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Diet and feeding behaviour of albacore *Thunnus alalunga* in the western Mediterranean Sea during the spawning period

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Running title: Diet of spawning albacore

Abstract

Mature albacore tuna (*Thunnus alalunga*) are expected to have high energy requirements at the time of breeding. However, in the Mediterranean Sea, there are no descriptions of the diet of albacore that can help to understand if such requirements can be obtained from feeding during reproduction. In this study, we analysed the stomach contents of reproductively active albacore captured from 2010 to 2015 in the oligotrophic waters of the western Mediterranean Sea, one of their main spawning grounds. Estimates of stomach fullness revealed intense feeding activity, and prey composition indicated important consumption of mesopelagic fish, including barracudinas, myctophids, and small pelagic crustaceans. Plastic debris occurred in 25%–53% of the stomachs sampled across all years. Prey composition was not different between males and females. However, females fed at higher rates and had higher hepatosomatic index values than males suggesting that increased feeding could contribute to meet their higher energy demand associated with offspring production. We observed a diet shift from small crustaceans to fish prey along fish size. During the spawning period, albacore showed a specialist feeding behaviour by preying on aggregations of vertically-migrating myctophids and small crustaceans, probably when they were near the surface. This study provides information and biological data to support ecosystem modelling, and to increase the understanding of albacore ecology.

Introduction

Albacore *Thunnus alalunga* (Bonnaterre 1788) is one of the most captured temperate tuna species globally (FAO, 2019; Nikolic *et al.,* 2017). This species is widely distributed around the world. Many aspects of its life history, such as its feeding behaviour, are poorly known, in particular for spawners (Bertrand *et al.,* 2002; Nikolic *et al.,* 2017). Food availability, spawning and physiological constraints related to body temperature control have been hypothesized as drivers of differences in global distribution across life stages of this species (Bertrand *et al.,* 2002; Pusineri *et al*., 2008; Sund *et al.,* 1981).

Albacore concentrate at cool and highly productive areas, foraging on abundant epipelagic prey (Goñi *et al.,* 2011; Watanabe *et al.,* 2004; Williams *et al.,* 2015). Similar to the other *Thunnus* species, albacore perform long migrations, from feeding grounds to spawning areas (Muhling *et al.,* 2017; Nikolic *et al*., 2017), where temperature and not food may be the main driver of population distribution (Dragon *et al.,* 2015). During the reproductive season, adults concentrate in warm, low chlorophyll areas (Alemany *et al.,* 2010; Arrizabalaga *et al.,* 2015), foraging on aggregations of epi- and mesopelagic prey (Goñi *et al.,* 2011; Romanov *et al.,* 2020). However, Mediterranean albacore tuna is a population that inhabits the Mediterranean Sea year round (Alemany *et al*., 2010), and where different spawning grounds have been identified. In the western Mediterranean Sea, the Balearic Islands are recognized as essential spawning habitats for albacore as well as for other large pelagic species (Alemany *et al.,* 2010). The International Commission for the Conservation of Atlantic Tunas (ICCAT) views the Mediterranean Sea albacore stock as an independent stock from the Atlantic Ocean. The Mediterranean Sea population of albacore is currently classified as a data-poor stock, and little is known about its life cycle period (Juan-Jordá *et al.,* 2013). Therefore it is important to improve our knowledge of this species, particularly in the Mediterranean Sea, to understand their population variability.

The few studies conducted in the Mediterranean Sea show that adults feed on cephalopods (Bello, 1999; Salman and Karakulak, 2009), fishes (Consoli *et al.,* 2008), and crustaceans (Goñi *et al.,* 2011). However, most of these studies were based on limited sample sizes, and on a low number of non-empty stomachs, probably because animals were caught at night when feeding activity is low (Aloncle and Delaporte, 1973; Watanabe *et al.,* 2004; Young *et al.,* 2010). One additional study by Salman and Karakulak (2009) analysed spawning albacore, but focused only on cephalopod prey,

and did not perform a complete diet analysis. No prior studies have assessed the albacore diet in the Mediterranean Sea spawning grounds during the spawning season.

Tuna species can store energy when food is plentiful and consume energy reserves when food is scarce (Olson *et al.,* 2016). Different patterns of energy storage are necessary to cope with the high energetic demands and changes in environmental conditions experienced during migration and spawning (McNamara and Houston 2008). Capital breeding allows feeding and spawning grounds to become dissociated in time and space, thus enabling adults to reproduce using stored energy. By contrast, income breeders use concurrent energy intake in response to higher resource demand during breeding (Jönsson, 1997). Nonetheless, some species adopt a mixed strategy, using energy stored previously in somatic tissues and food resource income, as has been described for female albacore (Dhurmeea *et al.,* 2018), as well as for yellowfin tuna (Zudaire *et al.,* 2014) in the Indian Ocean. Incorporating a capital breeding strategy can help female albacore to reproduce under conditions of limited productivity and unreliable food availability (Jönsson 1997; Stephens *et al.,* 2014). Very little is known about the albacore diet during reproduction; this information can help to elucidate the albacore strategy for offspring production in the Mediterranean Sea.

In the present study, we have investigated the diet and feeding strategy of albacore through stomach content analysis of albacore caught by a recreational fishery in their major spawning ground in the western Mediterranean Sea during the spawning season (Saber *et al*., 2015). Our specific objectives were 1) to describe albacore's diet composition, 2) to explore the effect of biological traits (sex and fish size) on the diet, and 3) to identify the feeding strategy (through the rate of feeding and condition index) during the spawning season. Furthermore, the trophic position was estimated, and predator- prey relationships were also discussed, in order to understand albacore feeding behaviour during the spawning period.

Materials and methods

Data collection

Albacore were caught by recreational fishers at fishing tournaments around the Balearic Islands in the western Mediterranean Sea. Fish were caught using rod and reel gear by trolling, which targets surface fish between 0 and 5 metres depth and consists of fishing lines (30 or 50 lb) with hooks and artificial lures. Fishing tournaments take place in June and July during the daytime (approximate effective fishing time 08:00–17:00);

fish were kept on ice until landing. Fishing grounds extend 60 nautical miles around the base ports (Figure 1). From 2010 to 2015 a total of 170 albacore (60–95 cm, standard fork length (SFL)), were randomly collected and were analysed for stomach contents (Table 1). Immediately after landing, fish were weighed (kg, round weight) and measured (cm, SFL). Gonads were removed and their weights (GW) were recorded (in grams). Sex and macroscopic maturity were determined by visual inspection of the gonads. Macroscopic maturity phases of ovaries and testes were assigned modifying the criteria suggested by Diouf (1981) and Brown-Peterson et al. (2011) (Table 2). Additionally, the gonadosomatic index (GSI) was calculated as follows: $GSI = GW / SFL^3 * 10^4$ (Kume and Joseph 1969). Liver and stomach were excised, weighed to the nearest 0.1 g (n = 90 individuals), stored individually in plastic bags, and frozen at -20 °C.

Stomach contents sampling and analysis

The stomach contents were washed through a sieve with 0.2 mm mesh size; the stomach lining was weighed after rinsing, and then blotted dry. The difference between the weights of the excised stomach and the stomach lining was recorded as the total stomach contents weight. Prey items were examined under a binocular microscope and classified into six broad categories: Crustacea, Mollusca, Pisces, Tunicata, Cnidaria, and Annelida. The unidentifiable remains and litter (pieces of paper, plastic, and wood) were also recorded. Prey items were identified to the lowest possible taxonomic level in all the stomachs (n = 170). Prey counts were performed on 146 stomachs, but prey weight was registered only in 71 individuals because of sampling constraints. Prey weight was recorded as a percentage of the total stomach contents weight that was assigned for each prey item using a trophometrer (calibrated device) as in previous studies (Olaso et $al.,$ 1998; Valls et $al.,$ 2011).

The state of prey digestion was classified into one of three stages: A) undigested or fresh; B) in process of digestion; and C) highly digested. The identification of highly digested prey was performed through otoliths for fish, beaks for cephalopods, and the exoskeleton for crustaceans; identification was performed according to available guides (Lombarte et $al.,$ 2006; Trégouboff and Rose, 1957) and to our reference collections. To identify the fraction of the prey population consumed by albacore during their spawning season, undigested or fresh prey, or prey in the process of digestion (TL, Total Length

for fish and crustaceans; ML, Mantle Length for cephalopods, all in mm) was analysed. Digital measurements of otoliths were performed through image analysis; the fish body length was then calculated using the known relationship between otolith length (mm) and fish length (mm) for Mediterranean Sea species (Giménez *et al.,* 2016).

Statistical analysis

Albacore were grouped by size (5 cm length categories) and sex; size distributions were plotted and mean size compared for sexes (Kruskal-Wallis test). To verify if the relationship between sex and size class followed a 1:1 ratio, a Chi-square test (p level < 0.05) was carried out using Statistica v10 (StatSoft Inc., 2011).

