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Evidence of density-dependent cannibalism in the diet of wild Atlantic bluefin tuna larvae (*Thunnus thynnus*) of the Balearic Sea (NW-Mediterranean)

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ABSTRACT

The heavy exploitation rates of Atlantic bluefin tuna (ABFT) during the nineties propitiated research into the larval ecology of ABFT and its associated species. The transition from a planktivorous to a piscivorous diet is considered a major bottleneck in the survival of ABFT larvae observed in aquaculture experiments. Although larval piscivory was reported in the Gulf of Mexico (GOM), the most important spawning grounds of this species in the W Atlantic, trophic studies have not been able to reveal piscivory in Mediterranean ABFT larvae. This study analyzes the trophic behavior of Mediterranean ABFT larvae by stomach content analysis. The results show that more than 90% of the larvae had at least one prey in their stomachs. The diet shifted from copepods and cladocerans to gastropod larvae in pre-flexion stages and to ABFT larvae in post-flexion stages. This is the first time that cannibalism is reported for wild ABFT larvae from the Mediterranean Sea. Intracohort cannibalistic feeding was observed when the requisite density-dependent processes aligned, namely the spatio/temporal overlap of a wide range of ABFT larval cohorts of different size class. Moreover, stomach contents of ABFT larvae revealed the ingestion of microplastic fibres. Whether these plastic contaminants were passively or actively ingested, they may affect the condition of larvae. The presence of microplastic strands in fish larvae undoubtedly raises concern because its impact on the survival of ABFT larvae still remains uncertain and is open to scientific experimentation.

1. Introduction

The Atlantic bluefin tuna (ABFT), *Thunnus thynnus* (Linnaeus, 1758), is a migrating large predator that spawns in the Mediterranean Sea (MED), the Gulf of Mexico (GOM), and from recent findings, the Slope Sea (Fromentin and Powers, 2005; Laiz-Carrion et al., 2015; Richardson et al., 2016; Muhling et al., 2017). ABFT larval ecology has been studied mostly in the Balearic Sea (MED) and the GOM. Spatial distribution of larvae has been assessed in relation to environmental variables (Teo et al., 2007; Alemany et al., 2010). Trophic ecology of ABFT larvae was studied from a comparative ecosystem viewpoint using stable isotope analysis (Muhling et al., 2013; Laiz-Carrion et al., 2015). Both MED and GOM spawning areas are characterized by their marked oligotrophy with mesoscale hydrographic features driving ABFT larval distributions. In the GOM, larvae are more abundant in the borders of anticyclonic gyres (Lindo-Atichati et al., 2012; Muhling et al., 2017), whereas in the Balearic Sea (MED) their distribution is influenced by frontal zones resulting from the convergence of recent and resident Atlantic surface waters (Alemany et al., 2010; Muhling et al., 2017). Such mesoscale features enhance particle food concentrations increasing the probability of survival of larvae in an oligotrophic environment.

Differences in diets of tuna larvae reflect the local availability of prey items (Muhling et al., 2017). To assess diet composition,

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traditional methods rely on morphological identification of prey. At present, many studies of this nature also assess diet composition by analyzing species-specific variable genetic markers (barcodes) in the DNA extracted from recovered prey items. DNA-based molecular approaches are very useful when prey are too digested to be identified visually (Carreon-Martinez et al., 2011), and allow simultaneous identification of multiple prey by multiplex PCR (King et al., 2008) or next generation sequencing (metabarcoding) (Pompanon et al., 2012). However, barcoding or NGS-based metabarcoding are unable to distinguish between predator and prey DNA when cannibalistic feeding occurs. Diet studies in marine animals are currently focusing on the ingestion of microplastics, which are a potential threat to marine life, as they are ingested by a wide variety of species (Frias et al., 2014; Lima et al., 2014; Setälä et al., 2014; Beer et al., 2017). An experimental study has shown the potential transfer of plastic microparticles via planktonic organisms from one trophic level (mesozooplankton) to a higher one (macrozooplankton) (Setälä et al., 2014). In tuna larvae, diet composition has been studied by traditional visualization of stomach contents. A comparative trophic ecology study of *ABFT* larvae between GOM and MED (Laiz-Carrion et al., 2015) showed a lesser degree of oligotrophy in the GOM than in the MED, particularly in the significantly higher biomass of mesozooplankton in the GOM. This fact indicates that food availability in the Balearic MED waters is comparatively much poorer (Laiz-Carrion et al., 2015). The trophic position estimated by bulk stable isotope analysis was lower in ABFT larvae from the GOM where the significantly higher temperatures can influence metabolic rates. A comparative ABFT daily growth study showed that the GOM ABFT larvae observed higher growth rates than MED spawned larvae (Malca et al., 2017).

Tuna larvae have high energy requirements for growth. Successful feeding during the early development of ABFT is key for maintaining growth and meeting the metabolic needs for larval survival (Llopiz et al., 2010; Laiz-Carrion et al., 2015; García et al., 2017; Muhling et al., 2017). ABFT larvae are characterized by their rapid somatic growth and development of internal organs, including the digestive system (Yúfera et al., 2014). With larval development, larvae augment their mouth gape, enabling the ingestion of larger prey (Catalán et al., 2007, 2011; Llopiz et al., 2010), including fish larvae (Morote et al., 2008; Llopiz et al., 2010). In growth and survival studies of cultured Atlantic and Pacific bluefin tuna larvae, an early transition to piscivorous feeding is shown to be beneficial for survival by increasing growth rates during larval development (Reglero et al., 2014; Tanaka et al., 2014, 2017). Furthermore, exposing reared Pacific Bluefin tuna to a single day of starvation causes an almost immediate delay in growth and contributes to high larval mortality (Tanaka et al., 2008). Piscivory is essential for ABFT larvae cultured under controlled conditions; in order to reach adequate survival rates, post-flexion larval stages rely on lecitotrophic stages of other fish species and on cannibalism (Reglero et al., 2014; Uriarte et al., 2017; Tanaka et al., 2014, 2017). Piscivory was reported in wild caught ABFT larvae from the GOM (Llopiz et al., 2010; Llopiz and Hobday, 2015), whilst this feeding strategy has not been observed to date in ABFT larvae spawned in the MED. However, piscivory has been reported in early post-flexion stages of albacore (*T. alalunga*) (Catalán et al., 2007) and in *A. rochei* larvae upon attaining 5 mm in length (Morote et al., 2008). Stable isotope trophic analysis attributed the increase of $\delta^{15}N$ signatures after post-flexion in tuna species to piscivory (Uriarte et al., 2016; García et al., 2017). The present study aims to use stomach content analysis and genetic identification of consumed larvae to confirm the occurrence of intraspecific piscivory in post-flexion of ABFT larvae spawned in the MED. Life trophodynamics during ontogeny and predator-prey relationships are explored as well as ABFT trophic specialization of larvae, the latter being addressed through selectivity indices and the trophic niche breadth.

2. Materials and methods

2.1. Field sampling

Thunnus thynnus (ABFT) larvae were collected during the Bluefin 2014 ichthyoplankton survey carried out in the Balearic Sea (Western Mediterranean) from June 17 to July 3, coinciding with the peak of the ABFT spawning season. A total of 98 systematic stations were sampled onboard the R/V SOCIB. Hydrographic data (temperature, salinity, fluorescence) was collected at each sampling station during daylight hours using a Seabird 19 + CTD profiler cast to 350 or 650 m depth, though in shallower stations a depth of 5 m was used. Ichthyoplankton samples were collected using a squared-mouth 90-cm Bongo net fitted with 500 µm meshes and General Oceanic flowmeters, carrying out oblique tows from around 30 m to surface. A Bongo net of 20 cm diameter fitted with nets of 55 and 200 µm meshes, each equipped with General Oceanic flowmeters, was attached 1 m above the Bongo 90 frame to sample micro-and mesozooplankton fractions. ABFT larvae were sorted on board from the ichthyoplankton samples. In some stations positive for ABFT, subsets of the sorted ABFT larvae collected in the Bongo 90 tows and subsamples of mesozooplankton (> 200 µm) from the Bongo 20 tows were preserved on board in 90% ethanol. To catch larger larvae, additional plankton tows were carried out with a Bongo 90-cm fitted with a black tinted mesh of 1000 µm in the station that recorded the highest larval density. The selected stations where larvae were collected for this study are shown in Fig. 1.

