Growth of Mediterranean young-of-the-year bluefin tuna *Thunnus thynnus* (Scombridae): regional differences and hatching periods

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**Summary:** This study analyses growth rates of bluefin tuna young-of-the-year in the Mediterranean. Potential differences in growth rates were examined between years (2013 and 2016) and regions (eastern, central and western Mediterranean). A total of 134 specimens were aged by analysing otolith microstructure. Fish sizes ranged between 14.7 and 57 cm fork length, and estimated ages varied between 45 and 192 days. The annual growth models explained more than 90% of growth variability. The observed differences in the growth rates between 2013 (3.2 mm d$^{-1}$) and 2016 (2.7 mm d$^{-1}$) were not significant, whereas the daily growth rate was significantly faster in the eastern region (4.01 mm d$^{-1}$) than in the western (2.52 mm d$^{-1}$) and central (2.75 mm d$^{-1}$) regions. Larval hatching windows were consistent with the known spawning periods but lasted longer than previously reported in the central and eastern regions. In the central region the hatching period showed two peaks in mid-June and mid-July, consistent with previous studies pointing to two distinct spawning pulses. These pulses might be due to the existence of different bluefin tuna contingents spawning at different times, the Mediterranean residents and the Atlantic migrants, but further research is needed to support this hypothesis.

**Keywords:** daily growth; young-of-the-year; juveniles; Mediterranean; Atlantic bluefin tuna; *Thunnus thynnus*.


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INTRODUCTION

The Atlantic bluefin tuna (Thunnus thynnus) is a highly migratory species with a wide distribution throughout the North Atlantic Ocean and the Mediterranean Sea. The species comprises two different populations, one spawning in the Mediterranean and one in the Gulf of Mexico (Rooker et al. 2008, Rodriguez-Ezepeleta et al. 2019). These populations share common feeding grounds and exhibit a wide spatial overlap throughout the Atlantic, as revealed by satellite tags and genetic and microchemistry analyses (Boustany et al. 2008, Rooker et al. 2014, Rodriguez-Ezepeleta et al. 2019). Despite the complex population structure of Atlantic bluefin tuna (ABFT), the International Commission for the Conservation of Atlantic Tunas (ICCAT) manages it as two different stocks separated by the 45°W meridian. The complexity of the ABFT structure is becoming increasingly conspicuous as knowledge of it progresses. The assumption that there are only two spawning areas has been questioned (Lutcavage et al. 1999). Indirect evidence based on reproductive studies (Goldstein et al. 2007) and tag data (Block et al. 2005, Walli et al. 2009, Galuardi et al. 2010) point to the existence of undocumented spawning grounds which, at least for the Slope Sea, was definitively confirmed by Richardson et al. (2016). In addition, several authors have pointed out the possibility of additional spawning areas in the Atlantic, such as in the Canary and Azores Islands (Lutcavage et al. 1999, Di Natale and Idrissi 2012), but the lack of direct studies in these areas prevents us from drawing conclusions on the subject for the time being. However, a recent identification of ABFT larvae in the Cantabrian Sea (Rodríguez et al., 2019) is an additional piece of evidence of the high degree of complexity of the ABFT spawning structure.