To assess the adequacy of the number of stomach samples analysed, the mean and standard deviation of the cumulative number of total prey items (Sobs ± standard deviation) was plotted against the cumulative number of non-empty stomachs (Ferry and Caillet, 1996) after a maximum of 999 permutations. The asymptotic stabilization of the curve indicated the minimum number of stomachs required for the analysis to obtain an accurate and reliable diet description (Ferry and Cailliet, 1996). The slope (b) of the linear regression, obtained from the last four randomly sampled stomachs, was used as an objective criterion; $b \leq 0.05$ determined that the curve had reached an acceptable asymptote (Bizzarro *et al*., 2007). Since diet composition was described using the number and weight of prey, based on different sample sizes (146 stomachs and 71 stomachs with contents), prey curves were generated for each dataset. The cumulative prey curves were performed using PRIMER 6 + PERMANOVA (Anderson *et al.,* 2008).

To characterize the diet of albacore during their spawning season in the western Mediterranean Sea, the following trophic indices were calculated: 1) frequency of occurrence (%F = number of stomachs containing prey i / total number of stomachs containing food * 100); 2) numerical composition (%N = total number of individuals of prey i / total number of individuals * 100); 3) weight composition (%W = total weight of prey i / total weight of stomach contents * 100); 4) index of relative importance, which integrates all of the indices above (IRI = %F * (%N + %W)) standardized as %IRI = (IRI/∑IRI * 100); 5) the mean percentage number (%MN) and 6) mean percentage weight (%MW) were calculated according to the proportion of each prey item found in the individual stomach and then averaged for each prey in all stomachs (including zeros), in order to obtain variance estimates, and to allow indices comparison

across studies (Chipps and Garvey, 2007); 8) the percentage prey specific index of relative importance (%PSIRI) (Brown *et al.*, 2012) was calculated as %PSIRI = ((%F * (%PNi + %PWi))/2, where %PNi and %PWi represent numerical prey specific abundance and weight values, respectively, but only for stomachs that contained prey i (Brown *et al.*, 2012). Both %PNi and %PWi were only used to calculate %PSIRI and were not used for overall stomach contents description. Loose otoliths that were accumulated (i.e. eroded) in the stomachs were excluded to avoid overestimating the number of fish prey. Unlike fishes, the majority of cephalopods were found in an advanced stage of digestion (i.e. most beaks were loose and had no remnant of flesh attached). To account for all contents related to this common but highly digested prey type, beaks were taken into account for occurrence, number, and weight prey composition estimations (Glaser *et al.*, 2015). Basic metrics (%N, %F and %W) were also reported excluding loose beaks. Although prey items were identified to the lowest possible taxon, higher taxonomic categories (22 prey items at the order level and above) were used for diet quantification (in terms of weight and compound indices) and statistical analyses.

To analyse the ontogenetic shift in diet, albacore were grouped into three length groups: small-sized albacore, G1, 60-69 cm SFL; medium-sized, G2, 70-79 cm SFL and; large-sized, G3, 80-95 cm SFL. Differences in diet composition by sex and size (fixed factors) and their crossed effects were tested on the abundance matrix (fourth root data transformation) following a distance-based permutation analysis of variance (PERMANOVA test) and Bray-Curtis distances. Similarity percentage (SIMPER) analysis was performed among standardized abundances to identify the main prey items contributing to any significant factor. Among the 22 prey categories, those with a frequency of occurrence below 5% (Octopodidae, Gastropoda, Argentinidae, Carangidae, Centrolophidae, Clupeidae, Gobiidae, and Polychaeta) were not taken into account for multivariate analyses. Unidentifiable remains and litter were excluded from all analyses. A final matrix of 14 prey items based on abundance data was thus obtained.

To analyse the feeding strategy of albacore during the spawning season, different indices were taken into account. Firstly, the diet breadth was calculated using the Levin's standardized index: $B_i = \dfrac{1}{n-1}\left(\dfrac{1}{\sum_j p_{ij}^2} - 1\right)$, where p_{ij} is the proportion of the diet of predator i that is composed of prey j, and n is the number of prey categories. This index

ranges from 0 to 1, low values indicate diets composed of few prey items (specialist predators), and higher values indicate generalist diets. Niche breadth was calculated using Ecological Methodology software version 7.0 (Krebs, 1999). Secondly, to investigate the rate of feeding activity, the index of stomach fullness (Hyslop, 1980) was calculated as follows: $SF = (SW/EW) * 100$, where SW is the stomach contents weight (in g) and EW is eviscerated wet weight (in kg); to avoid the effect of viscera and gonad weight, we calculated this index based on the eviscerated weight (Lloret *et al.,* 2014). Finally, the condition of fish was assessed according to the hepatosomatic index: $HSI = (LW/W) * 100$, where LW and W represent liver and eviscerated wet weights, respectively (all in g). The liver serves as a major energy storage site in albacore (Dhurmeea *et al.,* 2018), thus indicating nutritional state. Stomach contents and liver weights were available for 90 individuals (2010, n = 29; 2011, n = 27; 2012, n = 17; 2014, n = 17).

To assess the influence of sex on stomach fullness and hepatosomatic index, a Kolmogorv-Smirnov two-sample test (p level < 0.05) was carried out using Statistica v10 (StatSoft Inc., 2011). Due to the relationship between albacore feeding rate and size (Goñi *et al.,* 2011; Williams *et al.,* 2015; Young *et al.,* 2010), both indexes were plotted by sex and by 5 cm size classes.

All multivariate analyses were performed using PRIMER 6 + PERMANOVA software package from Plymouth Marine Laboratory, UK (Anderson *et al.,* 2008). The significance level was set at $\alpha = 0.01$ and obtained by using 9999 permutations. In the case of significant results, pairwise comparisons were performed. Distance-based tests for homogeneity of multivariate dispersions were carried out for each factor, to know if there was a dispersion effect (PERMDISP using distances to centroids using permutations, Anderson *et al.,* 2008). All graphs were plotted using SigmaPlot (Systat Software, San Jose, CA).

Finally, data on diet composition were also used for the estimation of the trophic level of albacore. TROPH expresses the position of organisms within the food webs that largely define aquatic ecosystems. The definition of TROPH for any consumer species (i) is: $TROPH_i = 1 + \sum_{j=1}^{n} DC_{ij} * TROPH_j$, where $TROPH_j$ is the fractional trophic level of prey j, DC_{ij} represents the fraction of j in the diet of i and n is the total number of prey species. TROPH value was calculated from the dataset using TrophLab (Pauly *et al.,* 2000) which is a stand-alone application for estimating TROPH and its standard

error using the weight contribution and the trophic values for various prey (based on data in FishBase; Froese and Pauly, 2020).

Results

Data collection

The size of females ranged from 60 to 84 cm SFL (mean ± SD = 68.4 ± 5.7 cm, n = 81); the size of males ranged from 61.5 to 95 cm SFL (mean ± SD = 75.8 ± 7.0 cm, n = 89). Significant differences in size were found (Kruskal-Wallis test, H = 42.28, p < 0.001), females being smaller than males. The sex ratio by size class was significantly different from the expected 1:1 (Chi-square test, χ^2 = 39.52, p < 0.001) (Figure 2). Females outnumbered males within the size range 60–70 cm SFL (p < 0.05), but males were more abundant in size classes over 80 cm SFL (p < 0.05). No sex ratio differences were found in individuals 70–80 cm SFL. Of the total number of females, 58 ovaries were classified as being in the spawning capable maturity phase, and 23 were in the spawning phase. Females in developing stage were absent. Regarding males, only one testis was classified as being in the developing maturity phase, 73 in the spawning capable maturity phase, and 15 were in the spawning phase. The mean GSI values (±SD) in females corresponding to each gonad maturity phase (spawning capable and spawning) were 9.8 ± 1.9, and 10.3 ± 2.0, respectively. The mean GSI values (±SD) in males to each gonad maturity phase were: 3.8 in the developing phase, 5.9 ± 1.6 in the spawning capable phase, and 6.4 ± 1.4 in the spawning phase. Therefore, all the individuals analyzed in this study were reproductively active except the one male in the developing phase.

Diet composition

A total of 170 albacore, 81 females and 89 males were analysed for stomach contents. Only five individuals had empty stomachs, that is, 97% of reproductively active albacore had prey in their stomachs. The cumulative curves of observed prey items indicated that the number of fish used for stomach contents analysis was sufficient to properly characterize the albacore diet (b<0.01 for both curves) (Figure 3). The diet composition is summarized in this paragraph, taking into account both the lowest identified taxonomic levels and the broad taxonomic categories (Table 3). Overall, 63 prey items were identified through the analysis of albacore stomach contents. The most

important prey in terms of both %IRI and %PSIRI were Pisces (56–57%) and Crustacea (33–35%), while Mollusca (5.5 %), Tunicata (2.6–5.4%), Cnidaria (0.01%), and Annelida (< 0.01%) were of minor importance. Pisces were the main prey found in the stomachs in terms of number (%N = 42.9; %MN = 36.1 ± 0.3) and mass (%W = 70.1; %MW = 45.5 ± 0.4). Although 21 fish species belonging to nine families were identified, myctophidae and paralepididae were the main diet items of reproductively active albacore. Myctophids showed the highest values for all indices (%N = 31.7, %W = 29.7, and %F = 57.0), with *Lampanyctus crocodilus* and *Hygophum benoiti* being the most frequent and abundant fish prey, followed by *Ceratoscopelus maderensis*. The Paralepididae fish family, mainly represented by *Arctozenus risso*, made the second-largest contribution in terms of biomass (%W = 32.3) and occurrence (%F = 54.5). Other fish prey such as the stomiiform *Vinciguerias attenuata*, and the demersal fish *Centracanthus cirrus* were also identified in stomach contents, but were less frequent (13.3% and 10.3% respectively).