2.2. Laboratory procedures

Selected ABFT larvae were measured for standard length (SL) and lower jaw length (LJL) using the ImageJ analysis software. To analyze stomach contents, the entire alimentary canal of each larva was first removed via dissection, and the stomach content was examined with a Leica M205 stereomicroscope, following the method described by Llopiz et al. (2010). Each prey found in ABFT larvae stomachs was counted, identified, and photographed for morphometric measurements. Zooplankton species were identified mainly consulting Trégouboff and Rose (1957), while ichthyoplankton were identified with the larval fish guide of Rodríguez et al. (2017). All microplastic particles observed in the stomach contents were measured and classified by color and type (fibres or fragments), using established criteria for visual characterization (Enderson et al., 2015). Fish larvae found in stomach contents were cleaned of predator tissue residues and stored in ethanol prior to carrying out morphological and genetic analyses.

The analysis of the composition of the mesozooplankton fraction (> 200 µm) was done in the 6 stations where highest ABFT larval catches were observed. The sample conserved in ethanol was divided into aliquots for taxonomical categorization: both species' identification and family grouping (Fig. 2). The density of each individual group was documented as individuals m^{-3} with respect to volume of water filtered during the plankton haul.

2.3. Genetic analysis of fish larvae

Genetic identification of consumed larvae species was carried out according to sequence similarity and to DNA character attributes as described by Puncher et al. (2015). Briefly, DNA was extracted from the six prey fish larvae recovered from five ABFT larvae and, subsequently, fragments of mitochondrial cytochrome C oxidase I (COI) and nuclear internal transcribed spacer region 1 (ITS1) were amplified by PCR and sequenced. The stomach contents recovered from one ABFT larvae were not genetically analyzed as they were too digested and the absence of visible predator tissue residues could not be ensured. To exclude DNA cross contamination, remaining predator and prey larvae tissues were genotyped for six microsatellites previously described for ABFT: Ttho-1 and Ttho-4 (Takagi et al., 1999), Tth-34 (McDowell et al., 2002), Tth

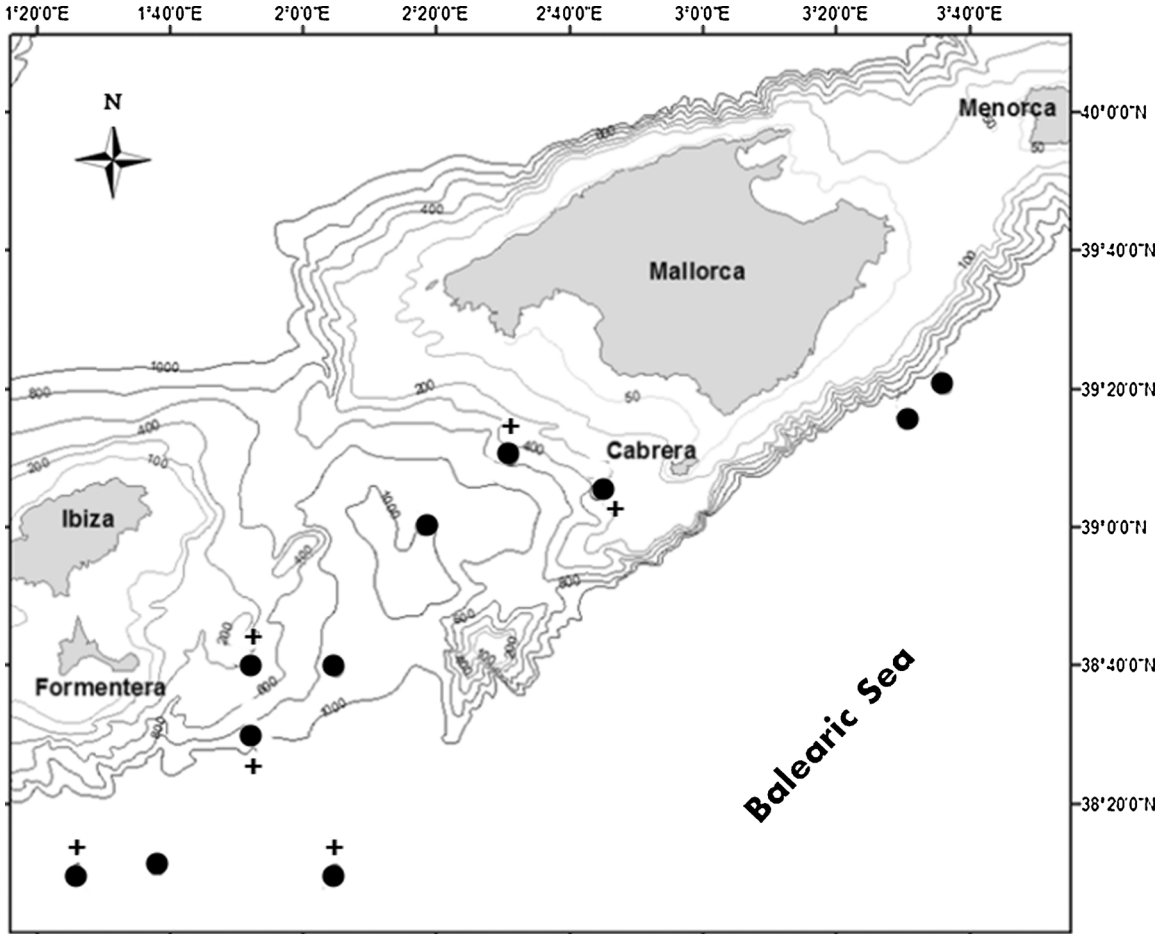

Fig. 1. Location of the stations in the Balearic Sea, where ABFT larvae were selected for this trophic study are shown with black circle. The stations selected for zooplankton sampling are shown with black crosses.

1–31, Tth 208 and Tth 157 (Clark et al., 2004). All six microsatellites were multiplexed in a PCR reaction performed with a Q5® High-Fidelity PCR kit (New England BioLabs, Inc.) including 50 ng template DNA and 25 pmol primer mix. The forward primer for each locus was fluorescently labeled for laser detection as follows: Ttho-1 and Tth 1–31 were labeled with NED™, Ttho-4 was labeled with 6-FAM™, Tth-34 was labeled with VIC®, and Tth 157 and Tth 208 were labeled with PET™. Multiplex PCR conditions were: 97 °C 3 min; 10 cycles (denaturation at 94 °C for 30 s, annealing with 1 °C decrease per cycle from 64 °C to 54 °C for 30 s, extension at 72 °C for 30 s), 25 cycles (94 °C 30 s, 54 °C 30 s,