The complexity of ABFT population structure is further exacerbated when the Mediterranean component is considered; this has been a subject of discussion for years, as it could have important management implications (Viñas et al. 2011, Arrizabalaga et al. 2019). The potential existence of discrete bluefin tuna subpopulations or contingents within the Mediterranean has been widely discussed (Renzi et al. 1998, Morales-Nin and Fortuño 1999, Fromentin and Powers 2005), because the presence of bluefin tuna in the Mediterranean is not seasonal as in the Gulf of Mexico but permanent throughout the year. Genetic studies on bluefin tuna within the Mediterranean have shown different views on the existence of genetic subpopulations. Some studies have found regional differences within the Mediterranean (Viñas et al. 2003, Carlsson et al. 2004, Riccioni et al. 2010), but the most recent ones based on single nucleotide polymorphisms and using larvae and age 0 fish as reference samples did not detect any genetic structure (Rodriguez-Ezepeleta et al. 2019). In addition, e-tagging studies have identified both resident individuals (Fromentin and Lopuszanski 2013, Cerméneo et al. 2015) and fish that just use the Mediterranean to reproduce (e.g. Aranda et al. 2013, Abascal et al. 2016). A recent review suggests that more populations or contingents might exist than was previously thought, and it seems more likely that ABFT that originated in the western Mediterranean migrate to the Atlantic for feeding more intensively than those that originated in the eastern Mediterranean (Arrizabalaga et al. 2019).

Additional information also reveals differences between Mediterranean regions. The spawning grounds in the Mediterranean, determined through histological analysis and larval findings, are well known: the Balearic waters in the western Mediterranean (Medina et al. 2002), the Levantine Sea in the eastern Mediterranean (Karakulak et al. 2004), and the South Tyrrhenian Sea and the waters around Malta and off the Tunisian coast and off the eastern coast of Sicily in the central Mediterranean (e.g. Nishida et al. 1998, Corriero et al. 2003, Giovannardi and Romanelli 2010). The spawning migration path from the Atlantic to the Mediterranean has shown a strong connection with all Mediterranean spawning grounds except the Levantine Sea (Block et al. 2005, Walli et al. 2009, Arrizabalaga et al. 2019), which places the origin of the eastern spawners into question. In addition, several studies have reported differences in the fish size structure between Mediterranean spawning regions (Karakulak et al. 2004, Heinisch et al. 2008, Addis et al. 2016). The spawners in the eastern Mediterranean seem to be smaller than those entering the Mediterranean and those from the western Mediterranean spawning grounds, while sizes in the central region had a wider distribution, which might be representative of a mixing spawning ground of fish coming from the Atlantic and eastern Mediterranean (Heinisch et al. 2008).

Differences in spawning periods among Mediterranean regions are known: spawning takes place in June-July in the western basin (e.g. Susca et al. 2001, Medina et al. 2002, Corriero et al. 2003) and one month earlier, May-June, in the eastern basin (Duclerc et al. 1974, Karakulak et al. 2004, Oray and Karakulak 2005). These differences are attributed to temporal differences in water warming between regions. In the Mediterranean Sea the differences in temperature and chlorophyll between the eastern and western basins are permanent throughout the year (d’Ortenzio and Ribera d’Alcalá 2009, Skliris et al. 2012, Shaltout and Omstedt 2014). Temperature and food availability can affect growth and survival during the early stages of fish development (Anderson 1988), as evidenced in Pacific and Atlantic bluefin tuna larvae (García et al. 2013, Satoh et al. 2013, Ishihara et al. 2019). Consequently, the oceanographic differences within the Mediterranean may cause differences in the growth rate during the earlier stages of development of the species. In spite of this, no comparative studies on the larval or young-of-the-year (YOY) growth within the Mediterranean have yet been carried out. So far, studies on ABFT larval growth are limited to the larvae collected in the western spawning ground in the Balearic waters (García et al. 2006, 2013), whereas the studies on YOY are performed with juveniles caught in the central Mediterranean (Santamaria et al. 2009 La Mesa et al. 2005).

The objectives of the present study were to analyse the growth rates of ABFT YOY in the Mediterranean basin in 2013 and 2016 and investigate potential differences in growth rates between eastern, central and western regions. We analysed the microstructure of ABFT otoliths using the daily increment counts to estimate the age and growth of juveniles collected in the framework of the Grand Bluefin Tuna Year Programme (GBYP). The GBYP was officially adopted by the ICCAT Commission.
in 2008 and aims to improve basic data collection through data mining, understanding of key biological and ecological processes and assessment models, and provision of scientific advice on stock status.

