining identified by full body or half-digested body, otoliths and eyes (*Figure 10*). 52.3% of the full stomachs contained cephalopods identified by their beaks in a variety of different sizes as well as full size cephalopods (*Figure 11*). 47.6% of the

Table 2: Frequency of food types in stomach contents of *Thunnus thynnus* (NS number of occurrences in stomach contents, %FO percentage of occurrence frequency)

Groups	NS	%FO
Teleost	17	80.9
Cephalopods	11	52.3
Crustaceans	10	47.6
Plastic	8	38
Empty stomach	3	14.3
Total stomachs examined	24	

full stomachs contained Crustaceans were identified by their exoskeleton. Most of them were classified as some species of Amphipod that are known to be epibenthic species (*Figure 12*)

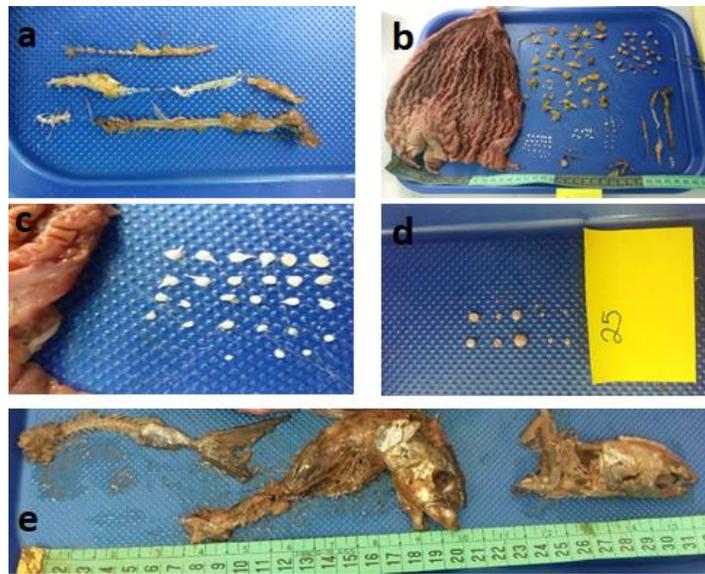


Figure 10: Teleost sampled from ABFT stomach. a) half-digested fish b) full stomach and total items found inside c) fish otolith d) fish eyes e) full body fish.



Figure 11: Cephalopods sampled from ABFT stomach: a) small cephalopods beaks b) big cephalopods beaks c) full size cephalopods .



Figure 12: Crustaceans sampled from ABFT stomach. a) Exoskeleton remains of Crustaceans b) Amphipods c) full stomach and total items found inside.

Another non-pelagic species that was identified in one of the stomachs was a sea horse (*Hippocampus sp.*) found with its structure intact within the stomach (*Figure 13*). In addition, 38% of the full stomachs contained plastic pieces like tube, strews, large plastic bags and wraps that broke up into smaller pieces (*Figure 15*). Additionally, parasites were observed in the abdominal space both outside the stomach and some within, like the *Diginea* flatworm (*Figure 14*).

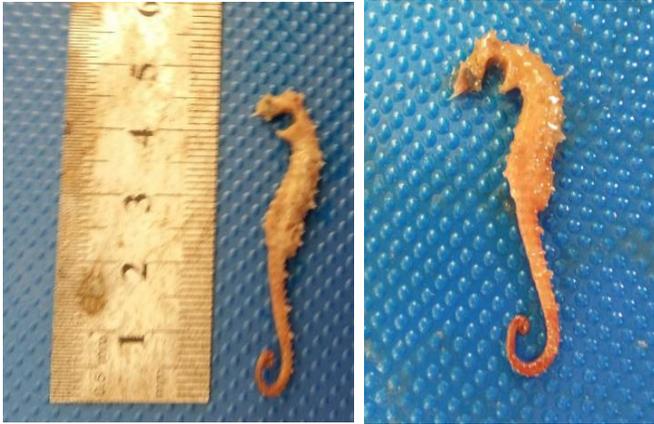


Figure 13: *Hippocampus sp.* sampled from ABFT stomach.



Figure 14: *Diginea* flatworm

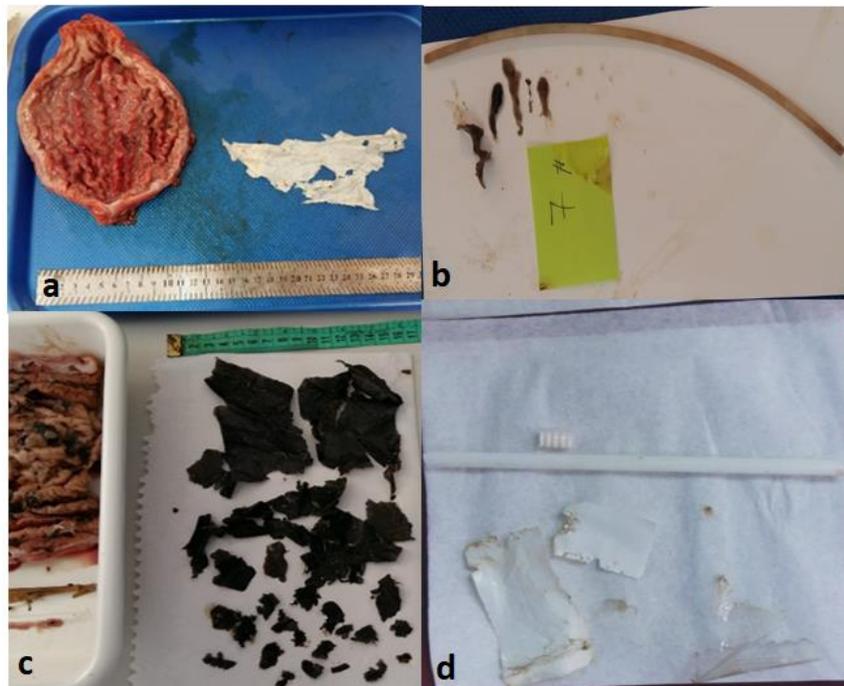


Figure 15: Plastic items sampled from ABFT stomach. a) Full stomach contain only plastic b) plastic tube c) large plastic bag broken into small pieces d) strew and plastic wrap.