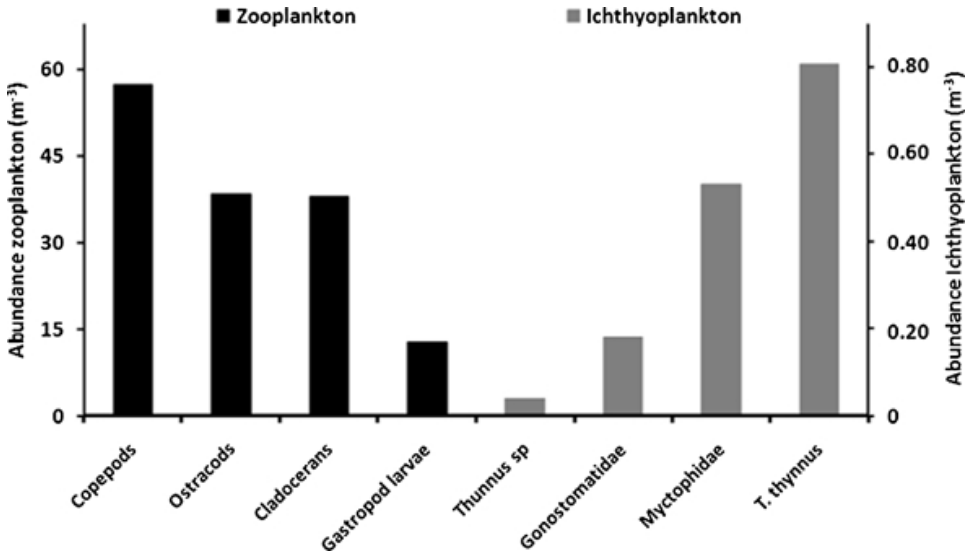

Fig. 2. Abundance of the main planktonic groups (zooplankton; icthyoplankton) in the six stations where a high density of ABFT larvae were found in the Balearic Sea.

72 °C 30 s), and final extension at 72 °C for 3 min. Alleles were separated by capillary electrophoresis on an Applied Biosystems 3730xl Genetic Analyzer (Stabvida, Caparica, Portugal), and scored after double manual inspection using GeneMapper® software v4.0 (Applied Biosystems).

2.4. Trophic analysis

Feeding analyses was performed for a total of 105 ABFT larvae collected from 11 stations (Fig. 1). A plankton composition analysis was carried out among 6 stations which showed highest ABFT larval densities. Descriptions of the diet for each size class of ABFT larvae were constructed, and the importance of the different prey items in the diets was calculated using the index of relative importance IRI = (N + V)F defined by Pinkas et al. (1971), where N = numeric frequency of the prey, V = volumetric-weight of prey, and F = percentage occurrence in the gut. However, owing to the small sizes of prey items (< 2 mm), it was not possible to estimate prey weight. Therefore, the index of relative importance (IRI) was estimated following the approach applied by Morote et al. (2008) in which: %IRI = (%N*%F), where %N is number individuals of prey item multiplied by 100/total number of all prey items, and %F is the frequency of the number of stomachs containing a prey item multiplied by 100/total number of non-empty stomachs.

Feeding incidence was calculated as the percentage of the total number of larvae examined having at least one prey item in the stomach. Size-related shifts in dietary composition (%N) of ABFT larvae were evaluated by permutational multivariate analysis of variance PERMANOVA and the differences among groups (size classes of ABFT larvae) by PAIR-WISE test, the homogeneity of dispersion of data was tested using permutational analysis of multivariate dispersions PERMDISP after performing a square-root transformation. The degree of similarity of dietary composition among groups was evaluated using Bray- Curtis similarity matrix estimated from ABFT larvae categorized into 5 size classes. All permutation-based tests were conducted using 999 permutations. Analyses of similarity, PERMDISP, and PERMANOVA were run in PRIMER version v6.1.13 statistical package (PRIMER-E Ltd, Plymouth, UK).

The dietary niche width was analyzed for each ABFT larval size class through the standardized Levin´s index (B) expressed as: $B = \left[\frac{1}{n-1}\right]\left[\left(\frac{1}{\sum P^2}\right) - 1\right]$, where n is the number of prey categories, and P is the proportion of IRI for each size class. This index ranges from 0 to 1, where low values indicate a specialist feeding behaviour and high values indicate a generalist feeding behaviour (Krebs, 1999).The relationship between morphometric parameters of larvae vs preys were represented by regression analysis. The trophic niche breadth with size was studied as the log standard deviation (SD) of prey width, plotted with the SL of larvae.The dietary niche analysis established a preferential size range of 6–8.5 mm SL (n = 26) to study the relative dietary preferences. The trophic niche width corresponding to post-flexion larvae was analyzed through Chesson's selectivity index (∝) expressed as: $\propto = (ri/pi)\sum_{i=1}^{n}(ri/pi)^{-1}$ where *ri* and *pi* are the percentage abundances of prey item *i* in the larval diet and in plankton samples, respectively (Chesson, 1978).

3. Results

3.1. Bluefin tuna larval distribution and abundance of potential prey

The ABFT larvae were mostly observed in the southern part of the Balearic archipelago within the frontal region where recent and resident surface Atlantic waters meet (Fig. 1). A total of 105 ABFT larvae suitable for feeding analyses were collected from 11 stations (Fig. 1) where larval sizes ranged from 2.7 to 8.5 mm SL recording on the whole an average of SL 5.11 mm (SD 1.35) (Table 1). Station 1282 recorded the highest ABFT larval concentration (N = 35) and widest size range (3.7–8.5 mm SL), thereby grouping different age/size cohorts in a single location. At this station, six post-flexion piscivorous ABFT larvae were found (Table 1).

Table 1
Number and size range of ethanol-preserved ABFT larvae by station examined for gut contents and the number of larvae observed to be piscivorous.

Station	N	SL (mm)	Mean SL (mm) ± SD	Piscivory
1146	5	3.2 – 5.6	4.29 ± 1.14	–
1194	5	4.03 – 4.58	4.36 ± 0.20	–
1234	8	4.77 – 5.69	5.39 ± 0.30	–
1282	35	3.68 – 8.47	6.56 ± 0.96	6
1323	5	3.37 – 4.98	3.98 ± 0.68	–
1499	5	3.82 – 4.89	4.33 ± 0.44	–
1501	6	3.39 – 3.71	3.57 ± 0.13	–
1595	8	3.50 – 4.27	3.87 ± 0.27	–
1795	9	3.41 – 4.21	3.85 ± 0.29	–
1797	8	2.69 – 6.05	4.16 ± 1.26	–
1801	10	4.72 – 5.47	5.03 ± 0.24	–

The analysis of the planktonic community was carried out in six stations where larval densities were higher (Fig. 1). Copepods (57.39 m^{-3}) were the most abundant zooplankton group, followed by ostracods (38.51 m^{-3}) and cladocerans (37.98 m^{-3}). Also, gastropod larvae (12.84 m^{-3}) were frequently found in the plankton community. Within the category of ichthyoplankton, total fish larval density was 1.56 m^{-3}, among which the most abundant family corresponded to species of the Scombridae, in which, ABFT (0.81 m^{-3}) represented 53% of the total. Species of the Myctophidae (0.45 m^{-3}) was next in order of abundance, followed by species of the Gonostomatidae represented by *Cyclothone* spp. (0.18 m^{-3}).