MATERIALS AND METHODS

Sampling

The otoliths of ABFT YOY were taken from the GBYP biological data bank; they were sampled within the GBYP biological sampling programme in several Mediterranean regions. During the sampling, catch date was recorded and fish fork length (FL) was measured to the nearest 0.1 cm. Each sagittal otolith was carefully extracted and cleaned. After drying, the otoliths were stored in plastic vials and kept in the GBYP data bank. The samples were requested to the GBYP for two years, 2013 and 2016, and were selected according to the specific objectives for each year. In 2013, the objective was to analyse the growth rate of YOY in the whole Mediterranean basin; a total of 60 otoliths were selected, 20 per region (eastern, western and central) and from samples caught close together in time (Fig. 1). In 2016, the objective was to analyse the growth rates for each Mediterranean region, which required a wide range of sizes in each region; consequently, for each region the otoliths were selected from two subsets of samples separated in time. The length frequency distribution of each sample, recorded in the GBYP data bank, was examined to ensure that the otoliths selected for each location represented the whole size range of the fish caught (Table 1). The juveniles collected in the Tyrrhenian Sea in August 2016 displayed a wider size range, and consequently the sample size was larger than that of those from the other regions. Another particularity of 2016 was that

<table>
<thead>
<tr>
<th>Year</th>
<th>Area</th>
<th>Location</th>
<th>Catch date</th>
<th>Sample size</th>
<th>Fork length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>Western</td>
<td>Balearic Islands</td>
<td>19 October</td>
<td>20</td>
<td>32.5 – 41.0</td>
</tr>
<tr>
<td></td>
<td>Central</td>
<td>Malta</td>
<td>10-23 September</td>
<td>20</td>
<td>19.4 – 27.6</td>
</tr>
<tr>
<td></td>
<td>Eastern</td>
<td>North Cyprus</td>
<td>15-20 August</td>
<td>20</td>
<td>21.0 – 26.4</td>
</tr>
<tr>
<td></td>
<td>Western</td>
<td>Balearic Islands</td>
<td>10-30 September</td>
<td>10</td>
<td>20.4 – 33.1</td>
</tr>
<tr>
<td></td>
<td>Eastern</td>
<td>Balearic Islands</td>
<td>2-18 November</td>
<td>11</td>
<td>35.0 – 48.0</td>
</tr>
<tr>
<td></td>
<td>Central</td>
<td>South Tyrrhenian</td>
<td>19-29 August</td>
<td>20</td>
<td>18.4 – 52.6</td>
</tr>
<tr>
<td></td>
<td>South Tyrrhenian</td>
<td>4-17 December</td>
<td>13</td>
<td>43.0 – 57.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>North Cyprus</td>
<td>22-29 July</td>
<td>5</td>
<td>14.7 – 17.1</td>
<td></td>
</tr>
<tr>
<td>2016</td>
<td>Central</td>
<td>South Tyrrhenian</td>
<td>12 October</td>
<td>2</td>
<td>29.5 – 32.6</td>
</tr>
<tr>
<td></td>
<td>North Cyprus</td>
<td>13-31 August</td>
<td>5</td>
<td>16.1 – 30.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>North Cyprus</td>
<td>2-10 September</td>
<td>10</td>
<td>20.5 – 35.8</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. – Summary of collected data: dates, locations, number of individuals and size ranges.

Fig. 1. – Sampling sites. Western locations (diamonds), central locations (squares) and eastern locations (circles). Symbols filled with grey and black outlines represent the years 2013 and 2016, respectively.
only 27 juvenile individuals were collected in the eastern region, so the analysed subsample (20) represented practically all the fish available in the GBYP data bank for that year and region.

Age and growth

The ageing of 2013 and 2016 samples was carried out in two different laboratories (AZTI and CEAB) three years apart, following the same technique. When both otoliths were available, the left ones were chosen preferably for age reading; otherwise the right one was used, because no significant differences between left and right sagittal otoliths have been reported (Rooker et al. 2003, Megalofonou 2006).