4.2.4. ABFT routes in the Mediterranean Sea

A total of 4 electronic tagging sessions were conducted in Israel during 2017-2018 and in addition 3 electronic tagging sessions were conducted in Spain in 2017 (*Table 3*). Tag number 166395 recorded for 150 days, which it then released prematurely by 90 days before programmed to do so. The tag popped up 35 km off the coast of Libya. Tag data shows that for the first two months, the fish stayed in the eastern side of the Mediterranean Sea, but during August it started swimming westward along the African coast, directly to the area around Malta where it stayed from mid-September until mid-November. In the last days of recorded information the fish started swimming towards Libya where according to the tag “behavior” it is assumed the fish was caught during a fishing operation (*Figure 16*). While fish weight was not recorded, the length-weight relationship table of ACPR indicates that this specimen weighted about 44 kg and was 4.5 years old

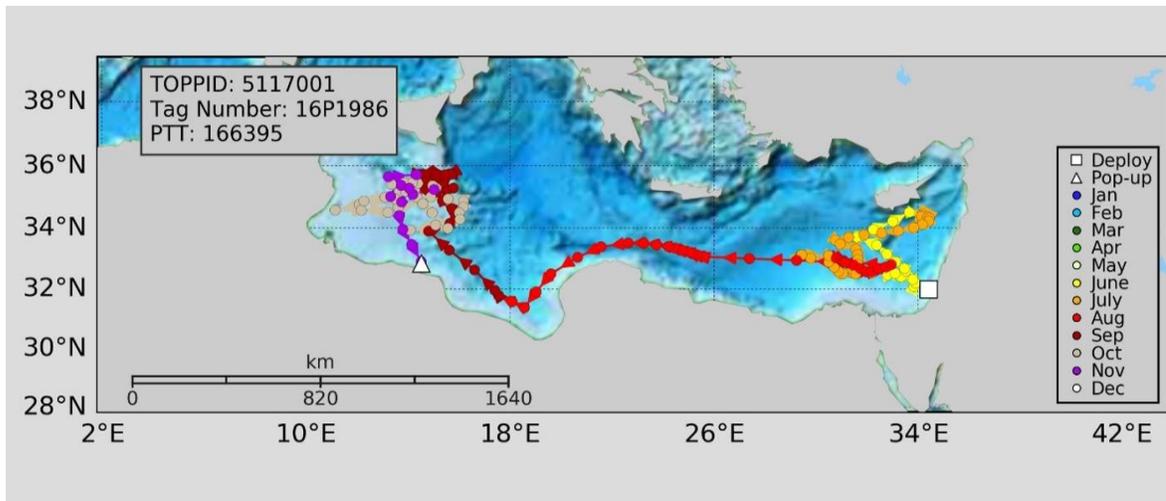


Figure 16: Map 1# of ABFT route tagged in Israel. Representative tracks showing movement patterns of ABFT electronically tagged of the coast of Ashdod, Israel. 150 days of movement was recorded. Tagging locations shown as white squares, Pop-up locations shown as white triangles. Different colors represent the different month of the year.

Tag number 152985 recorded for 181 days and released according to plan. The tag popped up 18.5 km off the coast of Italy, a short distance from Rome. The tag was washed ashore and was recovered by a colleague from Spain. For the first two and a half months, the fish crossed the Mediterranean Sea from east to west, swimming directly to the area around the Balearic Island where it remained for three months (September – November). In the beginning of December it started swimming towards Italy crossing Corsica from north (*Figure 17*). This fish weighted

around 35 kg and was about 4 years old. Tag number 61842 was tagged in 2018 and popped-up premature after only 13 days. It is assumed dead probably as a result of the tagging operation. We believe this is the case as the tag reached the sea floor (shown in the Meta data of the tag) and did not move for 3 days. The lack of movement resulted in a forced pop-up of the tag. Tag number 61845 is still recording as for this day of writing and is planned to pop-up in January 2019.