3.2. Feeding incidence and diet composition

A total of 99 larval samples contained at least one prey item in the stomach, which resulted in a feeding incidence of 94%. The maximum number of prey observed was 11 cladocerans (*Evadne sp*) in a single ABFT larva. The average number of prey found was 2.98 ± 2.23 (SD) and fullness tended to increase with larval size. The index of relative dietary importance (%IRI) varied during ontogenic development of ABFT larvae, showing changing preferences for prey types at different development phases (Table 2). The number of prey categories tend to increase in the larger size classes analyzed (Fig. 3). The most abundant and important prey category for pre-flexion larvae were copepods (*Farranula sp, Oithona sp*) (78% IRI), followed by cladocerans (*Evadne sp*). The largest number of prey per larvae was found in the notochord flexion stage (5–6 mm SL), where copepods and cladocerans were predominant (50% IRI). A dietary shift occurs after reaching 6 mm SL, which corresponds to the ontogenic stage of early post-flexion (Fig. 3) as ingestion of gastropod larvae was shown to be an important component of the consumed prey (46% IRI) (Table 2). Beyond 7 mm SL, the main fraction of the consumed prey was that of piscivorous nature (50% IRI) (Fig. 3). In addition, microplastic fibres (1–2 mm) were also found in 4 of these larger (> 7 mm) larvae (Table 2).

PERMDISP showed no significant ($p > 0.05$) differences in dispersions in prey data among size classes. The PERMANOVA analysis showed differences in the dietary composition with larval growth expressed by SL gain (PERMANOVA, $p = 0.001$). Posterior pair-wise test comparisons revealed significant differences between the group of the largest size class (> 7 mm) with each of the smaller size classes ($p < 0.001$) (Table 3). The Similarity Bray-Curtis test shows that the first four size groups have similar trophic patterns (Table 3). Alternatively, the largest size group (> 7 mm) show a differentiated trophic diet in relation to the smaller size groups (Table 3; Fig. 3).

Table 2
Thunnus thynnus larval diet derived from non-empty guts. Index of numeric frequency of the prey (%N), percentage occurrence in the gut (%F) and index of relative importance of the prey category (%IRI).

SL (mm) Larvae	< 3–4			4–5			5–6			6–7			> 7			Total (mean)		
Number of larvae	30			24			24			16			11			105		
Mean ± SD	3.6 ± 0.3			4.5 ± 0.3			5.5 ± 0.3			6.6 ± 0.2			7.5 ± 0.5			5.11 ± 1.35		
Non-empty guts	27			22			24			16			10			99		
Prey Category	**%N**	**%F**	**%IRI**	**%N**	**%F**	**%IRI**	**%N**	**%F**	**%IRI**	**%N**	**%F**	**%IRI**	**%N**	**%F**	**%IRI**	**%N**	**%F**	**%IRI**
Tintinnid	1.5	3.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0	2.0	6.2	0.8	0.0	0.0	0.0	0.7	1.1	0.0
Cladocerans	25.8	66.7	21.2	38.2	63.6	46.6	40.6	58.3	50.2	12.0	18.7	9.4	5.3	13.5	1.3	24.4	28.5	27.6
Nauplii	3.0	7.4	7.9	2.9	9.1	8.5	3.8	16.7	17.0	2.0	6.2	9.4	0.0	0.0	0.0	2.4	5.1	0.8
***Calanoida* sp**	1.5	3.7	0.1	0.0	0.0	0.0	3.8	12.5	1.7	2.0	6.2	0.8	0.0	0.0	0.0	1.5	2.9	0.3
***Oithona* sp**	18.2	25.9	11.0	27.9	50.0	44.7	20.7	41.7	31.1	10.0	18.7	12.8	0.0	0.0	0.0	15.4	17.7	18.3
***Farranula* sp**	45.4	55.6	59.0	29.4	45.4	0.0	28.3	50.0	33.7	22.0	50.0	0.0	0.0	0.0	0.0	25.0	25.7	43.3
Harpacticoide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.3	10.0	1.2	1.0	0.6	0.0
Decapoda larvae	1.5	3.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.6	0.0
Unidentified copepods	3.0	7.4	0.5	1.5	4.5	0.2	2.8	13.6	0.0	12.0	25.0	20.5	26.3	40.0	23.8	9.1	8.0	4.9
Gastropods larvae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	36.0	37.5	46.1	26.3	40.0	23.8	12.5	5.7	2.5
Larval fish	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	6.2	0.8	36.8	49.9	50	7.8	4.0	2.1
Microplastic fibres	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.3	25	13.7	0.0	0.0	0.0	2.2	2.3	0.14
Prey items (N)	66			68			106			50			19			309		

3.3. Identification of preyed fish larvae

Fish larvae extracted from larval ABFT stomach contents showed the morphological attributes of *Thynnus thunnus* larvae. However, genetic identification was needed to assure the species' identity. The piscivorous ABFT larvae were found in the stations where the ABFT larvae were most abundant. All the prey fish larvae that were genetically identified were confirmed to be ABFT larvae. In addition, DNA analyses of predatory and prey larvae genotypes for six microsatellite loci demonstrated that DNA cross-contamination did not occur. Electropherograms for a representative set of consumed larvae and corresponding predator are shown in Fig. 4.

3.4. Prey selectivity and trophic niche breadth

The average prey sizes consumed by ABFT larvae show a significant exponential relationship with increased LJL size and SL (Fig. 5a, b). However, the number of prey per larva did not increase with SL. Food consumption is compensated by larger prey size observed with larval growth.

Despite being significant, the trophic-niche breadth did not exhibit a clear difference with the SL of ABFT larvae ($y = -0.0769 + 0.0851x$-0.009×2; $r = -0.17$, $p < 0.05$) (Fig. 6). The regression analysis showed a high dispersion pattern. The greatest dietary niche breadth occurs in pre-flexion larvae at 5.1 ± 1.35 mm. On the other hand, post-flexion larvae (> 7 mm SL) observed a narrower trophic-niche breadth (Fig. 6) showing preference for larger sized prey (Fig. 5b).

Levin's index showed that ABFT larval size classes ranging from 4 to 6 mm have a lesser degree of trophic specialization (B > 0.5) in comparison to the larger ABFT larval size classes (> 6 mm) (B < 0.3). Nevertheless, the relative dietary preferences (Chesson´s index) for

Table 3
Results of PAIR-WISE PERMANOVA and Bray-Curtis Similarity test estimated from standard length of ABFT larvae for 5 size classes. Groups by size class; a: < 3-4 mm, b: 4–5 mm, c: 5–6 mm, d: 6–7 mm, and e: > 7 mm, respectively.

Groups Size class SL (mm)	Similarity Bray-Curtis	Significance of difference by PAIR-WISE Test
a vs b	47.60	NS
a vs c	49.26	NS
a vs d	42.83	NS
a vs e	7.26	*
b vs c	47.14	NS
b vs d	37.32	NS
b vs e	7.03	*
c vs d	42.39	NS
c vs e	6.34	*
d vs e	16.02	*

NS: No Significant differences. Significant differences* ($p < 0.01$).

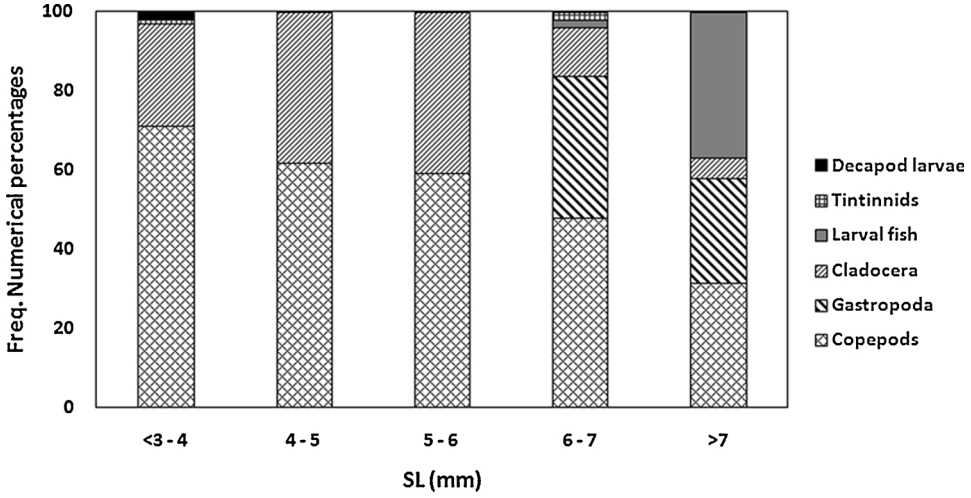

Fig. 3. Numerical percentages of the diet by prey categories for *Thunnus thynnus* larvae grouped by size class intervals.