In order to obtain a transverse section, the otoliths were mounted on the edge of a glass slide using a thermoplastic glue (Crystal Bond), placing the primordium just before the edge of the slide. Both the anterior and posterior ends were sequentially grinded down using wet lapping films (30 and 12 microns), resulting in a section containing the nucleus. Then, the otoliths were placed side down in the centre of the slide and the polishing procedures continued. The increment sequence was continuously checked under a compound microscope. A further, finer smoothing was done with a 1 micron lapping film until a plane including all the rings and nucleus could be observed. Grinding and polishing procedures were carried out with a Metaserv 3000 variable speed grinder-polisher. Finally, the samples were brushed with immersion oil to enhance the contrast. Each assigned age was corrected by adding four days to the total counted increments (Brothers et al. 1983, Itoh et al. 2000). Two different readings were made. In 2013, when counts differed by more than 10%, a third reading was performed. If the difference in counts was greater than 10%, that otolith was considered unreadable. In 2016 the third reading was considered necessary when counts differed by more than 5%.

Simple linear regression models were used to determine the daily growth of YOY in the Mediterranean. Potential growth differences between years (2013 and 2016) and between regions (eastern, western and central) and basins (eastern vs western & central) were examined by comparing the slopes of the regression lines based on a Student t-test after examining the homogeneity of their variances (F-test). In order to examine the possible effect of fish size range on the estimates of growth rates, an additional analysis was carried out. The western & central growth rate was recalculated without the larger individuals (>380 mm), which were absent in the samples collected from the eastern basin.

In addition, the individuals’ hatching dates were back-calculated from the age estimates and date of capture to estimate the hatching windows of our samples.

RESULTS

The otolith microstructure showed a concentric pattern of increments from the core region until around the sixth increment, and then became larger and elongated along the postero-anterior axis, which was gradually reduced (Fig. 2). In 2013, 83.3% of the readings differed

Fig. 2. – Otolith pattern of daily growth.
from each other by less than 5%, whereas 16.7% of the readings differed by 5% to 10% and no age estimate differed more than 10%. In 2016, 87% of the readings differed by less than 5% and the differences between the remaining percentage were below 9%.

A total of 134 specimens between 14.7 and 57 cm FL were aged. This is the largest sample size on YOY bluefin tuna otolith microstructure analysis so far. Age estimates ranged from 45 to 192 days. An overview of the results (Fig. 3A) showed some abnormal observations: young individuals (<80 days) with extraordinarily large sizes, even above 50 cm. These observations corresponded to YOY caught in the Tyrrhenian Sea in August 2016. The catch was comprised of individuals with a wide length range, spanning 18.4 to 52.6 cm FL, but with a narrow age range (59 to 78 days). The age and length of the exceptional observations were double-checked. First, the age was re-read and the new readings confirmed the previous age estimates. Next, the reported fork lengths of the YOY captured in 2016 were examined with the size of their otoliths based on the known linear relationship between fish length (FL) and otolith length (e.g. Jenkins and Davis 1990, La Mesa et al. 2005, Gunn et al. 2008). The radius length (LR) was measured for each otolith and the linear regression between FL and LR were estimated. The model fitted with the data holding the exceptional observations only explained 46% of FL variability but increased to 92% when the sample was excluded (Fig. 3B). These results indicated that the extraordinarily large sizes in that specific sample were wrong. Consequently, every FL observation outside the 99% prediction interval was discarded from the growth analysis; a total of 13 fish were excluded from the growth analysis because their sizes

Fig. 3. – Relationship between young-of-the-year fork length and A) age in days; B) otolith radius (m). Linear regressions and confidence intervals fitted with all the observations (in red) and without the southern Tyrrhenian samples taken in August 2016 (in black).