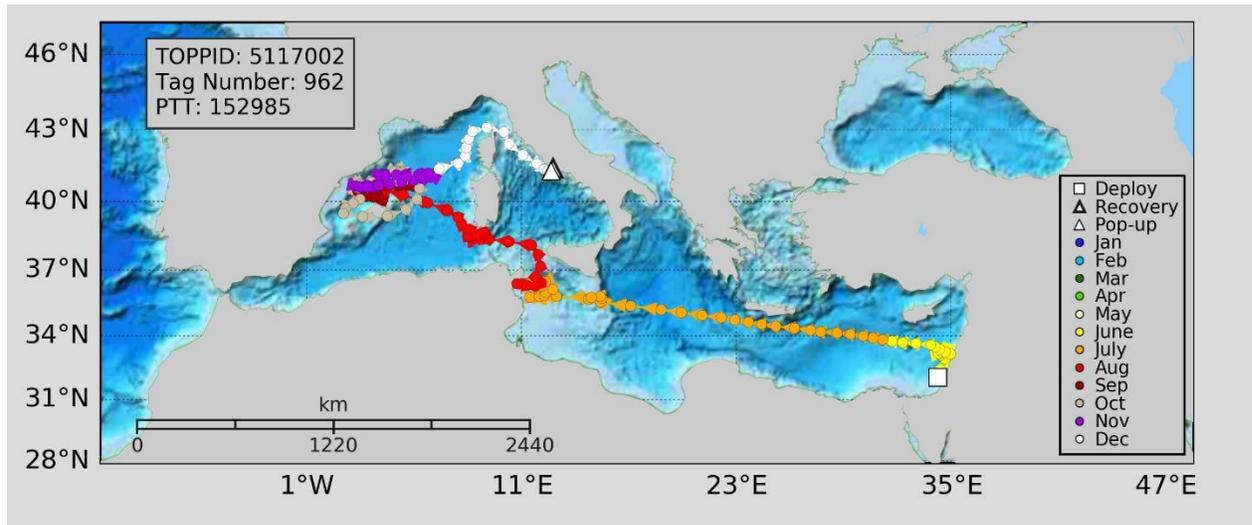


Figure 17: Map 2# of ABFT route tagged in Israel. Representative tracks showing movement patterns of ABFT electronically tagged of the coast of Ashdod, Israel. 181 days of movement was recorded. Tagging locations shown as white squares, Pop-up locations shown as white triangles. Different colors represent the different month of the year.

Out of the three tagged deployed in Garraf, Spain during the “Great Tuna Race” two tags failed to pop-up and no information was obtained. Tag number 33924 popped-up premature and recorded only for 37 days. For 27 days this fish was swimming around the Balearic Island, but in the last 10 day of recording the fish started swimming outside the Mediterranean Sea, and the tag popped-up off the Atlantic coast of Spain.

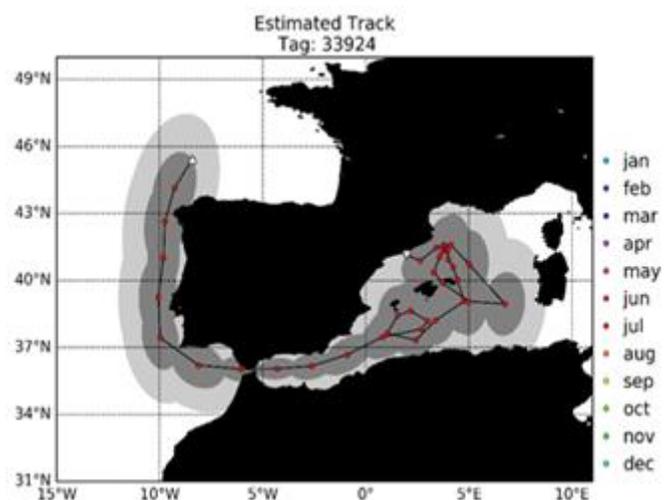


Figure 18: Map 3# of ABFT route tagged in Spain. 37 days of recorded movement by a tag deployed in Garraf, Spain during the “Great Tuna Race” event organized by ACPR team.

Table 3: Summary information on the deployments of electronic tags from 2017–2018

Year	Pop-up ID	Area	Deployment position	Deployment Date	CFL (cm)	Days prog.	Pop-off position	Pop-off date	Days at sea
2017	166395	Asdod, Israel	31° 98' N 34° 41' E	15/06/2017	135	240	32° 838' N 14° 519' E	12/11/2017	150
2017	152985	Asdod, Israel	32° 04 N 34° 28' E	15/06/2017	125	180	41° 278' N 12° 65' E	13/12/2017	181
2018	61842	Asdod, Israel	32°03 N 34° 29 E	14/06/2018	160	210	32° 09' N 33° 04' E	27/06/2018	13
2018	61845	Asdod, Israel	32°13 N 34°32 E	11/06/2018	141	330	-	-	-
2017	33930	Garraf, Spain	41° 01' N 01° 96' E	04/06/2017	158	365	-	-	-
2017	33925	Garraf, Spain	41° 12' N 01° 95' E	04/06/2017	224	365	-	-	-
2017	33924	Garraf, Spain	41° 14' N 01° 98' E	04/06/2017	184	365	45° 432' N 08° 405' E	11/07/2017	37

4.3. ABFT Fisheries in Israel

4.3.1. Fishing Vessels and Methods

Tuna fishing in Israel takes place outside the territorial waters, between 12 to 25 nm away from shore for recreational boats, and can reach up to 40 nm away from shore for the Commercial boats. From the 72 ABFT sampled in Israel during 2016-2018, only 14% ($n=10$) were caught using trolling and the other 86% ($n=62$) were caught using longline

Tuna fishing in Israel is divided between the commercial fisheries and recreational fishing boats. Commercial tuna fishermen in Israel use either a floating or drifting longline (LL) fishing method. The longline consists of a mainline kept near the surface, by means of regularly floats, and are spaced every 300 meters. Relatively long snoods and baited hooks are then evenly spaced every 50 meters. Tuna fisherman usually use dead mackerels or sardines as bait. Floating LL in an Israeli fishing boat can range between a length of 12 km to 25 km long, and contain between 200-

400 hooks with bait. A line usually stays in the water for 16-24 hours before being collected back into the boat. As of to date, according to the Israeli Ministry of Agriculture, there are 13 fishing license for long liners in Israel (*Supplements 3*). During the three year of this project (2016-2018) only 4 commercial boat were active throughout the tuna season.