Small-sized crustaceans, including Amphipoda, Decapoda, Euphausiacea, and Isopoda, were identified as secondary prey in the diet of albacore; although the relative weight of crustacean prey greatly increased after averaging for individual stomachs (%MW = 44.8 ± 0.3), and had similar biomass as fishes prey. Among crustaceans, amphipods were the most important prey group; they were as frequent (84.2%) as fish (83.6%) in the stomach contents, but their number (%N = 25.7; %MN = 26.3 ± 0.2; (%N$_{fish}$ = 42.9; %MN$_{fish}$ = 36.1 ± 0.3) and weight (%W = 21.9; %MW = 37.0 ± 0.3; %W$_{fish}$ = 70.1; %MW$_{fish}$ = 45.5 ± 0.4) proportions were lower. At the species level, the Hyperiidae *Brachyscelus crusculum*, *Phronima sedentaria* and *Phrosina semilunata* were the most frequent prey (50.9%, 47.3%, and 36.4% respectively). Euphausiids, namely *Meganycthiphanes norvegica*, were the most important crustacean prey, after amphipods, accounting for 13.6% and 26.7% in terms of number and frequency, respectively.

Similarly to fishes and amphipods, molluscs (primarily cephalopods), were frequently consumed by albacore (83%) but were in low abundance (%N = 10; %MN = 15.1 ± 0.1) and, due to their advanced state of digestion (> 75% were beaks without flesh; Figure 4), made a very low contribution in terms weight (%W = 1; %MW = 1.3 ± < 0.1). The percentage values were clearly lower (%F=14.5; %N=0.7; %W=0.8) when loose beaks were not considered. The ommastrephid squid *Todarodes sagittatus* (%F = 21. 2) and the sepiolid *Heteroteuthis dispar* (%F = 26.1) were the most commonly

identified cephalopod prey species. Salps (Thaliacea) were also frequently found (40%) in the stomachs of albacore and accounted for 4.7% of the total prey weight composition.

The size spectrum of the consumed fishes, e.g., *L. crocodilus* (TL = 45–86 mm), *Notoscopelus* sp. (TL = 28–79 mm) and *C. maderensis* (TL = 30–53 mm) revealed the small size of the individuals consumed (Table 4). Similar results were observed in cephalopods; small-sized individuals of the squid species *Ancistrotheutis lichtensteinii* (ML = 31–58 mm), *I. coindetii* and *T. sagittatus* (both species ML = 25–80 mm) were found (Table 4).

Regarding the biological factors that influenced albacore diet (sex and size), size differences were detected (PERMANOVA pseudo-F = 2.508, $p < 0.01$). Pairwise comparisons indicated diet differences between the small-sized albacore group (G1) and the medium-sized individuals (G2) (PERMANOVA pseudo-F = 1. 8564, $p < 0.01$). No differences in diet composition were found between females and males (PERMANOVA pseudo-F = 0.9237, $p = 0.4638$). No interaction effect between sex and size was detected (PERMANOVA pseudo-F = 1.6716, $p = 0.0916$). The SIMPER analysis showed that the smallest individuals (G1) consumed higher abundance of euphausiids and amphipods, in comparison to the medium-sized specimens (G2), which showed a higher preference for fish prey (myctophids and paralepids) (Table 5). Homogeneity of dispersion was confirmed for both sex and size factors (PERMDISP: $p = 0.855$ and $p = 0.580$ respectively). Plastics were frequently found (37.6% of the stomachs sampled) across the sampling period (Table 1).

Feeding strategy and trophic level

Levin's standardized index was 0.108 for the abundance of 63 prey; *H. benoiti*, *L. crocodilus*, *B. crusculum*, euphausiids, and salps, were frequent (cut-off proportion 0.05), indicating a specialist diet. Stomach fullness differences were found between the sexes (Kolmogorv-Smirnov t-test, $p < 0.001$). Females (5.09 ± 0.77 g/kg, n = 45) had higher mean values relative to males (1.57 ± 0.33 g/kg, n = 45) across all size groups (Figure 5). Sex differences were also found for the hepatosomatic index (HSI) (Kolmogorv-Smirnov t-test, $p < 0.001$), with higher values in females (1.37 ± 0.06, n = 44) compared to males (0.66 ± 0.02, n = 45) across all size groups (Figure 6). The

estimated TROPH value (from TropLab default prey TL), for albacore in the western Mediterranean Sea, was 4.19 ± 0.71.

Discussion

Diet composition

During the spawning season, albacore rely on mesopelagic fish species as major food-energy sources, as indicated by the high percentage of occurrence, number, and weight of these fishes. The most important mesopelagic fish prey was the paralepid *Arctozenus risso*, and three species of the Myctophidae family, represented by *Lampanyctus crocodilus*, *Hygophum benoiti*, and *Ceratoscopelus maderensis*, which are among the most abundant species in the study area in summer (Olivar *et al.,* 2012). Similarly, paralepids were identified as the main fish prey in terms of biomass for albacore tuna in two other areas of the Mediterranean Sea (Tyrrhenian and Adriatic Seas) (Consoli *et al.,* 2008; Goñi *et al.,* 2011). By contrast, in the northeast Atlantic Ocean and the western tropical Indian Ocean, epipelagic and reef-associated fishes, rather than mesopelagic fishes, are the main food source for albacore tuna (Goñi *et al.,* 2011; Romanov *et al.,* 2020). Mesopelagic fish species in the Mediterranean Sea are abundant (Lam and Pauly, 2005) and energy-rich (Spitz *et al.,* 2010), representing a valuable and predictable food resource for many demersal (Cartes *et al.,* 2009; Valls *et al.,* 2017) and pelagic (Battaglia *et al.,* 2013; Karakulak *et al.,* 2009; Piccinetti and Piccinetti Manfrin, 1970) predators. Owing to the opportunistic feeding behaviour of tuna species, regional prey availability was identified as the main driver of albacore diet variability at a global scale (Duffy *et al.,* 2017).

Although albacore mainly consumed fishes, mesopelagic crustaceans (mainly Hyperiidean amphipods *B. crusculum*, *P. sedentaria*, and *P. semilunata*) were also important prey. Similar results have been published recently for both spawning capable and post-spawning albacore caught around anchored fish aggregating devices in the western Indian Ocean, with higher values in terms of weight (40%), and even with the same dominating species (i.e., *B. crusculum*) (Dhurmeea *et al.,* 2020). Amphipods have been generally reported as accessory prey for adults in the Mediterranean Sea (Consoli *et al.,* 2008; Goñi *et al.,* 2011), and for young specimens in oceanic areas (Pusineri *et al.,* 2005; Williams *et al.,* 2015; Young *et al.,* 2010). However, in the work of Ortiz de Zarate (1987), hyperiids were identified as an important food source (%W = 31. 4) for juvenile albacore caught in the Bay of Biscay (Norteast Atlantic Ocean). Other pelagic

predatory fish such as lancetfish or snake mackerel also prey on hyperiids, making up a large fraction of their diet (Choy *et al.,* 2013). Small-sized crustaceans, such as vertically-migrating euphausids, have been reported to dominate the diet of juvenile albacore from the Atlantic and Pacific Oceans (Pusineri *et al.*, 2008; Duffy *et al.,* 2017; Williams *et al.,* 2015). In the Mediterranean Sea, macroplankton groups (i.e., amphipods, euphausiids, and salps), which show peaks of abundance in summer (Cartes, 1998), might provide a complementary and low-cost food resource for reproductively active albacore.

Although mesopelagic fishes and hyperiids were identified as main food resources in terms of frequency and occurrence, cephalopods (particularly squids) were as frequent as fishes and crustaceans (around 85%). Nonetheless, the inclusion of loose beaks in this study makes the estimates of cephalopods consumed by albacore less conservative than if cephalopod beaks were excluded (%F=14.5). While beaks were frequent in the stomachs of albacore, they appeared in small numbers with cephalopod prey contributing only to 1% of the total mass of the overall diet. Cephalopods have been reported as an important food source in other oceanic regions (Duffy *et al.,* 2017; Dhurmeea *et al.,* 2020; Romanov *et al.,* 2020). Similarly, cephalopods were also frequently consumed by albacore in the eastern Mediterranean Sea (95.1%; Salman and Karakulak, 2009). In the central Mediterranean Sea, Bello (1999) reported the same low proportion of cephalopods by weight (1%) as the present study; however, cephalopods were more abundant in Bello's report (29%) than in our study (9%). Moreover, squids accounted for 51.6% of the total mass of the diet of albacore of the central Mediterranean Sea (Goñi *et al.,* 2011). Comparisons with previous studies have to be interpreted with caution, because of the low number of non-empty stomachs analysed (< 60) and the differences in the temporal scale of sampling. Considering the reported albacore digestion rate (Aloncle and Delaporte 1973), the existence of cephalopod diel migrations, their advanced stage of digestion, and fishing time, it is likely that most cephalopods found in the stomach of albacore had been ingested the night before capture (Bello, 1999; Pusineri *et al.*, 2005).