Fig. 4. – Fraction of fish born per week, area and year.

Fig. 5. – Linear regressions fitted to the age-length data of young-of-the-year born in 2013 and 2016.
were outside the prediction interval but their ages were included in the birth date estimations. The birth dates found for each region varied from 24 May to 11 July in the eastern region, from 31 May to 4 August in the central region and from 2 June to 29 July in the western region. The extension of the birth intervals in the eastern and western regions was close to two months (Fig. 4). The main differences between regions (Fig. 4) were: 1) the eastern region consistently showed earlier ending and earlier hatching, with the peak around the first fortnight of June for both sampled years, 2) the hatching interval was wider in the central region than in the other regions and showed two peaks, in mid-June and in mid-July, and 3) the peak observed in the western region in late June took place between the two peaks observed in the central region.

The growth models fitted for 2013 and 2016 observations explained more than 90% of the daily growth variability of YOY in the whole Mediterranean basin (Fig. 5). The t-test analysis showed no significant differences between the growth rates of 2013 (3.25 mm d\(^{-1}\)) and 2016 (2.70 mm d\(^{-1}\)). In contrast, the results of the growth models fitted for each region separately displayed high variability, from 2.52 mm d\(^{-1}\) to 4.01 mm d\(^{-1}\) (Table 2). While the western and central regions showed no significant differences between each other, the daily growth rate of the eastern region (4.01 mm d\(^{-1}\)) was significantly faster. These differences in the growth rates between basins remain after the additional analysis in the western and central basin, in which the largest fish (>380 mm) were excluded (Table 2).

**DISCUSSION**

Spawning onset of *T. thynnus* seems to be temperature-related for all tuna species (Schaefer 2001). In the Mediterranean, the reported temperatures during the ABFT spawning period range from 19.5°C to 26.5°C (García et al. 2005, Teo et al. 2007, Gordoa and Carreras 2014), but neither spawning nor water warming are simultaneous along its almost 4000 km of length. The time differences in spawning between regions assumed to be related to the warming time lag range from June to July in the western basin (e.g. Susca et al. 2001, Medina et al. 2002, Corriero et al. 2003) and from May to June in the eastern basin (Duclerc et al. 1974, Karakulak et al. 2004, Oray and Karakulak 2005). These spawning windows in the Mediterranean spawning regions are to a certain extent in accordance with the hatching time intervals shown in this study. However, they do not support the hypothesis of a 2016 winter spawning event based on the anomalously large YOY caught in the Tyrrhenian sea (Di Natale et al. 2017), which have been shown here to be errors. It should be highlighted that the estimated hatching time intervals shown here may be only indicative of the minimum spawning window expected in each region. Notwithstanding this limitation, the observed hatching interval in the central Mediterranean, which spanned from the end of May to the beginning of August, was wider than the spawning window reported by previous studies. The sampling periods reported by these studies, either based on gonadal development or larval survey, were limited to May–July (Corriero et al. 2003, García et al. 2005, Heinisch et al. 2008). We must go back to 1932 to find the first and last indication of bluefin tuna spawning in August, when some larvae were found around Sicilian waters (Sanzo 1932). Currently, there is no information to infer whether spawning events in August are permanent or occasional. Similarly, in the eastern region (Levantine Sea), the hatching interval lasted until mid-July, which is well beyond the previously reported spawning period (May–June).

The results of the central region covered a wide hatching period (late May to early August) with a bimodality in the hatching frequency in mid-June and mid-July. The observed pattern could be an artefact of a weak sampling coverage. However, this is unlikely given that the expected peak under a progressive spawning process would be found in late June and/or early July, which is precisely when we observed the minimum frequency. Furthermore, these results are consistent with the length frequency bi-
modality of YOY found by Relini et al. (1995), who suggested two different spawning pulses in the central region. The existence of different spawning pulses might be due to the existence of different bluefin tuna contingents (e.g. residents and Atlantic migrants) using the central spawning region at slightly different times, but further research is needed to support this hypothesis.