The second type of tuna fisherman is the recreational fishing boats which use a trolling method. A trolling line consists of a line with natural or artificial baited hooks and is trailed by a vessel near the surface or at a certain depth. Several lines are often towed at the same time. It is hard to assess the amount of recreational tuna fishing boats in Israel as no special license is required for this type of fishing, and there does not exist any limitation on quotas per season. However, the based on this field work, it is estimated that during the tuna season between 10 to 15 recreational boats may be going out regularly (2-3 times a week), with an additional 20 boats going out occasionally (2-10 times during the entire season). A majority of recreational tuna fisherman do not catch any ABFT fish at all during the season, and their focus is mainly on that of albacore tuna.

Fishing activity targeting the ABFT in Israel is mostly limited to the pre-spawning and spawning periods when BFT group in schools and swim in surface waters. However, there are exists off-season catches (October-February) of recreational fisherman not falling within the known fishery period, and operate much closer to shore than that of the main seasons (1 nm from shore). During the period time of this work, several evidence for off-season catches were reported to me and approved the



Figure 19: ABFT catches outside the main fishing season. Left) ABFT caught in November 2018 off the coast of Ashdod weighted. Right) ABFT caught in January 2017 off the coast of Haifa.

4.3.2. Bycatch of longline boats in Israel

The longline (LL) fishing method is known as non-selective method concluding with high rates of non-target species catches. A total of five pelagic fish species were identified during fishing trips by our team or reported through telephone interviews. The most common one was swordfish (*Xiphias gladius*) being caught almost in every fishing trip; this was a desirable catch even though it was not the main target by the fisherman. The other four species were protected species and in all cases were carefully released from the hook and thrown back into the sea. Two species of sharks: bigeye thresher sharks (*Alopias superciliosus*) (Figure 20), shortfin mako shark (*Isurus oxyrinchus*) as well as a pelagic stingray (*Pteroplatytrygon violacea*) and a sunfish (*Mola mola*) were observed in some of the longline catches.



Figure 20: LL boat bycatch of bigeye thresher sharks (*Alopias superciliosus*) in a tuna longline fishing boat.

4.3.3. Fishing effort and total catch

Number of individuals, total weight (kg) and average effort are summarized in Table 4 with the full data shown in Supplements Table 2. Fishing effort presented for each one of the commercial boats (A-D) (Figure 21). In 2018 data receiving from commercial fisherman was more detailed and accurate than in 2017 due to an improvement in collaboration with the fisherman. On the contrary, most of the data receiving from recreational fishermen in 2018 was reported only by interviews that were conducted at the end of the season, and contained less details.

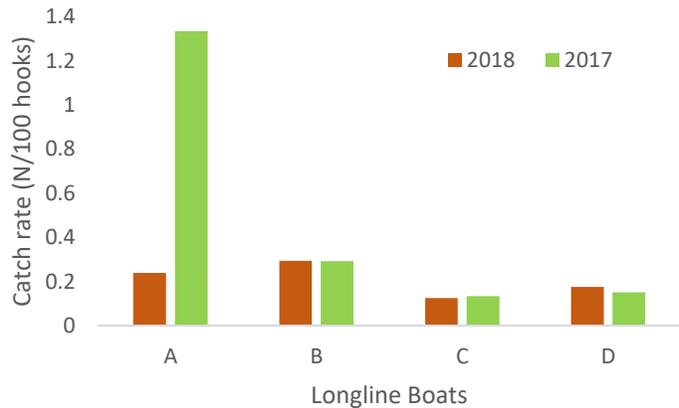


Figure 21: ABFT catch rates of Israeli longline boats. A-D represent the four commercial boats in the years of 2017-2018.

Table 4: Summary information on tuna fisheries in Israel 2017–2018 number of individuals, total weight and average effort for commercial and recreational fisherman.

		Number of individuals	Total weight (kg)	Avg. effort (days)
2017	commercial	125	10300	29.75
	recreational	14	125	16.4
	total	139	10900	
2018	commercial	111	9386	36.75
	recreational	11	350	
	total	122	9736	

5. Discussion

While ABFT have been studied for the past 60 years both ecologically and biologically, little is known about this fish in regard to the easternmost part of the Mediterranean Sea. During a study spanning over three consecutive years (2016-2018), we initially characterized this population sampled in Israel providing base line data in order to continue long term monitoring and future research in this field.

5.1. *ABFT size*

Most of the fish sampled in this study were relatively small fish (<100kg, n=57), with the majority being very young specimen (<50kg, n=35) (*Figure 4*). Recent Mediterranean studies demonstrated that individuals below 100 kg do not exit the Mediterranean and remain to forage in areas of high primary production in the Mediterranean Sea (Block et al., 2005; Fromentin and Lopuszanski, 2013; Metrio et al., 2005), supporting the hypothesis that the Levantine basin may function as a habitat for young ABFT. As the fish in this study were caught using longline, a non-selective fishing method, it is believed a representative sample was taken. Still, a larger sampling size is required to better understand the nature of the population in the Levantine basin.

5.2. *Genetic Population structure*

As collecting samples and information for this research was conducted with the assistance of many individuals not considered experts in tuna biology, misidentification of the desirable species is expected. Therefore, the COI and the mtDNA CR segments were found to be a reliable method for distinguishing between tuna species in order to avoid errors in any other related analysis.

Three main groups showed some repetitive genetic variation within the ABFT population. In order to avoid errors in sequences, individuals were considered a group if more than two samples could be group together. Groups I and II (*Figure 7*) were restricted to Israeli samples. In addition, Group I contained only young fish samples (n>40), further supporting the Levantine basin hypothesis mentioned previously.