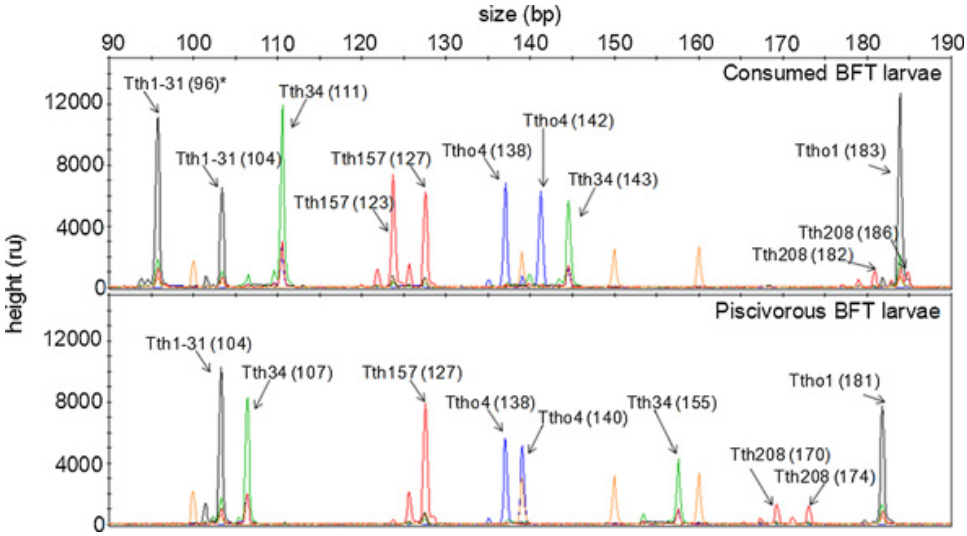

Fig. 4. Different genotypes for one consumed BFT larvae (upper panel) and its predator piscivorous BFT larvae (lower panel). Electropherograms show alleles (indicated in brackets) for six BFT microsatellite loci: Ttho-1, Ttho-4, Tth-34, Tth 1–31, Tth 208 and Tth 157. Multiplex PCR reactions were performed with forward primers labeled as follows: Ttho-1 and Tth 1–31 were labeled with NED™ (shown in black), Ttho-4 with 6-FAM™ (blue), Tth-34 with VIC® (green) and Tth 157 and Tth 208 with PET™ (red). DNA fragments comigrated with an internal size standard (orange) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

post-flexion larvae (n = 26) indicated that fish larvae were the most important prey ($\alpha = 0.6$) in relations to copepods and gastropods ($\alpha < 0.3$).

4. Discussion

This is first study reporting evidence of piscivorous feeding in wild ABFT larvae captured in the Balearic Sea. Moreover, the piscivory documented was in fact intra-cohort cannibalism. The stomach contents of ABFT larvae showed a high incidence of feeding (94%), within the range of those previously described in the Balearic Sea and the GOM (Catalán et al., 2011; Llopiz et al., 2015). Despite the oligotrophy of the study area, ABFT larvae are rather successful planktonic predators because 94% of the larvae were positive for at least one food item.

Diet composition revealed that smaller size classes of ABFT larvae showed preference first for copepods and later, after further growth, cladocerans. Prey sizes increased with SL, in agreement with previous trophic analysis studies carried out in ABFT larvae (Catalán et al., 2011; Llopiz et al., 2015), and other tuna species (*A. rochei*; *T. alalunga*) (Catalán et al., 2007; Morote et al., 2008; Llopiz et al., 2010). A dietary shift is observed when larvae reach post-flexion stages (> 6 mm), as this is the earliest stage when consumption of gastropod larvae is documented (Fig. 3). Despite the aforementioned reported literature, it this study represents the first time that gastropod larvae were shown to be an important part of the diet of post-flexion ABFT larvae. Gastropod larvae showed a significant presence within the zooplankton composition of the study site selected. This prey is an attractive food source for fish larvae and has been used in aquaculture (May, 1970). Lasker et al., (1970) showed that gastropod larvae proved to be an excellent food source when combined with a dinoflagellate sources for promoting optimal growth in anchovy larvae cultured under laboratory rearing conditions.

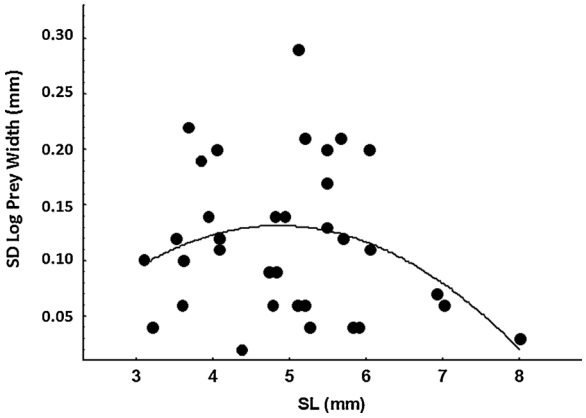

Fig. 6. Relationship of trophic-niche breadth, expressed as the SD log of prey width, plotted with SL (mm) of larvae.

The greatest dietary niche breadth was observed in the pre-flexion and flexion phase of ABFT larvae (3–6 mm SL). Within this SL range, a significant number of preys per larva were also found. However, the only prey categories represented are small zooplankton groups (copepods and cladocerans). With ontogenic development, and particularly with the increase of LJL, ABFT larvae can ingest larger prey without having to increase the number of prey consumed (Catalán et al., 2011). On the other hand, larger larvae showed a decrease in trophic niche breadth due to their preference for larger prey. These results, together with the Levin´s standardized index and relative dietary preference (Chesson´s index) showed that fish larvae is the most important prey

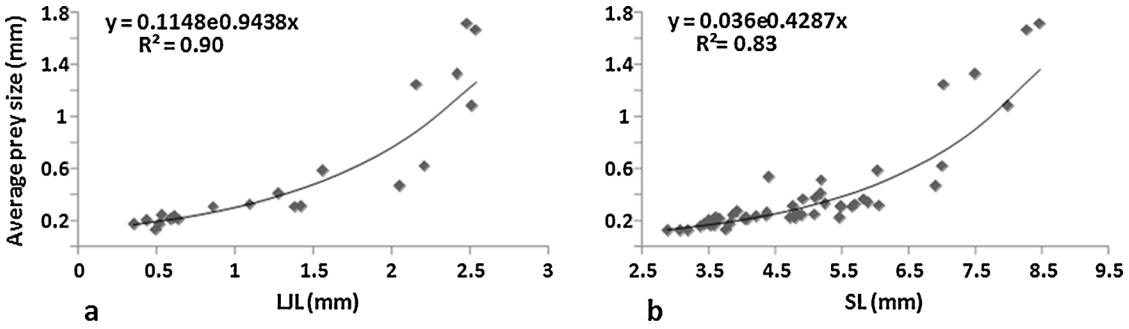

Fig. 5. Relationship between average prey size with LJL (a) and SL (b) of *Thunnus thynnus* larvae.

category in post-flexion ABFT larvae. Consequently, larvae select highly energetic prey of larger sizes that can efficiently support an energy investment needed for the development of search and capture stratagems in early ontogeny (Tanaka et al., 2008; Catalán et al., 2011; Reglero et al., 2011; Uriarte et al., 2016, 2017).