Plastics found in the stomach of albacore varied between 25% and 53% of the fish, depending on the year; these values are higher than those previously reported for albacore (13%; Romeo *et al.,* 2015) and other large pelagic predators in the Mediterranean Sea (*Coryphaena hippurus*, 7%, Massutí *et al.,* 1998; *Thunnus thynnus*, 17%, Karakulak *et al.,* 2009; *Xiphias gladius*, 12. 5% and *T. thynnus*, 32%, Romeo *et*

al., 2015). Around 20% of albacore sampled five decades ago in the northeast Atlantic Ocean already contained plastic debris in their stomachs (Aloncle and Delaporte, 1973), but at much lower values than those reported recently (3.2%, Romanov *et al*., 2020).

Feeding strategy and trophic level

Tunas are recognized as opportunistic predators that feed on a broad spectrum of prey. Reproductively active albacore caught in the western Mediterranean Sea foraged on a broad spectrum of prey (63), but only a few species dominated their diet (i.e., *H. benoiti*, *L. crocodilus*, *B. crusculum*, and euphausiids) resulting in a low value of Levin's index. This result is in line with the feeding strategy of albacore of the central Mediterranean Sea (Consoli *et al.,* 2008). Similarly, previous studies conducted on other temperate waters described a feeding strategy based on a few prey that accounted for a large proportion of their diet (Glaser *et al*., 2015; 2010; Goñi *et al.,* 2011; Pinkas *et al.,* 1971; Pusineri *et al.,* 2008; Watanabe *et al.,* 2004). In Balearic waters, Olivar *et al.* (2012) reported the dominance of few myctophid species on the mesopelagic assemblages both in near-surface and deeper layers during both day and night. This observation supports the presence of monospecific myctophid aggregations which might be available for albacore. Based on previous studies, the high consumption of small crustaceans in adult albacore specimens is striking. Unfortunately, precise data on the abundance of pelagic invertebrates are not available for our study area. However, albacore spawning areas in the western Mediterranean are related to oceanographic fronts (Alemany *et al.,* 2010), and thus to sites where prey aggregation occurs. Euphausiids were the predominant prey in albacore (Goñi *et al*., 2011) and skipjack tunas from the Balearic Sea (Varela *et al*., 2019). The high abundance of few prey items (i.e., amphipods and myctophids) supports the hypothesis of albacore preference for ram filter-feeding on dense prey aggregations (Romanov *et al*., 2020). Although crustaceans (e.g., amphipods and euphausiids) provide less energy than fishes (Dhurmeea *et al.,* 2020; Spitz *et al.,* 2010), they move slowly and form dense aggregations, which make them an easy target. Furthermore, in addition to fish consumed by albacore, the lipid composition of crustaceans was also rich in different lipids and essential fatty acids (Dhurmeea *et al.,* 2020) that have important roles in fish physiology, including the reproductive process (Ortega and Mourente, 2010).

The reported pattern of small-sized-prey preferences in the albacore diet (Bello, 1999; Pusineri *et al.,* 2008; Romeo *et al.,* 2011; Salman and Karakulak, 2009) is also

found for species consumed by reproductively active individuals in the western Mediterranean Sea. Albacore foraged on vertically-migrating cephalopod juveniles (*I. coindetii* and *T. sagittatus*) moving to the upper layers at night (Quetglas *et al.,* 2014). Chasing and capturing small prey, instead of fast-moving cephalopod adults, would increase the net energy gain obtained from the prey. Energy optimization in the foraging strategy (Romanov *et al.,* 2020), together with trophic niche segregation for reduction of interspecific competition (Young *et al.,* 2010), have been suggested to explain albacore small prey size selectivity.

Macroscopic examination of gonads and GSI values of albacore from the western Mediterranean indicated that fish were able to spawn. The estimated high GSI values were similar to values observed in the same area (Saber *et al*., 2015), and in the eastern Mediterranean Sea for males (Akayli *et al*., 2013) and females (Karakulak *et al*., 2016). A thorough analysis of daily energy consumption estimated from stomach contents is beyond the scope of this study; however, albacore showed clear signs of intense feeding during spawning. Stomach fullness of albacore was within the range of the values observed in fish of the Pacific and Atlantic Oceans (Williams *et al.,* 2015; Young *et al.,* 2010). This is surprising, as Balearic waters are considered oligotrophic and therefore prey-limited, compared to adjacent areas and oceanic waters (Alemany *et al.,* 2010). This fact indicates that the plankton biomass around the Balearic archipelago would enhance not only larvae growth and survival (Doty and Oguri, 1956) but also feeding opportunities for adults (Boehlert and Mundy, 1994).

Adult albacore need large amounts of energy for displacement to the breeding areas and reproduction (egg production and spawning behaviour). However, dimorphic foraging rates were observed in this study, indicating higher feeding rates per body unit in females than in males. Spawning females displayed feeding rates similar to the higher values described for the young albacore from oceanic populations (≈ 6 g/kg; Williams *et al*., 2015), whereas male's feeding rates were within the range of the adult oceanic albacore (≈ 1 g/kg; Young *et al*., 2010). Previous studies identified several factors that influence stomach fullness, such as water temperature (Aloncle and Delaporte, 1973; Williams *et al.,* 2015), inshore-offshore distribution (Young *et al.,* 2010), productivity, and different growth rates between immature and mature specimens (Goñi *et al.,* 2011). In fact, the highest values of stomach fullness were reported for young specimens of the northeast Atlantic Ocean (Goñi *et al.,* 2011), where productivity is higher than in other areas (Moriarty and O'Brien, 2013). To our knowledge, this is the first study to

highlight sex differences in albacore stomach fullness.Our data showed that females dominated in the small length classes (between 60 and 70 cm SFL) while a greater abundance of males of larger length classes (above 80 cm FL) were found. The predominance of males in larger length classes has been reported in the Mediterranean albacore stock (Karakulak *et al*., 2011; Saber *et al*., 2015) as well as in the oceanic stocks (Farley *et al*., 2013; Dhurmeea *et al*., 2016; Ashida *et al*., 2020). The difference in sex ratio among length classes has been related to differences in natural mortality and vulnerability to capture (Schaefer 2001; Schaefer *et al*., 2005) and by the growth difference due to the discrepancy in reproductive investment which is higher in females than in males (Chen *et al*., 2012; Farley *et al*., 2013).

The high hepatosomatic index values and feeding ratios observed, especially in females, indicated that reproductively active albacore have access to a large abundance of prey in the area which in turn leads to intensive feeding to help meet the higher energy demand of females during reproduction. Relative to spawning-capable albacore from the Indian Ocean (Dhurmeea *et al*., 2018), we found higher values of female liver weights. Skipjack and yellowfin tunas have been described as income-capital breeders, relying mostly on food intake (Grande *et al.,* 2016; Zudaire *et al.,* 2014, 2015); in contrast, albacore have been described as capital-income breeders, mostly relying on stored reserves (Dhurmeea *et al.,* 2018) to acquire energy for reproduction. This flexibility might reduce the extent to which reproductive success is dependent on environmental conditions (i.e., prey availability) at the time of breeding (Stephens *et al.,* 2014). An individual's position along the capital-income continuum may shift with ontogeny or with environmental conditions, thus breeding patterns are a conditional reproductive strategy (McBridge *et al.,* 2015). Whether albacore from the western Mediterranean Sea rely mainly on current food intake or previously stored energy reserves (e.g. liver, Dhurmeea *et al.,* 2018; perigonadal fat, Ratty *et al*., 1990) requires further research, and would help to understand albacore plasticity to adapt to different environmental conditions.

In oceanic regions, factors such as depth, water column structure, primary production, diel behaviour, and size influence albacore's spatial distribution (Cosgrove *et al.,* 2014; Logan *et al.,* 2020; Nieto *et al.,* 2017; Williams *et al.,* 2015). Food availability and physiological capacities are hypothesised drivers of the albacore vertical behaviour (Briand *et al.,* 2011; Pusineri *et al*., 2008). Whether reproductively active albacore perform downward vertical displacements in Mediterranean Sea waters

remains to be fully investigated. Albacore spawn near the surface around midnight and the early hours of the morning (Farley *et al.,* 2013), therefore a preference for day feeding appears to be a probable foraging strategy, as has been described in previous studies (Watanabe *et al.,* 2004; Young *et al.,* 2010). We found that most of the albacore prey were mesopelagic species that undertake vertical diel migration moving to the upper layers at night (Olivar *et al.,* 2012; Quetglas *et al.,* 2014). Predation on those prey could probably occur during sunrise and/or sunset when prey are still accessible near the surface. However, several mesopelagic fish prey (i.e., *A. hemigimnus*, *D. holti*, *E. risso*, and *V. attenuata*) have been reported in the study area during both day and night in the Deep Scattering Layer (400–600 m), while being absent from the surface (Olivar *et al.,* 2012). The frequency of occurrence of mesopelagic fish prey in the stomach of albacore suggests that they occasionally dive to feed in deep layers. This behaviour has been documented in albacore by analysing the composition of prey species (Romanov *et al.,* 2020; Young *et al.,* 2010) and by tagging experiments (Childers *et al.,* 2011; Cosgrove *et al.,* 2014).