The Israeli, Spanish and Italian samples presented a unique haplotype (H_5 in *Figure 6*), was supported by the mtDNA CR phylogenetic tree results where they were grouped together again (III in *Figure 7*). Since this group contained both Spain and Israel samples, this could suggest that

central Mediterranean Sea is a mixing point for resident ABFT arriving from the Levantine Sea with ABFT arriving from the Atlantic Ocean as suggested in Heinisch 2008. Additional evidence for this assumption is demonstrated through the results of a tagged specimen. One of the fish that demonstrated this genetic variance (i.e. group III and H_5) was the same fish that was tagged in 2017. This fish showed in its route (Figure 16) a long duration in the area of Malta, in the central Mediterranean, perhaps representing resident ABFT arriving from the Levantine Sea to this central mixing point.

In spite of this evidence, representative sequences from outside the Mediterranean were not available for comparison which could confirm or reject this hypothesis. In addition, the assumption that group I and II are restricted to Israel may be biased due to the relatively lower sample size of the Spanish samples. Genetic variation requires further investigate using a larger sample size from more locations within the Mediterranean combined with genetic markers with increased sensitivity, like SNPs and microsatellites, as to represent greater genetic variability.

5.2. Trophic levels

Working with the GC/C/IRMS machine in order to identify trophic levels of ABFT raised several issues. First, most TL measured for ABFT samples from Israel and Spain did not correspond with the literature data. It was expected to receive TL values of 4 and above which will be resemble to most adult ABFT documentation from the Mediterranean (Estrada et al., 2005; Stergiou and Karpouzi, 2002). But, result yielded surprising low TL values (≤ 3). Considering 3 was the lowest TL recorded for young larvae of ABFT (Sara and Sara, 2007), those values are more resemble to vegetarian fishes TL (Stergiou & Karpouzi 2002) and not to any kind of a predator.

Furthermore, large variation of TL within the same size group was observed in contrary to Sara 2007, which found significant relationships between TL and size. In addition, Samples analysis using the GC/C/IRMS machine was distributed over a wide time period due to different constrains (e.g., timing of fishing season, continual calibration of the machine and technical difficulties). However, samples that were analyzed within the same day resulted in a lower standard error. Therefore, while logistically challenging at times, as fluctuations appear to accrue over time, future analysis through GC/C/IRMS should be carried out within a short consecutive time. Moreover, Spanish and Israeli samples did not show any differences in TL, however, the number of samples from Spain was very low compare Israel. Therefore, more samples collected from the

western basin of the Mediterranean Sea need to be examined. In addition, due to a modification in calibration method, carbon isotopic ratios were not properly. Comparison of the TL inferred from the nitrogen isotopes to the species main food sources inferred from the carbon isotopes and to stomach content was not possible. Carbon isotopic ratio should be measured in future works as such information could lead to a better understanding of the food web each individual is leaning on.

5.3. Stomach content

Stomach content examination presented the distribution of food sources of the ABFT and was mostly similar to that found by other authors in Mediterranean research which contained fish and squids (Battaglia et al., 2013; Karakulak et al., 2009; Stergiou and Karpouzi, 2002). While it is assumed that the species making up the stomach contents are mostly pelagic organisms (Sorell et al., 2017), identified to the lowest possible taxon is being done using specific identification keys which require extensive experience. Therefore species identification did not go further in depth due to lack of experience and time limits.

In addition, there were the presence of non-pelagic species observed as well (*Figures 12, 13*). Even though ABFT were hypothesized to occupy mostly the surface waters area, especially in reproductive period (Mather et al., 1995), archival tagging and ultrasonic telemetry have revealed that ABFT frequently dive to depths of 500– 1000 m (Fromentin and Powers, 2005) that may explain the presence of some benthic organisms inside their stomach.

In our study 14% of the stomach were empty, showing similar result to Battaglia et al. 2013. Empty stomach assumes to be related with spawning season when there is a tendency to reduce, and ultimately stop, feeding as the volume of the gonads increases (Estrada et al., 2005). Then again, much evidence indicates that significant numbers of these fish do feed during the spawning season (Mather et al., 1995).

In addition to food items, high numbers of stomach contained plastic items were found (38%) with higher percent compared to other EMS work (Karakulak et al., 2009). Not once plastic items were the main or the only items found inside a stomach. However, in most diet composition studies of the ABFT plastic subject under “unknown” or exclude from results. It can be assumed that plastic and marine debris in other areas of the Mediterranean are not as common as it is in the EMS. But As a major contamination factor in the marine environment, plastic may have a direct

effect on the entire food web and on apex predator in particular and should be monitored (Ferreira et al. 2018)

5.4. ABFT routes in the Mediterranean Sea

ABFT tuna are hypothesized to migrate to the Mediterranean Sea from the Atlantic Ocean during spawning season (Medina et al., 2002) and back again to feed in the ocean when season is over. Even though this is a well-documented movement pattern in the literature (Block et al., 2005; Lutcavage et al., 1999; Metrio et al., 2005, 2004) our preliminary tagging result from Israel reveal residency for long durations (>150 days) in the Mediterranean. In addition, these tags are the first to be deployed in this easternmost side of the Mediterranean. These results are similar to other recent electronic tagging efforts reported residency in the Mediterranean (Cermeño et al., 2015; Fromentin and Lopuszanski, 2013; Natale and Idrissi, 2015; Tudela et al., 2011).