In contrast with previous studies on the trophic ecology of ABFT larvae (Catalán et al., 2011; Llopiz et al., 2010, 2015, Tilley et al., 2016), our results show that gastropod larvae constitute an important prey item in larvae with SL 6–7 mm (46% IRI). In fact, this type of prey has been reported as an attractive food source for larval fish (Lasker et al., 1970; Tuner 1984). Furthermore, because of its availability and small size, it has been used successfully to feed young larvae in aquaculture (May, 1970). Despite the abundance of gastropod larvae in the zooplankton community, they were only found in early post-flexion larvae. Compared with larvae of other co-existing tuna species of the NW of the MED, gastropod larvae are a frequent prey in *A. rochei* larvae < 4 mm (Morote et al., 2008), but have not been observed in the digestive tracts of *T. alalunga* (Catalán et al., 2007). Nevertheless, our results help to suggest that gastropod larvae should be considered as important prey during the transition from planktivorous to a piscivorous feeding in the Mediterranean.

Piscivory, including that of cannibalistic nature has been documented in large ABFT larvae (> 7 mm) in the GOM (Llopiz et al., 2015). Until now this behavior has never been reported for ABFT in the Mediterranean. In a previous analysis of ABFT stomach contents, Catalán et al. (2011) researchers were not able to identify piscivory due to the small number of large post-flexion larvae. In a comparative stable isotope analysis of the four co-existing tuna species of the Balearic Sea, García et al. (2017) argued that the increase in $\delta^{15}N$ signatures with post-flexion development in *T. alalunga* and *A. rochei*, indicated potential piscivory. However, an increase of $\delta^{15}N$ was not observed in ABFT post-flexion stages. It was hypothesized that this difference was attributable to a delayed shift to piscivory with respect to the former species. However, this study is able to demonstrate that six ABFT post-flexion larvae contained fish larvae in their stomach contents. The morphological traits of these prey corresponded to ABFT, and the genetic barcoding and genotyping of these prey larvae confirms cannibalism. A recent study in northern Gulf of Mexico on the invasive red lionfish also applied barcoding followed by genotyping of microsatellite loci to assess cannibalism that appears to be size- and density-dependent (Dahl et al., 2018).

The advanced degree of trophic specialization observed in ABFT larvae is key for survival in the oligotrophic environments in which they develop. The generally low amount of available food for the initial developmental stages is compensated by ABFT's spawning strategy and use of specific hydrographic features responsible for particle food concentrations, e.g. fronts and gyres (Alemany et al., 2010; Bakun and Broad, 2013). Reglero et al. (2011) infer from ABFT larval energetics models that larvae should become piscivorous at post-flexion stages to be able to survive. As much as 71% of the GOM ABFT larvae in 8–10 mm size classes test positive for fish larvae consumption, including *Thunnus* spp larvae (Llopiz et al., 2015). Due to the asynchronic egg production of ABFT (Tyler and Sumpter, 1996), the coexistence of cohorts of different sizes may favour, and thereby, propitiate cannibalistic feeding behavior (Perry and Roitberg, 2005; Reglero et al., 2011). Therefore, cannibalism among different size classes can help decrease starvation induced mortality and improve survival of larval tuna in oligotrophic ocean areas.

Density-dependent factors are evidently relevant to the potential for piscivory among ABFT larvae. A spatial and temporal match of older and bigger potentially piscivorous larvae with smaller larval subcohorts of the same or other tuna species is necessary. Through piscivory, highly energetic trophic resources are made accessible, aiding the post-larval stages growth and survival to recruitment. Nonetheless, the spatial and temporal overlap of size differentiated larval cohorts of ABFT, allowing density-dependent piscivory, is rather rare. In fact, it was observed in only one of the 11 stations sampled. The fact that this station recorded the highest abundance of ABFT larvae with a wide range of sizes (3.68–8.47 mm SL), as well as the largest ABFT larvae captured (average SL = 6.56 ± 0.96), suggests that piscivory and cannibalism are likely to be density-dependent among sizes classes. For piscivorous feeding to be possible, the range of larval size classes that coincide in one area must be broad enough to include post-flexion larvae as well as smaller larvae (Reglero et al., 2011).

The concurrence of high ABFT larval abundance distributed over a wide size range allowed the discovery of piscivorous/cannibalistic larval specimens among Mediterranean ABFT larvae. The reasons why piscivory was not observed prior to this study could be the consequence of the paucity of ABFT larvae in earlier years. ABFT larval abundance and spawning extension have been shown to be increasing since 2011 and suggest the recovery of the MED ABFT (García et al., 2013). ABFT larval abundances have increased greatly since the TUNIBAL project period (2001–2005) and have enabled the implementation of a fishery independent larval index for the estimation of stock size (Ingram et al., 2017). On the other hand, the relative larval abundance of other tuna species (*Thunnus alalunga, Auxis rochei, Euthynnus alleteratus* and *Katsuwonus pelamis*) have shown a significant decrease during the same period, while that of ABFT has increased greatly from 2011 onwards, reaching as high as 95% of total composition of tuna larvae. In the aforementioned TUNIBAL surveys (2001–2005), ABFT represented around 20% of the total tuna larval catch (Alemany, pers. com.). As a result of the increased ABFT larval abundances during the recent years, food partitioning with other competing tuna associated species is practically inexistent, thereby increasing the chances of finding piscivory in the ABFT MED spawning grounds.

Although cannibalism may be seemingly detrimental for population growth, it has been shown to be beneficial for survival and development for a number of fish species that resort to this trophic behavior (Payne et al., 2002; Reglero et al., 2011). ABFT larvae cannot solely survive on zooplankton diets, which do not provide the necessary energy requirements for metabolic growth. At some point after post-flexion, ABFT tuna larvae become voracious (observed in aquaculture) and consequently resort to preying on fish larvae, which have the needed bioenergetic requirements for continued ontogenic development (Reglero et al., 2011; Tanaka et al., 2014; Llopiz et al., 2015; Uriarte et al., 2016, 2017).

It was also considered relevant in this study to report the finding of microplastic fibres in the gut of 4 ABFT larvae (6–7 mm SL). The microplastic fibres found seems to correspond to bluish nylon type strands like those found in the cordage of the fishing and navigation industry. Prior to this study, plastic debris found in alimentary canal of tuna larvae has not been reported. The majority of studies on plastic pollution in marine ecosystems have mainly concentrated on large debris, which may be hazardous to marine mammals, birds, or adult fish. In planktivorous fish from the North Pacific Central Gyre, it has been observed that approximately 35% of their diet was plastics (Boerger et al., 2010). In wild fish larvae from the western English Channel 2.9% had ingested microplastics, of which 66% were fibres (Steer et al., 2017). In an experimental study with samples of zooplankton collected in the Baltic Sea, Setälä et al. (2014) showed the microplastic transfer from one trophic level (mesozooplankton) to a higher level (macrozooplankton) in planktonic food webs. A recent study carried out by Alomar et al. (2016) showed a high proportion of microplastic filaments in the Balearic Sea. Not surprisingly, the morphological description of these filaments matches those recovered in this study from the larval stomach contents. Since plastic concentration in marine ecosystems constitutes a growing global problem that poses a threat to a variety of marine organisms, additional studies are needed to determine the residence time of ingested plastics and their effects on fish health as well as on food chain implications (Boerger et al., 2010).