Better knowledge of the spatial distribution of Mediterranean Sea albacore prey could aid in determining the vertical distribution of albacore. Understanding how and why highly mobile species utilize and move along their vertical and horizontal habitat is vital to determining how successful stocks are managed (Evans *et al.,* 2015).

Based on stomach contents, estimation of albacore trophic position was 4.19, which was similar to that reported for albacore in the Tyrrhenian Sea (4.47; Consoli *et al.,* 2008) and other large pelagic predators in the Mediterranean Sea (*Euthynnus alletteratus*, 4.44; *Xiphias gladius,* 4.42; Karachle and Stergiou, 2017).

Conclusion

This study presents new information on the diet and feeding behaviour of albacore during the spawning period showing that reproductively active albacore predominately consume energy-rich mesopelagic fish species, which account for more than half of their diet. This result provides further evidence of the importance of mesopelagic species in the Mediterranean Sea's pelagic food webs. A better knowledge of the micronekton and the trophic structure of the pelagic ecosystem would improve the reliability of the models in predicting stock abundance and fishing changes in climate change scenarios (Olson *et al.,* 2016). Furthermore, foraging on aggregations of prey (i.e., myctophids and small crustaceans), together with a preference for small-sized

prey, leads us to hypothesize that this foraging strategy may result in increased net energy gain for mature albacore. Future work is needed to link feeding and reproduction to better understand which prey are particularly important for albacore reproductive success. Estimates of stomach fullness were higher than previously reported for adult specimens from the Mediterranean Sea and sex differences observed for these estimates, including feeding rates, could be linked to the higher energy requirements associated with offspring production in females. The new biological information on this large pelagic species provides new insights on their trophic ecology and can be used to improve albacore stock management and conservation.

Authors' contributions M.V. and S.M. designed the study. M.J.G., S.M. and S.S. collected the albacore and the survey data. M.V. analysed the stomach contents. M.V elaborated the statistical analyses and figures. M.V. wrote the manuscript with the contribution of all the authors.

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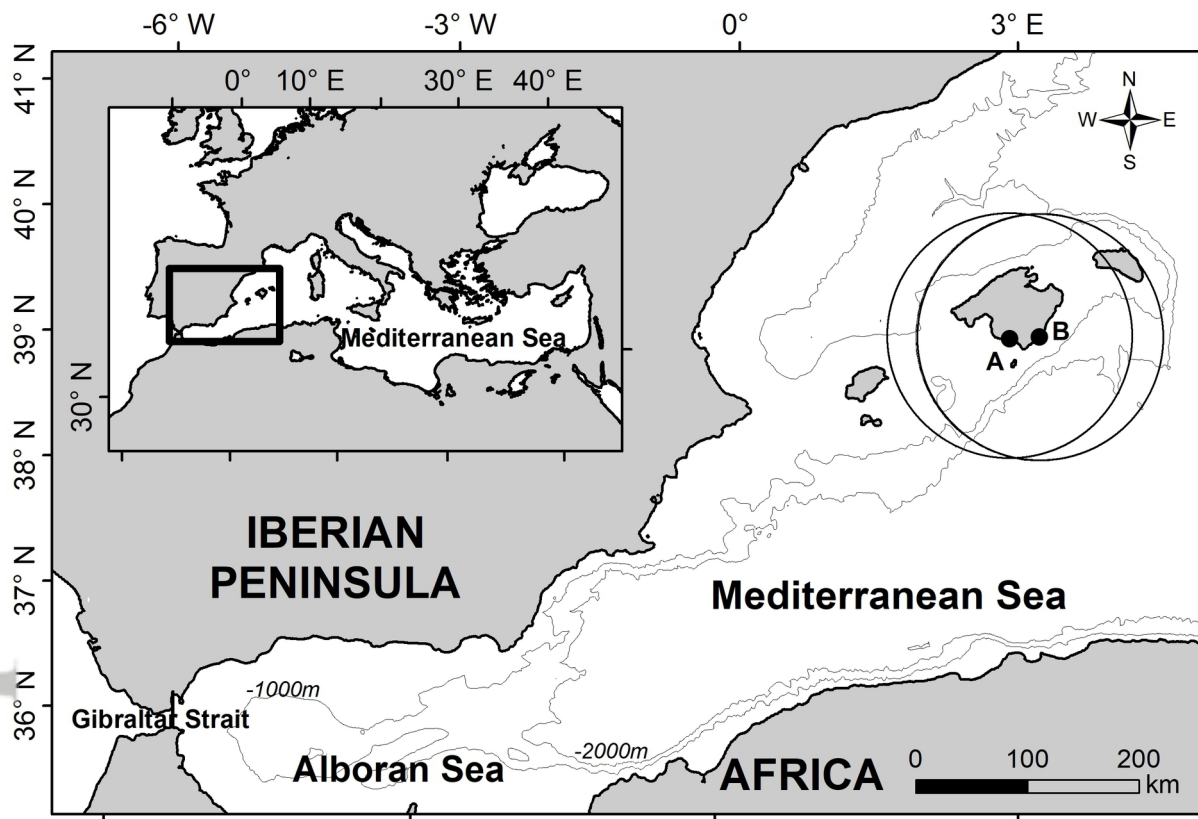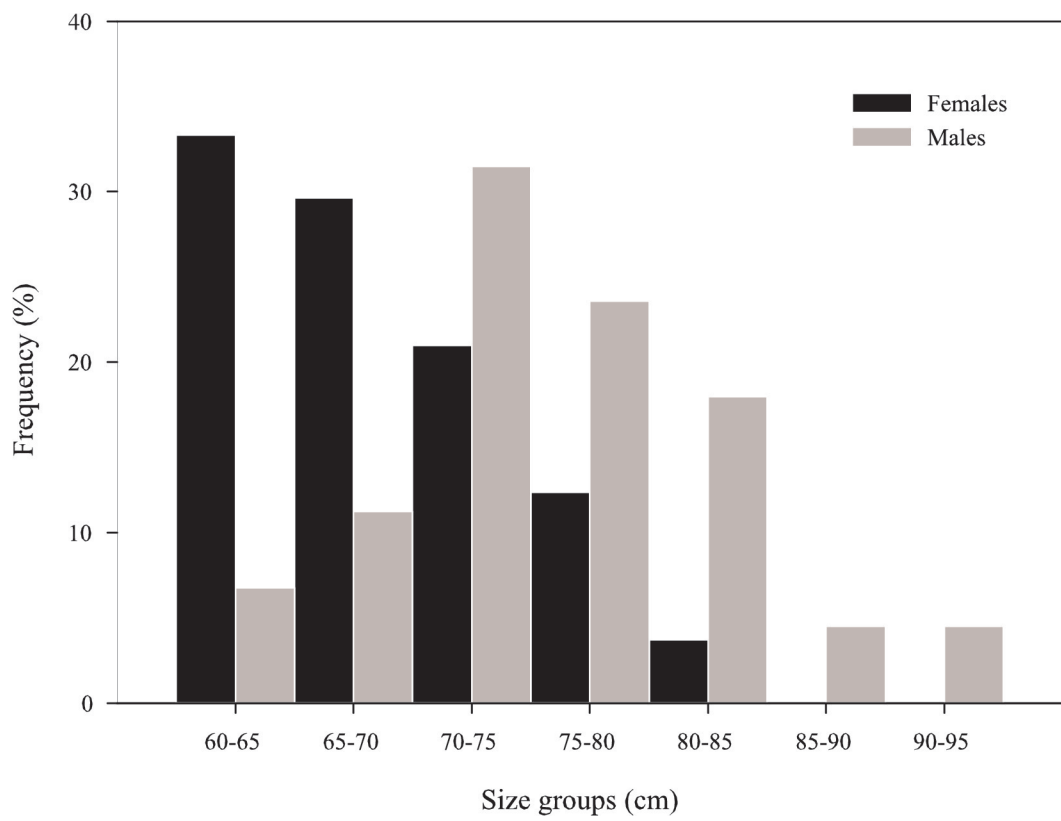
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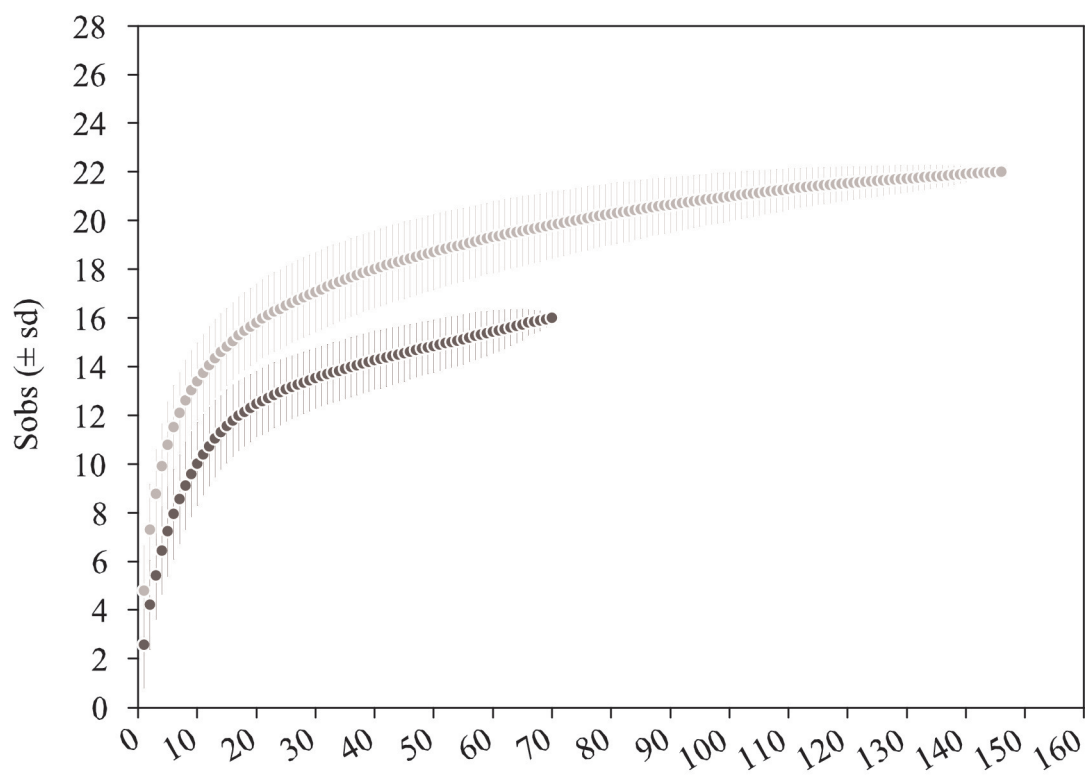
Number of full stomachs