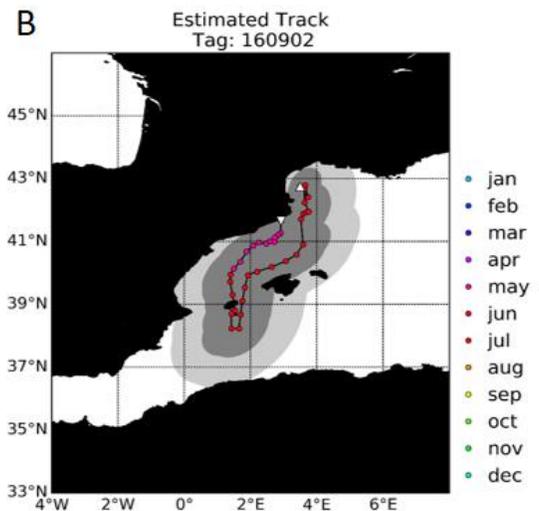


Figure 22: Map 4# of ABFT route tagged in Spain. B) 36 days of recorded movement by a tag deployed in, Blanes, Spain during the “Great Tuna Race” event organized by ACPR team.

Residency in the Mediterranean is reported as well by the ACPR. During five years of tagging in the “Big Tuna Race” program in Spain, none of the fish tagged in the Mediterranean ever exited it. In fact, most of the fish that they tagged display fidelity to the area in which it was tagged. After a couple of weeks, where they spend around the Balearic Island for spawning season, they head back for the area where they were tagged. A fish tagged in Blanes in May 2017 during the ACPR program showed this kind of a route (*Figure 22*). Nevertheless the fish tagged in Spain during my trip (*Figure 18*) was recorded exiting the Mediterranean Sea. This specific pattern is not surprising since residential behavior is mostly observed for specimen below 100 kg (Block et al., 2005) while this fish weighed around 130 kg (equal to 184 cm CFL). However, in both case of tagging in Spain the time of recoding it not substantial enough to truly understand the fish route.

Both fish tagged in Israel were young fish but were assumed to be sexually mature (> 4 years). A better analysis of the depth records from the tag can imply on the presence of active

spawning for those fish (Boustany et al., 2008) This kind of analysis will be conducted in the near future together with Hopkins Marine Station team.

One main difference between the Israeli and the Spanish tagged fish was that the Israeli fish were observed migrating long distance to west and central Mediterranean after spawning season was over. Fish are assuming to move from the EMS that might function only as a spawning ground, into forage aggregation hot spots in west and central Mediterranean. This estimation is based on the fact that northwest Mediterranean and areas around Sicily displayed permanent frontal zones that are known to concentrate abundant vertebrate and invertebrate prey for ABFT and marine mammals (Druon, 2010; Gannier and Praca, 2007) while the EMS is a highly oligotrophic environment short with food supply (Azov, 1991)

In spite of this new information observed from satellite tags, still, no information about fish migration into the east side of the Mediterranean was received. Further tagging activity should be carried on pre-season outside the EMS in order to get such information. In addition, further tagging activity should be carried out in Israel in order to obtain more data and to better understand the migration routes of the ABFT in the EMS and the entire Mediterranean Sea.

5.5. ABFT Fisheries in Israel

In this work we collected and presented tuna fisheries data that was not documented and available until now. Data was collected based on our active participants in fishing trips, observations in marinas and port and interviewing collaborating tuna fisherman during the fishing season. Therefore, total fishing catches estimation is rough, yet representative, as most active tuna fisherman agreed to collaborate with us. Comparing catch rates of long line tuna fishing vessel in Israel to other areas is challenging since most fishing vessels targeting tuna in the Mediterranean use purse seine boats. In order to get a better idea about the ABFT fishing rates in Israel, data was compared to ABFT fishing quotas from neighboring countries (*Figure 23*). Since fishing catch data is not accessible to non-contracting parties of ICCAT, the only data available was fishing quotas. Nevertheless, it is assumed the actual yearly catch is even higher than those numbers (ICCAT asses 2017). This comparison demonstrates how minor tuna fishing is in Israel compared to other countries in the Mediterranean. Yet, understanding the Levantine basin as a spawning ground and

considering the evidence for young residency fish in our marine space calls for better management.

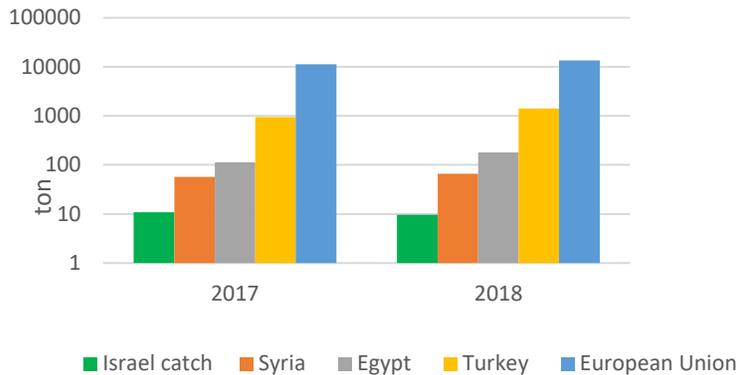


Figure 23: comparison of Israel total catch to Mediterranean countries tuna fishing quotas. Catch and quotas are presented in logarithmic scale.

5.6. Conclusions

Overall, my results provide first clues for understanding the ABFT population migration routes, genetic structure and feeding habits in the eastern most part of the Mediterranean Sea. This work indicates that there may be a larger population of young fish (>50) suspecting to be Mediterranean residence. However, a substantial amount of data regarding these population in the EMS is still missing. In order to produce reliable data that may translate into policy measures, management and conservation, sampling and documentation must continue as part of a long-term monitoring program.

In addition, for many years the fishing of tuna in Israel was unregulated. The big gap between this situations to the decision taken to immediately halt the ABFT fishing is unsustainable and is not performed in any other tuna fishing country. A more balanced approach could include numerous intermediate levels of regulation including personal fishing quotas, declaration of limited fishing season, minimum size regulation and close areas of spawning grounds using MPAs as a marine conservation tool. Those are consider to be best practice regarding to tuna fishing as mentioned in ICCAT recovery plan. However, all of the above should be based on local research and more substantial data. Finally, becoming a member of ICCAT could give Israel the support and knowledge to initiate this kind of a process.