In conclusion, this trophic study represents the first description of cannibalistic feeding behavior in wild ABFT from the MED through

molecular identification of ingested larvae. Additionally, the data show dietary shifts through ontogeny, where pre-flexion stages of ABFT larvae mainly ingest copepods and cladocerans, shifting during flexion phase to gastropod larvae, an important prey for the widening of niche breadth, and later shifting to piscivory, which was observed in post-flexion larvae. The data also suggest that cannibalistic behavior is related to density-dependence processes and spatial and temporal overlap among larval size classes. The main proximate advantage conferred by cannibalism is assumed to be nutritional (Smith and Reay, 1991), and therefore, in environments with low amount of available food for development, it would be a necessary resource for survival. The results also showed that microplastic fibres were ingested by ABFT larvae, raising concerns about the threats affecting the health of larval tuna.

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References

Alemany, F., Quintanilla, L., Vélez-Belchí, P., García, A., Cortés, D., Rodríguez, J.M., Fernández de Puelles, M.L., González-Pola, C., López-Jurado, J.L., 2010. Characterization of the spawning habitat of Atlantic bluefin tuna and related species Balearic Sea (Western Mediterranean). Prog. Oceanogr. 86, 21–38.

Alomar, C., Estarellas, F., Deudero, S., 2016. Microplastics in the Mediterranean Sea: deposition in coastal shallow sediments, spatial variation and preferential grain size. Mar. Env. Res. 115, 1–10.

Bakun, A., Broad, K., 2013. Environmental 'loopholes' and fish population dynamics: comparative pattern recognition with focus on El niño effects in the Pacific. Fish. Oceanogr. 12 (4–5), 458–473.

Beer, S., Garm, A., Huwer, B., Dierking, J., Nielsen, T.G., 2017. No increase in marine microplastic concentration overA the last three decades – a case study from the Baltic Sea. Sci. Total Environ. 621, 1272–1279. https://doi.org/10.1016/j.scitotenv.2017.10.101.

Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar. Pol. Bul. 60, 2275–2278.

Carreon-Martinez, L., Johnston, T.B., Ludsin, S.A., Heath, D.D., 2011. Utilization of stomach content DNA to determine diet diversity in piscivorous fishes. J. Fish Biol. 78, 1170–1182.

Catalán, I.A., Alemany, F., Morillas, A., Morales-Nin, B., 2007. Diet of larval albacore *Thunnusalalunga* (Bonnaterre, 1788) off Mallorca Island (NW Mediterranean). Sci. Mar. 71 (2), 347–354.

Catalán, I.A., Tejedor, A., Alemany, F., Reglero, P., 2011. Trophic ecology of Atlantic bluefin tuna *Thunnusthynnus* larvae. J. Fish Biol.. 78, 1545–1560.

Chesson, J., 1978. Measuring preference in selective predation. Ecology 5, 211–215.

Clark, T.B., Ma, L., Saillant, E., Gold, J.R., 2004. Microsatellite DNA markers for population-genetic studies of Atlantic bluefin tuna (*Thunnusthynnusthynnus*) and other species of genus *Thunnus*. Mol. Ecol. Notes 4, 70–73.

Dahl, K.A., Portnoy, D.S., Hogan, J.D., Johnson, J.E., Gold, J.R., Patterson, W.F., 2018. Genotyping confirms significant cannibalism in northern Gulf of Mexico invasive red lionfish, Pterois volitans. Biol. Invasions 1–14.

Enderson, K., Lenz, R., Stedmon, C.A., Nielsen, T.G., 2015. Abundance, size and polymer composition of marine microplastics ≥10μm in the Atlantic Ocean and their modelled vertical distribution. Mar. Pollut. Bull. https://doi.org/10.1016/j.marpolbul.2015.09.027.

Frias, J.P.G.L., Otero, V., Sobral, P., 2014. Evidence of microplastics in samples of zooplankton from Portuguese coastal waters. Mar. Environ. Res. 95, 89–95.

Fromentin, J.M., Powers, J.E., 2005. Atlantic bluefin tuna: population dynamics, ecology, fisheries and management. Fish Fish. 6, 281–306.

García, A., Laiz-Carrión, R., Quintanilla, J., Corregidor, F., Alemany, F., 2013. Report on abundant bluefin larval concentrations (*Thunnusthynnus*) found off the shelf/slope area in the Spanish Levantine coasts. Signs of recovery? Collect. Vol. Sci. Pap. ICCAT 69 (1), 292–296.

García, A., Laiz-Carrión, R., Uriarte, A., Quintanilla, J.M., Morote, E., Rodríguez, J.M., Alemany, F., 2017. Differentiated stable isotopes signatures between pre- and post-flexion larvae of Atlantic bluefin tuna (*Thunnusthynnus*) and of its associated tuna species of the Balearic Sea (NW Mediterranean). Deep Sea Res. II 140, 18–24.

Ingram, G.W., Alvarez-Berastegui, D., Reglero, P., Balbín, R., García, A., Alemany, F., 2017. Incorporation of Habitat Information in the Development of Indices of Larval Bluefin tuna (*Thunnusthynnus*) in the Western Mediterranean Sea (2001-2005 and 2012-2013) Deep-Sea Research Part II. https://doi.org/10.1016/j.dsr2.2017.03.012.

King, R.A., Read, D.S., Traugott, M., Symondson, W.O.C., 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol. Ecol. 17, 947–963.

Krebs, C.J., 1999. Ecological Methodology, 2nd edition. Addison-Wesley, New York.

Laiz-Carrion, R., Gerard, T., Uriarte, A., Malca, E., Quintanilla, J.M., Muhling, B., Alemany, F., Privoznik, S., Shiroza, A., Lamkin, J., García, A., 2015. Trophic ecology of Atlantic bluefin tuna (*Thunnusthynnus*) larvae from the Gulf of Mexico and NW Mediterranean spawning grounds: a comparative stable isotope study. PLoS One 10, e0138638.

Lasker, R., Feder, H.M., Theilacker, G.H., May, R.C., 1970. Feeding, growth, and survival of *Engraulismordax* larvae reared in the laboratory. Mar. Biol. 5, 345–353.

Lima, A.R.A., Costa, M.F., Barletta, M., 2014. Distribution patternes of microplastic within the plankton of a tropical estuary. Environ. Res. 132, 146–155.

Lindo-Atichati, D., Bringas, F., Goni, G., Muhling, B., Muller-Karger, F.E., Habtes, S., 2012. Variability of mesoscale structures with effects on larval fish distribution in the northern Gulf of Mexico during spring month; Marine. Ecology Progress Series 463, 245–257.

Llopiz, J.K., Hobday, A.J., 2015. A global comparative analysis of the feeding dynamics and environmental conditions of larval tunas, mackerels, and billfishes. Deep Sea Res. Part II 113, 113–124.

Llopiz, J.K., Richardson, D.E., Shiroza, A., Smith, S.L., Cowen, R.K., 2010. Distinctions in the diets and distributions of larval tunas and the important role of appendicularians. Limnol. Oceanogr. 55, 983–996.

Llopiz, J.K., Muhling, B.A., Lamkin, J.T., 2015. Feeding dynamics of Atlantic bluefin tuna (*Thunnusthynnus*) larvae in the Gulf of Mexico. Collect. Vol. Sci. Pap. ICCAT 71 (4), 1710–1715.

Malca, E., Muhling, B., Franks, J., García, A., Tilley, J., Gerard, T., Ingram, W., Lamkin, J.T., 2017. The first larval age and growth curve for bluefin tuna (*Thunnusthynnus*) from the Gulf of Mexico: comparisons to the straits of Florida, and the Balearic Sea (Mediterranean). Fish. Res. 190, 24–33.