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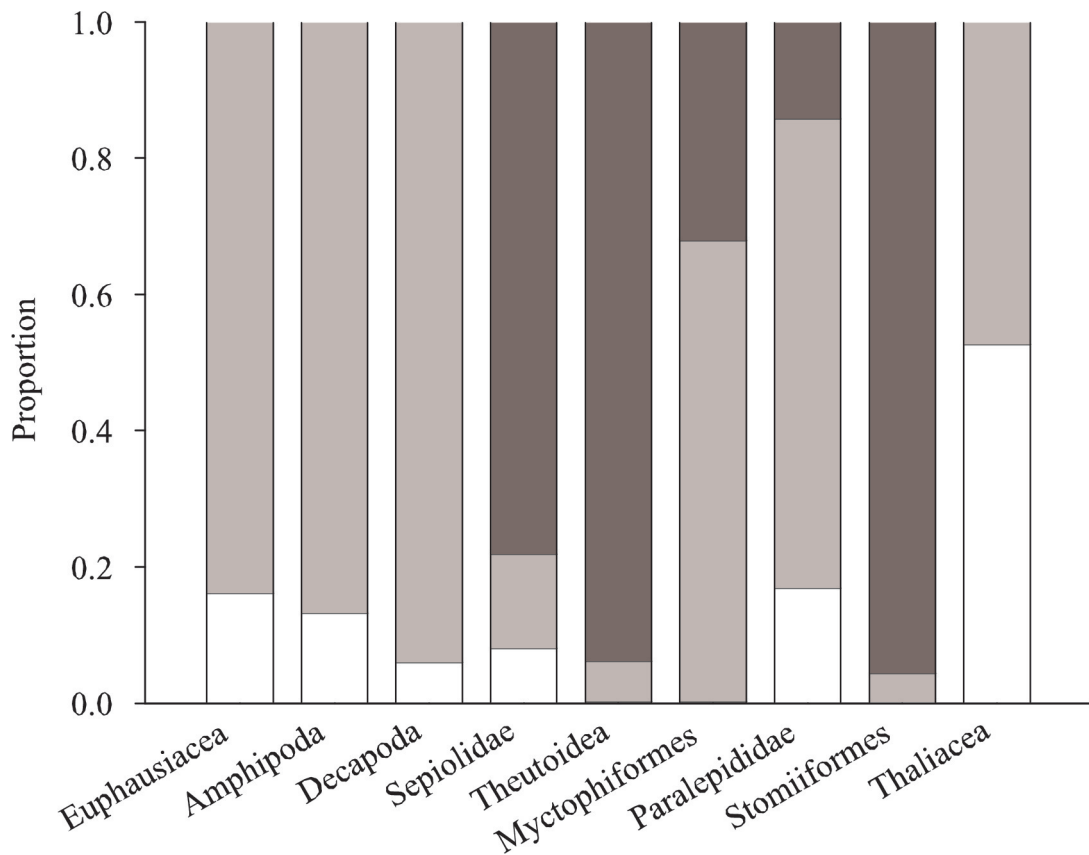
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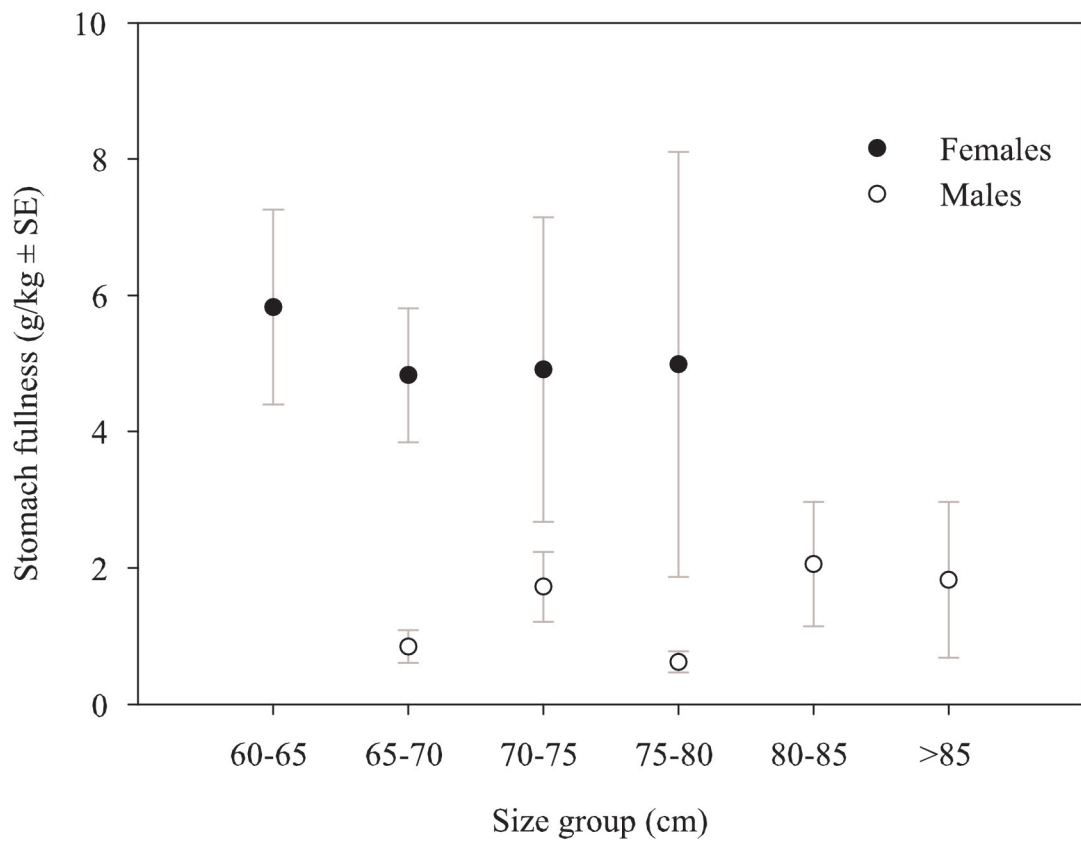
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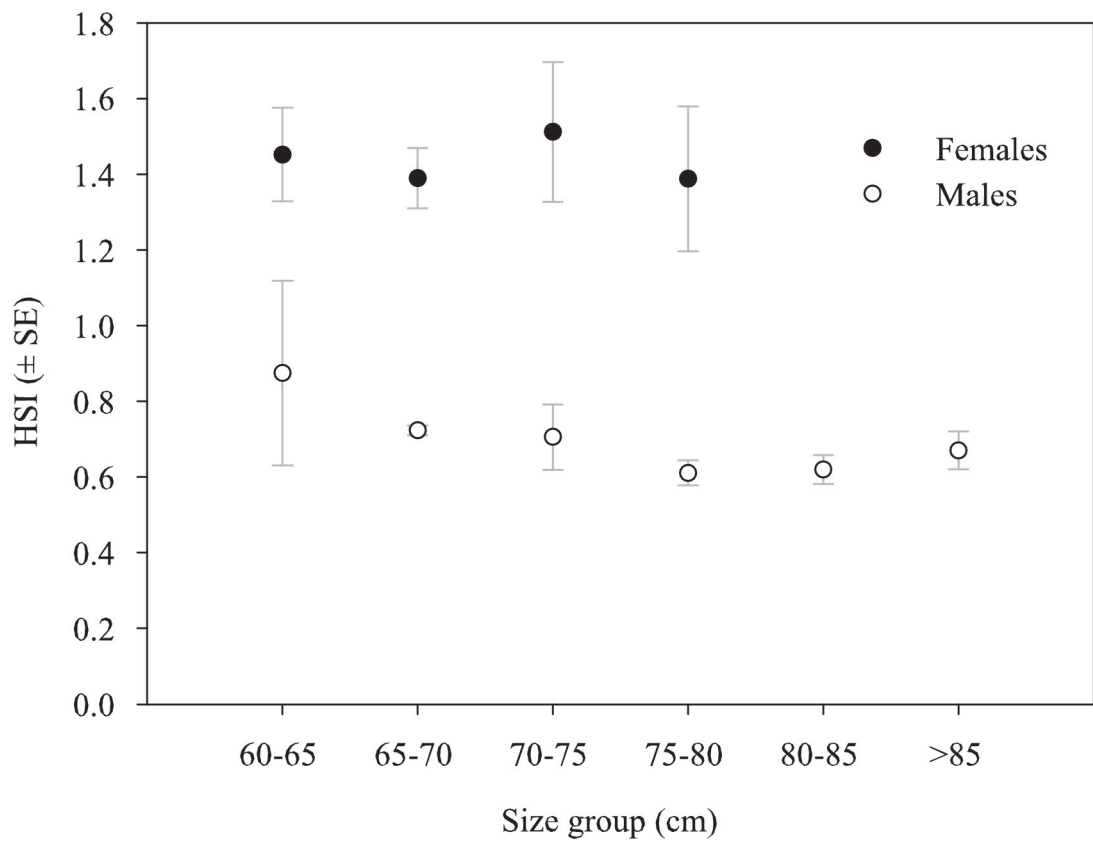
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FIGURE 1 Map of fishing grounds of albacore recreational fishery (60 miles circles around the base ports) during the fishing tournaments carried out in the Balearic Islands (western Mediterranean Sea). Port locations: A, S'Estanyol and B, Cala d'Or.