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7. Supplements

Supplements 1: Detailed information on all ABFT sampled during this work.

#	Sample ID	Date	location	Length (cm)	Weight (Kg)	Tissue								Fishing Method
						Muscle	Gonads	Blood	Parasite	Liver	Brain	Stomach	Otolith	
1	2	6.6.16	Israel		180	v								--
2	3	7.6.16	Israel		?	v								--
3	4	10.6.16	Israel		20	v				v		v		Trolling
4	5	17.6.16	Israel		20	v			v	v		v		Trolling
5	6	21.6.16	Israel		30	v	No gonads		v		v		v	Long-line
6	7	3.7.16	Israel		25	v		v						Long-line
7	8	3.7.16	Israel		140	v								Long-line
8	9	3.7.16	Israel		280	v								Long-line
9	10	13.7.16	Israel		20	v								Trolling
10	12	13.7.16	Israel		25	v								Trolling
11	13	13.7.16	Israel		35	v							v	Trolling
12	19	4.5.17	Israel	150	40	v				v		v		long-line
13	20	5.5.17	Israel	137	43	v				v		v		long-line
14	21	8.5.17	Israel	150	60.5	v				v		v		long-line
15	22	8.5.17	Israel	125	30	v				v		v		long-line
16	23	10.5.17	Israel	134	27	v								long-line
17	24	11.5.17	Israel	137		v				v		v		long-line
18	25	11.5.17	Israel	139		v						v		long-line
19	26	13.5.17	Israel		40	v								long-line
20	27	13.5.17	Israel		40	v								long-line
21	28	12.5.17	Israel		35	v								Trolling
22	29	13.5.17	Israel		40	v								Trolling
23	30	14.5.17	Israel		32	v								long-line
24	31	15.5.17	Israel	212	144	v								Trolling
25	32	25.5.17	Israel	115	30	v							v	long-line
26	33	27.5.17	Israel			v								long-line
27	34	27.5.17	Israel			v								long-line
28	35	27.5.17	Israel			v								long-line
29	36	27.5.17	Israel			v								long-line
30	37	29.5.17	Israel	247	231	v						v		long-line
31	38	29.5.17	Israel		67	v			v			v		long-line
32	39	29.5.17	Israel		37	v			v			v		long-line
33	40	02.06.17	Israel	120	48					v		v		trolling
34	41	5.6.17	Israel	140	45	v						v		long line
35	42	5.6.17	Israel	192	110	v				v		v		long line
36	43	17.5.17	Israel			v								long line
37	44	6.6.17	Israel	133	55	v			v					Trolling
38	45	15.6.7	Israel		125	v								long line
39	46	15.6.17	Israel		135	v			v					long line
40	47	15.6.17	Israel						v	v		v		long line
41	48	21.6.17	Israel	170		v				v		v		long line
42	49	21.6.17	Israel	40		v						v		long line
43	50	2.5.18	Israel	147	51	v								long line
44	51	14.5.18	Israel	164	62	v								long line
45	52	14.5.18	Israel	237	207	v								long line

Supplements 2: Detailed information on tuna fisheries in Israel 2017–2018

vessel	Numbers of individuals	Total weight per season(kg)	Day of fishing trips	Number of hooks	Avg. catch per day	Catch rate	Max W	
A	52	3000	13	300	4	1.333333333		2017
B	35	3000	30	400	1.166666667	0.291666667		
C	8	1500	30	200	0.266666667	0.133333333		
D	30	2800	50	400	0.6	0.15		
	125	10300	30.75	325		0.407		
1	2	200	10		0.2			
2	2	50	10		0.2			
3	3	80	17		0.176470588			
4	2	70	15		0.133333333			
5	5	200	30		0.166666667			
	14	600	16.4					
	139	10900						
A	30	2500	42	300	0.714285714	0.238095238	440	
B	41	2890	35	400	1.171428571	0.292857143	374	
C	5	727	20	200	0.25	0.125	213	
D	35	3269	50	400	0.7	0.175	270	
	111	9386	36.75	325		0.207		
1	1	30						
2	1	30						
3	1	40						
4	8	250						
	11	350						
	122	9736						

Supplement 3: List of fisherman boats permitted for longline fishing by the Israeli fishing ministry .

רשימת ספינות המורשות לדיג...

3 of 3

מדינת ישראל
משרד החקלאות ופיתוח הכפר
אגף בכיר לדיג וחקלאות מים



רשימת ספינות דיג עם מערך חכות צף המורשות לדוג בים התיכון
בתקופה שבין 1/4/2016 ועד 31/5/2016 * #

מספר רישיון דיג	שם בעלים	מספר רישום ספינה	מעגן
33114	מנסור אדהם	9021	ת"א-יפו
45810	חבורה שלום	72717	ת"א-יפו
53899	סלע יואל	71783	שדות ים
66609	פרץ סמי	56880	מרינה הרצליה
67009	הראל ים בע"מ	72760	ת"א-יפו
71104	יצקן עופר דן אייל	72064	אשדוד
72705	ניזרי חיים	70357	מרינה הרצליה
74708	סייג מיכאל	72286	קישון
76308	גרנות עידו	72704	מרינה אשדוד
76510	פוליבה אילן	72347	מרינה הרצליה
76611	נחום שלום שי	56424	נתניה
76712	גוקמן ויקטור	73011	קישון
76813	בניזרי אהרון	70359	קישון

* מלבד רישיון דיג משולם, כל ספינה חייבת גם בהצגת כושר שיט בתוקף מרשות הספנות, אחרת רישיון הדיג לא בתוקף.
 # לחלק מהספינות יש רישיון לדוג גם בשיטות נוספות מלבד הקפה, שאסורות בתקופה הזו (רשתות עמידה, מערך חכות קרקע, מלכודות, חכות).