May, R.C., 1970. Feeding larval marine fishes in the laboratory: a review. Calif. Res. Comm, CalCOFIRept 14, 76–83.

McDowell, J.R., Diaz-Jaimes, P., Graves, J.E., 2002. Isolation and characterization of seven tetranucleotide microsatellite loci from Atlantic northern bluefin tuna *Thunnusthynnusthynnus*. Mol. Ecol. Notes 2, 214–216.

Morote, E.M., Olivar, P., Pankhurst, P., Villate, F., Uriarte, I., 2008. Trophic ecology of bullet tuna *Auxisrochei* larvae and ontogeny of feeding-related organs. Mar. Ecol. Prog. Ser. 353, 243–254.

Muhling, B.A., Reglero, P., Ciannelli, L., Alvarez-Berastegui, D., Alemany, F., Lamkin, J.T., Roffer, M.A., 2013. Comparison between environmental characteristics of larval bluefin tuna *Thunnus thynnus* habitat in the Gulf of Mexico and western Mediterranean Sea. Marine Ecology-Progress Series 486, 257–276.

Muhling, B.A., Lamkin, J.T., Alemany, F., García, A., Farley, J., Ingram, W., Alvarez-Berastegui, Reglero, P., Laiz-Carrion, R., 2017. Reproduction and larval biology in tunas, and the importance of restricted área spawning grounds. Rev. Fish Biol. Fish 27 (4), 697–732. https://doi.org/10.1007/s11160-017-9471-4.

Payne, A.G., Smith, C., Campbell, A.C., 2002. Filial cannibalism improves survival and development of beaugregory damselfish embryos. Proc. R. Soc. Lond.B 269, 2095–2102.

Perry, J.C., Roitberg, B.D., 2005. Games among cannibals: competition to cannibalize and parental-offspring conflict lead to increased sibling cannibalism. J. Evol. Biol. 18, 1523–1533.

Pinkas, L., Oliphante, M.S., Iverson, I.L.K., 1971. Food habits of albacore, bluefin tuna and bonito in California waters. California Fishery Game 152, 1–105.

Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D., Jarman, S.N., Taberlet, P., 2012. Who is eating what: diet assessment using next generation sequencing. Mol. Ecol. 21, 1931–1950.

Puncher, G.N., Arrizabalaga, H., Alemany, F., Cariani, A., Oray, I.K., Karakulak, F.S., Basilone, G., Cuttitta, A., Mazzola, S., Tinti, F., 2015. Molecular identification of Atlantic Bluefin tuna (*Thunnusthynnus*, Scombridae) larvae and development of a DNA character-based identification key for Mediterranean Scombrids. PLOS One 10, e0130407.

Reglero, P., Urtizberea, A., Torres, A.P., Alemany, F., Fiksen, Ø., 2011. Cannibalism among size classes of larvae may be a substantial mortality component in tuna. Mar. Ecol. Prog. Ser. 433, 205–219.

Reglero, P., Ortega, A., Blanco, E., Fiksen, O., Viguri, F.J., De la Gandara, F., Seoka, M., Folkvord, A., 2014. Size-related differences in growth and survival in piscivorous fish larvae fed different prey types. Aquaculture 433 94–10.

Richardson, D.E., Marancik, K.E., Guyon, J.R., Lutcavage, M.E., Galuardi, B., Lam, C.H., Walsh, H.J., Wildes, S., Yates, D.A., Hare, J.A., 2016. Discovery of a spawning ground reveals diverse migration strategies in Atlantic bluefin tuna (*Thunnusthynnus*). Proc. Natl. Acad. Sci. U. S. A. 113, 3299–3304.

Rodríguez, J.M., Alemany, F., García, A., 2017. Aguide to the Eggs and Larvae of 100 Common Western Mediterranean Sea Bony Fish Species. Food and Agriculture Organization of the United Nations, Rome, pp. 242.

Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut. 185, 77–83.

Smith, C., Reay, P., 1991. Cannibalism in teleost fish. Rev. Fish Biol. Fish. 1, 41–64.

Steer, M., Cole, M., Thompson, R.C., Lindeque, P.K., 2017. Microplastics ingestion fish larvae in the western English channel. Environ. Pol. 1–10.

Takagi, M., Okamura, T., Chow, S., Taniguchi, N., 1999. PCR primers for microsatellite loci in tuna species of the genus *Thunnus* and its application for population genetic study. Fish Sci. 65, 571–576.

Tanaka, Y., Satoh, K., Yamada, H., Takebe, T., Nikaido, H., Shiozawa, S., 2008.

Assessment of the nutritional status of fieldcaught larval Pacific bluefin tuna by RNA/DNA ratio based on a starvation experiment of hatchery-reared fish. J. Exp. Mar. Biol. Ecol. 354, 56–64.

Tanaka, Y., Minami, H., Ishihi, Y., Kumon, K., Higuchi, K., Eba, T., Nishi, A., Nikaido, H., Shiozawa, S., 2014. Relationship between prey utilization and growth variation in hatchery-reared Pacific bluefin tuna, *Thunnusorientalis* (Temminck et Schlegel), larvae estimated using nitrogen stable isotope analysis. Aquac. Res. 45, 537–545.

Tanaka, Y., Kumon, K., Ishihi, Y., Eba, T., Nishi, A., Nikaido, H., Shiozawa, S., 2017. Mortality processes of hatchery-reared Pacific bluefin tuna *Thunnusorientalis* (Temminck et Schlegel) larvae in relation to their piscivory. Aquac. Res. 49, 11–18.

Teo, S.L.H., Boustany, A., Block, B.A., 2007. Oceanographic preferences of Atlantic bluefin tuna, *Thunnusthynnus*, on their Gulf of Mexico breeding grounds. Mar. Biol. 152, 1105–1119.

Tilley, J.D., Butler, C.M., Suarez-Morales, E., Franks, J.S., Hoffmayer, E.R., Gibson, D.P., Comyns, B.H., Ingram, G.W., Blake Jr, E.M., 2016. Feeding ecology of larval Atlantic bluefin tuna, *Thunnusthynnus*, from the central Gulf of Mexico. Bull. Mar. Sci. https://doi.org/10.5343/bms.2015.1067.

Trégouboff, G., Rose, M., 1957.Manuel de Planctonologie méditerranéenne. Edit. Centre National de la RechercheScientifique, Paris, Tome I: 1-587; Tome II (Planches): I-XXXIX + Tableau de diagnose des familles et des genres (Copépodes).

Tyler, J.R., Sumpter, J.P., 1996. Oocyte growth and development in teleosts. Rev. Fish Biol. Fish. 6, 287–318.

Uriarte, A., García, A., Ortega, A., De La Gándara, F., Quintanilla, J.M., Laiz-Carrión, R., 2016. Isotopic discrimination factors and nitrogen turnover rates in reared Atlantic bluefin tuna larvae (*Thunnusthynnus*): effects of maternal transmission. Sci. Mar. 80 (4), 447–456.

Uriarte, A., Johnstone, C., Laiz-Carrión, R., García, A., Llopiz, J.K., Quintanilla, J.M., Lozano-Peral, D., Reglero, P., Alemany, F., 2017. First report on cannibalistic feeding behaviour in post-flexion bluefin larvae (*Thunnus thynnus*) of the Balearic Sea (NW Mediterranean) collect. Vol. Sci. Pap. ICCAT 74.

Yúfera, M., Ortiz-Delgado, J.B., Hoffman, T., Siguero, I., Urup, B., Sarasquete, C., 2014. Organogenesis of digestive system, visual system and other structures in Atlantic bluefin tuna (*Thunnusthynnus*) larvae reared with copepods in mesocosm system. Aquaculture 426-427, 126–137.