FIGURE 2 Length-frequency distribution (SFL, cm) for males (n= 89) and females (n= 81) of *Thunnus alalunga* sampled for stomach contents analysis in the western Mediterranean Sea.

FIGURE 3 Prey items accumulation (Sobs) plot as an average (± SD) of 999 permutations by randomizing the full stomachs of *Thunnus alalunga* analysed for numeric (N; n=146) and weight (W; n=71) indices.

FIGURE 4 Digestion states of main prey items found in the stomachs of *Thunnus alalunga*: in process of digestion (white), highly digested (grey) and hard parts (dark grey).

FIGURE 5 Stomach fullness (g/kg, mean ± SE) of reproductively active *Thunnus alalunga* (n=90) by size group (SFL, cm) and sex.

FIGURE 6 Hepatosomatic index (HSI) (mean ± SE) of reproductively active *Thunnus alalunga* (n=90) by size group (SFL, cm) and sex.

TABLE 1 Data summary of *Thunnus alalunga* sampled for stomach content analysis showing: fishing date, location, total number of stomachs sampled and liver weighed, standard fork length (SFL) size range, mean SFL (± SD) and mean weight (± SD). Total number of plastics found in the stomachs and their frequency of occurrence ($F_{plastics}$) are also provided.

Date	Location	Stomachs	Liver	SFL range (cm)	Mean SFL (cm)	Mean weight (kg)	Plastics	$F_{plastics}$ (%)
09/07/2010	S'Estanyol	32	29	61.0-94.5	72.6 ± 9.5	8.2 ± 3.3	38	53.1
08/07/2011	S'Estanyol	35	27	60.0-95.0	74.3 ± 6.8	8.2 ± 2.2	24	37.1
29/06/2012	Cala D'Or	31	17	61.0-85.7	71.8 ± 6.6	7.7 ± 2.2	13	25.8
28/06/2013	Cala D'Or	32	-	60.6-84.0	72.1 ± 7.5	7.7 ± 2.3	25	31.3
27/06/2014	Cala D'Or	20	17	60.5-85.0	71.0 ± 7.3	7.7 ± 2.3	13	40.0
27/06/2015	S'Estanyol	20	-	60.5-83.7	70.4 ± 6.1	7.3 ± 1.7	11	30.0

TABLE 2. Macroscopic maturity classification of *Thunnus alalunga* gonads from the western Mediterranean Sea.

Phases	Other terminalogy	Macroscopic features
I. Immature (virgin)		Ovaries are small and cylindrical in shape. Colour: more or less translucent-pinkish or pink. Testes are small, thin, flattened and ribbon-like. Colour: more or less translucent -lightly pink.
II. Developing	Early developing, early maturing	Ovaries are increasing in size. Colour: creamy white to orange. External blood vessels start to develop around the gonads (vascularisation). Oocytes not visible to the naked eye. Testes are increasing in size and triangular in cross section. Colour: whitish - pinkish colour. Sperm does not flow with pressure.
III. Spawning capable	Late developing, late maturing	Ovaries are well developed, large and firm. Colour: pinkish - orange –lighty red colour. Opaque oocytes are visible. Testes are well developed. Colour: whitish - pinkish colour. Accumulation of sperm in the spermatic ducts, sperm flows with low pressure.
IV. Spawning	Ripe	Ovaries are greatly enlarged. Colour: orange - reddish with conspicuous superficial blood vessels. Opaque oocytes are visible and large translucent hydrated oocytes may be visible. Under very lightly pressure oocytes may be released. Testes are greatly enlarged. Colour: white or bloodshot (pinkish with conspicuous superficial blood vessels). Large amount of sperm flows freely under very light pressure.
V. Postspawning	Spent, spawned, regressing	Ovaries size is reduced from stage IV. Colour: dark red. They are bloody and flaccid, show a wrinkled wall. Some residual large clear or opaque oocytes may still present. Testes size is reduced from stage IV. Colour: dark pink. They appear shrivelled, thin, hardened, and bloodshot. Sperm may still flow (only small quantity) from collecting duct when cut and squeezed.

TABLE 3 Prey taxonomic composition of spawning *Thunnus alalunga* in the western Mediterranean Sea. Dietary indices calculated: frequency of occurrence (%F), proportion by number (%N) and mean proportion by number (%MN ± sd), proportion by weight (%W) and mean proportion by weight (%MW± sd), standardized Index of Relative Importance (%IRI) and the Prey-Specific IRI (%PSIRI). Unid.: species unidentified.