דרך המכבים ראשל"צ; ת.ד. 30 בית דגן 5025001; טל 03-9485426 פקס 03-9485735

Supplement 4: Table of stomach content of ABFT

Fish #	Plastic	Teleost	Cephalopods	Crustaceans	Otolith only	Other/Unknown	total
80#	1	3	5	1	0	2	12
97#	1	5	0	0	0	0	6
56#	0	1	1	1	0	0	3
55#	0	13	4	61	27	0	105
54#	0	0	0	0	0	0	0
53#	2	6	0	0	0	1	9
52#	0	2	1	0	0	10	13
47#	0	2	1	6	7	12	27
44#	0	1	0	0	0	0	1
43#	0	1	4	3	16	0	25
42#	0	3	0	3	0	0	6
41#	0	0	8	0	0	1	9
40#	0	0	0	0	0	0	0
39#	0	0	0	0	0	1	1
38#	1	2	3	0	0	1	7
37#	2	1	0	0	1	1	5
25#	0	1	9	1	0	11	22
24#	0	0	0	3	0	0	3
22#	0	0	0	21	0	3	24
21#	0	0	0	0	0	0	0
20#	2	18	0	1	1	1	23
19#	1	0	0	0	0	0	1
5#	1	3	1	0	1	0	5
4#	0	1	1	0	1	0	3

אפיון ראשוני של אוכלוסיית הטונות כחולות הסנפיר בחלקו המזרחי ביותר של הים התיכון

תקציר

הטונה כחולת הסנפיר הוא מין בעל חשיבות אקולוגית, סביבתית וכלכלית רבת עוצמה. לטונה פיזיולוגיה מרשימה המאפשרת לה לשמר טמפרטורת גוף גבוהה משל הסביבה בה היא נמצאת. תכונה ייחודית זו מאפשרת לה את היכולת לנדידה בין תנאי סביבה משתנים, החל מהאזור הצפוני והקר של האוקיינוס האטלנטי שם היא ניזונה ועד למים החמימים של מפרץ מקסיקו והים התיכון לשם היא מגיעה לצרכי רבייה. לאורך השנים הדרישה הגבוהה לברר טונה בכל העולם ובפרט בשוק היפני בשילוב שיפור ציוד וטכנולוגיות הדיג הביא את המין לסכנת הכחדה. על מנת לשקם את האוכלוסייה הפגיעה הזו נדרש ניהול דייג נוקשה אשר מתבצע על ידי ארגון ICCAT, אך ניהול אוכלוסייה נודדת הינו משימה קשה. לשם כך מאמץ מחקר גדול מושקע על מנת לאתר תת אוכלוסיות של הטונה כחולת הסנפיר באמצעים שונים. על אף מאמץ זה אזור מזרח הים התיכון נותר בגדר חידה בכל הנוגע לאוכלוסיית הטונה הכחולה. בעבודה זו נדגמה ואופיינה לראשונה אוכלוסיית הטונות שחולפות בשטחה הימי של ישראל. בשנים 2016-2018 נדגמו 73 דגים בישראל בעזרת השתתפות פעילה בהפלגות דייג, דיגום במרינות והנמלים לאורך הארץ וראיונות שבוצעו עם דייגי טונות אשר שיתפו פעולה עם הפרויקט. בנוסף נדגמו 28 דגים בספרד במסגרת השתתפות בתחרות תיוג טונות של דייגים ספורטיביים. הדגים אופיינו באמצעות חמש שיטות עיקריות: (1) תועדו ונמדדו גודל ומין הדג. (2) אופיין המבנה גנטי על בסיס גנים מיטוכונדריאליים (COI, mtDNA CR). (3) נמדדה הרמה הטרופית בעזרת היחס האיזוטופי של חנקן (בשריר). (4) אופיינו הרגלי אכילה של הדג על בסיס תכולות הקיבה שלו. (5) תועדו נתיבי הנדידה של האוכלוסייה בעזרת תגים לוויינים. אוכלוסיית הדגים שנדגמו מישראל השוותה לאוכלוסיית הדגים שנדגמו בספרד ולמקומות נוספים בים התיכון שנלקחו מן הספרות. בנוסף אופיינו כמויות, שיטות, ציוד ומאמץ דייג הטונות בישראל שכן נתונים אלו אינם תועדו מעולם על ידי רשויות הדיג. איסוף הנתונים הראה על יחס גבוה של דגים קטנים שנדגמו בישראל ($<100\text{kg}$) ($n=57$) ותמך בתוצאות הבדיקות הגנטיות והתיוג הלווייני אשר רימזו על היתכנות של קבוצות דגים צעירים שנשארים בתוך הים התיכון ואינם נודדים חזרה לאוקיינוס האטלנטי. מדידת הרמה הטרופית הראתה על חוסר אחידות ונדרשת לבדיקה מחדש. המידע אשר נאסף אודות פעילות דייג הטונות בישראל משמש כבר בימים אלו כעזר בקבלת החלטות הנוגעות לשימור המין בישראל. כל תוצאות המחקר תורמות יחדיו להבנה כי הכרחי להמשיך לאסוף נתונים אודות אוכלוסייה ולפעול למען ניטור ומחקר ארוך טווח.

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אוניברסיטת חיפה

הפקולטה למדעי הטבע

ביה"ס למדעי הים ע"ש לאון צ'רני

החוג לביולוגיה ימית

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