Phylum/Order/ Family	Species/prey item	%F	%N	%MN	%W	%MW	%IRI	%PSIRI
Crustacea		87.9	41.7	42.2 ± 0.3	24.3	44.8 ± 0.3	34.9	33.0
Amphipoda		84.2	25.7	26.3 ± 0.2	21.9	37.0 ± 0.3	35.4	23.8
	Anchylomera blossevillei	7.9	1.6	1.0 ± 0				
	Brachyscelus crusculum	50.9	12.0	12.5 ± 0.1				
	Hyperia sp.	2.4	0.1	0.1 ± < 0.1				
	Hyperiidea unid.	17.0	4.3	3.2 ± < 0.1				
	Phronima sedentaria	47.3	4.2	5.4 ± < 0.1				
	Phrosina semilunata	36.4	2.7	3.1 ± < 0.1				
	Platyscelus ovoides	4.8	0.8	0.8 ± < 0.1				
Decapoda		20.0	1.4	2.0 ± < 0.1	0.5	2.3 ± 0.1	0.3	0.9
	Acanthephyra pelagica	1.2	0.0	< 0.1 ± < 0.1				
	Brachyura megalopa	1.2	0.1	< 0.1 ± < 0.1				
	Decapoda unid.	2.4	0.1	0.1 ± < 0.1				
	Decapoda Larvae	1.8	0.1	0.2 ± < 0.1				
	Natantia unid.	7.9	0.4	0.4 ± < 0.1				
	Pandalidae unid.	2.4	0.5	0.6 ± < 0.1				
	Pasiphaea sp.	1.8	0.0	< 0.1 ± < 0.1				
	Sergestidae unid.	1.8	0.1	0.2 ± < 0.1				
	Scyllaridae larvae (phyllosoma)	2.4	0.1	0.1 ± < 0.1				
Euphausiacea		26.7	13.6	12.5 ± 0.2	1.7	5.2 ± 0.2	3.6	7.7
	Euphausiacea unid.	14.5	6.7	6.8 ± 0.1				
	Meganycthiphanes norvegica	12.7	6.9	5.6 ± 0.1				
Isopoda		9.1	1.0	1.2 ± < 0.1	0.2	0.3 ± < 0.1	0.1	0.6
	Eurydice sp.	0.6	<0.1	< 0.1 ± < 0.1				
	Idotea metallica	6.1	0.8	1.1 ± < 0.1				
	Idotea sp.	2.4	0.1	< 0.1 ± < 0.1				
Mollusca		83.0	10.0	15.1 ± 0.1	1.0	1.3 ± < 0.1	5.5	5.5
Gastropoda		1.2	<0.1	< 0.1 ± < 0.1	<0.1	< 0.1 ± < 0.1	<0.1	
	Gastropoda unid.	0.6	<0.1	< 0.1 ± < 0.1				
	Thecosomata unid.	0.6	<0.1	< 0.1 ± < 0.1				
Octopodidae	Octopodidae unid.	3.0	0.1	0.2 ± < 0.1	<0.1	0.4 ± < 0.1	<0.1	<0.1
Sepiolidae	*Heteroteuthis dispar*	26.1	1.1	1.2 ± < 0.1	0.4	0.4 ± < 0.1	0.3	0.7
Theutoidea		72.7	7.3	12.2 ± 0.1	0.6	0.5 ± < 0.1	5.1	4.0
	Ancistrotheutis lichtensteinii	15.8	1.0	2.5 ± 0.1				
	Abralia veranyi	1.2	0.1	< 0.1 ± < 0.1				
	Illex coindetii	7.9	0.6	0.9 ± < 0.1				
	Loliginidae	3.6	0.3	0.4 ± < 0.1				
	Ommastrephidae	17.0	1.2	2 ± < 0.1				
	Theutoidea unid.	25.5	2.0	2.4 ± < 0.1				
	Todarodes sagittatus	21.2	2.1	3.5 ± < 0.1				
Cephalopoda unid.	Cephalopoda unid.	9.1	1.4	1.4 ± < 0.1	<0.1	< 0.1 ± < 0.1	0.1	0.7
Pisces		83.6	42.9	36.1 ± 0.3	70.1	45.5 ± 0.4	56.9	56.5
Argentidae	*Glossanodon leioglossus*	1.2	0.1	0.1 ± < 0.1				
Carangidae	*Trachurus* sp.	1.8	0.1	0.2 ± < 0.1				
Centracanthidae	*Centracanthus cirrus*	10.3	0.7	1.6 ± < 0.1	0.3	0.3 ± < 0.1	<0.1	0.5
Centrolophidae	*Centrolophus niger*	1.2	0.0	< 0.1 ± < 0.1	<0.1	< 0.1 ± < 0.1	<0.1	<0.1
Clupeidae	*Sardinella aurita*	1.8	0.1	0.3 ± < 0.1	0.3	0.9 ± 0.1	<0.1	0.2
Gobiidae	*Leusuerogobius* sp.	0.6	<0.1	0.1 ± < 0.1	<0.1	< 0.1 ± < 0.1	<0.1	<0.1
Myctophiformes		57.0	31.7	20.3 ± 0.3	29.7	19.8 ± 0.4	30.9	30.7
	Benthosema glaciale	3.6	0.4	0.1 ± < 0.1				
	Ceratoscopelus maderensis	12.7	1.2	1.2 ± < 0.1				
	Diaphus holti	1.2	<0.1	< 0.1 ± < 0.1				
	Electrona risso	3.6	1.0	0.4 ± < 0.1				
	Hygophum benoiti	27.9	21.2	11 ± 0.2				
	Lampanyctus crocodilus	39.4	5.8	5.2 ± 0.1				
	Lampanyctus pusillus	4.2	0.1	0.4 ± < 0.1				
	Lampanyctus sp.	7.9	1.7	1.2 ± < 0.1				
	Myctophidae unid.	1.8	<0.1	< 0.1 ± < 0.1				
	Notoscopelus sp.	5.5	0.2	0.2 ± < 0.1				
	Notoscopelus elongatus	0.6	<0.1	< 0.1 ± < 0.1				
	Notoscopelus bolini	0.6	<0.1	< 0.1 ± < 0.1				

Paralepididae		54.5	4.7	8.4 ± 0.1	32.3	17.2 ± 0.3	17.8	18.5
	Arctozenus risso	53.9	4.7	8.1 ± 0.1				
	Lestidiops jayakari	1.8	0.1	0.1 ± < 0.1				
Pisces unid.	Pisces unid.	23.0	2.5	3.2 ± < 0.1	1.7	3.5 ± 0.1	0.9	2.1
Stomiiformes		20.0	2.9	2.5 ± < 0.1	5.8	3.9 ± 0.1	1.5	4.3
	Argylopelecus hemigimnus	1.8	<0.1	< 0.1 ± < 0.1				
	Maurolicus muelleri	4.8	0.8	0.3 ± < 0.1				
	Stomias boa	0.6	0.1	0.1 ± < 0.1				
	Vinciguerias attenuata	13.3	2.0	1.8 ± < 0.1				
Unidentified	Unidentified	1.8	0.1	< 0.1 ± < 0.1	<0.1	<0.1	<0.1	0.1
Thaliacea	Salpidae	40.0	6.2	6.4 ± 0.1	4.7	8.1 ± 0.1	3.8	5.4
Siphonophora	Diphydae	6.1	0.2	0.3 ± < 0.1	0.1	0.3 ± < 0.1	<0.1	0.1
Annelida	Polychaeta	0.6	<0.1	0.1 ± < 0.1	<0.1	< 0.1 ± < 0.1	<0.1	<0.1
Litter	Paper	2.4	0.1	0.2 ± < 0.1	-			
	Plastic	37.6	1.9	2.1 ± < 0.1	-			
	Wood	0.6	<0.1	< 0.1 ± < 0.1	-			
Total full stomachs		165	146		71			

TABLE 4 Summary of important prey found in the stomach contents of *Thunnus alalunga* in the western Mediterranean Sea. Frequency of prey occurrence in the stomachs (%F) and frequency of occurrence of these species reported in the studied area (%F$_{AREA}$) (Fish: Olivar *et al.*, 2012; Cephalopods: Quetglas *et al.*, 2014); Number (N) of measured (directly or estimated) prey, together with prey size range and their mean size (±SD) are shown. NA: not available.

Prey	Taxa	%F	%F$_{AREA}$	N	Size range (mm)	Mean size (mm)
Brachyscelus crusculum	Hyperiid	50.91	NA	71	15-22	17±2
Phrosina semilunata	Hyperiid	36.36	NA	3	20-22	21±1
Phronima sedentaria	Hyperiid	47.27	NA	NA	-	-
Salpa maxima	Thaliacea	40	NA	NA	-	-
Meganycthiphanes norvegica	Euphausiid	12.73	NA	70	20-38	30±7
Ancistrotheutis lichtensteinii	Theutoidea	15.15	10.26	3	31-58	45±14
Ommastrephidae (*Illex*/*Todarodes*)	Theutoidea	44.85	48.78	4	25-80	56±23
Heteroteuthis dispar	Sepiolid	26.06	25.64	8	5-20	13±5
Benthosema glaciale	Myctophid	3.64	50	4	17-21[a]	19±2
Lampanyctus crocodilus	Myctophid	39.39	22.20	21	45-86[a]	70±9
Notoscopelus spp	Myctophid	6.06	66.70	3	28-79[a]	49±26
Hygophum benoiti	Myctophid	27.88	66.70	23	22-62[a]	35±15
Ceratoscopelus maderensis	Myctophid	12.73	61.10	3	30-53[a]	38±13
Diaphus holti	Myctophid	1.21	44.40	NA	-	-
Electrona risso	Myctophid	3.64	27.80	NA	-	-
Arctozenus risso	Paralepid	53.94	27.80	7	65-170	116±33
Argylopelecus hemigimnus	Stomiform	1.82	50	1	15	-
Maurolicus muelleri	Stomiform	4.85	NA	NA	-	-
Vinciguerias attenuata	Stomiform	13.33	61.10	NA	-	-

[a] Estimated fish size length.

Table 5 Prey items contribution (SIMPER analysis; cut-off for low contribution at 80%) to the dissimilarity between the smallest (G1) and medium size individuals (G2) of *Thunnus alalunga* in the western Mediterranean Sea

Species	Av. abund (G1)	Av. abund (G2)	Contrib%	Cum%
Myctophidae	21.4	26.5	23.7	23.7
Amphipoda	26.2	21.9	18.0	41.7
Euphausiacea	16.8	9.4	15.2	56.9
Theutoidea	10.8	13.3	10.8	67.7
Paralepididae	6.4	11.6	10.0	77.7
Thaliacea (salps)	4.6	6.2	6.0	83.6

Av. Abund: average abundance; Contrib%: percentage contribution; Cum%: percentage cumulative contribution

Despite albacore are have to fulfill the highly energetic cost supporting offspring production, no diet study has yet assessed its feeding behaviour while reproducing in the oligotrophic Mediterranean Sea. Results show that reproductively active albacore display an intense feeding, preying on mesopelagic fishes, crustaceans and cephalopods. Females showed a high feeding ratio and condition suggesting that increased feeding could contribute to meet their higher energy demand associated with offspring production.