The sampled location of the YOY does not necessarily correspond to their hatching place. However, based on current knowledge, it is likely that sampling of YOY occurred close to their hatching places in the western and eastern regions. The Mediterranean is comprised of two basins that are connected by the strait of Sicily, but each of them has a counter-clockwise surface current (Millot 1999, Hamad et al. 2005) which, in addition to the long distance between the western and eastern spawning regions, makes the exchange of YOY between western and eastern regions unlikely. The central region represents a different scenario due to its large extension, from Sicily to the Aegean Sea, which could receive some YOY from the Levantine Sea. Unfortunately, little is currently known about YOY migration capability because of the lack of scientific tagging studies for this age class.

The few studies on age and growth of YOY in the Mediterranean (Table 2) showed much faster growth rates than those reported for YOY in the western Atlantic (Brothers et al. 1983, Arias et al. 2020), which were around 1.1 to 1.5 mm d⁻¹. The results of the growth model for the central Mediterranean showed no major differences from previous studies in this region (La Mesa et al. 2005, Santamaria et al. 2009), with the exception of the data reported by Megalofonou (2006), which differed from the rest. In the latter study, the model showed faster growth: even faster than our estimation for the eastern region. The YOY collected in that study came from a very large area, which included various seas of the central Mediterranean: the south Tyrrenian, the Ionian, the Adriatic and also the Aegean Sea. However, it is unlikely all these fish have their origin in the central Mediterranean spawning ground. In particular, the YOY collected in the Aegean Sea may have come from the Levantine Sea because this is the closest spawning ground and the anti-clockwise direction of the surface current from the Levantine to the Aegean Sea would favour the transport of these specimens.

The present study has dealt with YOY information, and, although only individuals surviving the recruitment phase may be analysed, one might assume that they are the best adapted for each scenario. There is evidence for the Pacific bluefin tuna that offspring survival depends largely on the growth rates during the late larval phase (Tanaka et al. 2006, Watari et al. 2017). Food availability and water temperature are the critical factors affecting the growth (Heath 1992) and survival rates (Campana 1996, Meekan and Fortier 1996) of marine fish larvae. For bluefin tuna, high water temperature seems to promote larval growth rates (García et al. 2013, Satoh et al. 2013). However, the results of a recent study on Pacific bluefin tuna pointed out that both temperature and food availability have an effect on larval growth but at different stages of development (Ishihara et al. 2019).

The findings of the current study showed a significantly faster YOY growth in the eastern Mediterranean, and the results ruled out the possibility that this was due to the size range of the YOY in this region. The warmer temperature in this region compared with the western region (Shaltout and Omstedt 2014) could be an important factor determining the observed differences in growth. It is worth remembering that the results of growth rates are skewed toward higher values because only surviving individuals are analysed (Le Pape and Bonhommeau 2015), because key survival factors might change between regions.

The population structure of ABFT has been the object of debate for decades, particularly regarding the eastern Mediterranean population. The presence of fish in the Mediterranean all year around (Cermeño et al. 2015), along with the potentially smaller connectivity between the Atlantic and the eastern Mediterranean (Walli et al. 2009, Arrizabalaga et al. 2019), raises the question of the existence of a single eastern Mediterranean population or contingent. Thus, the extent to which the faster growth in the eastern Mediterranean can be attributed just to the warmer environmental conditions, excluding biological potential differences, should be further investigated.

In synthesis, we can conclude that the growth rate of ABFT YOY is faster in the eastern Mediterranean, possibly because of the warmer conditions of the region, but ontogenetic differences cannot be excluded. We suggest the possibility of occurrence of two spawning pulses in the central Mediterranean, which might correspond to the existence of two different bluefin tuna contingents (e.g. residents and Atlantic migrants) using the central spawning region at slightly different times. However, further research is needed to support this hypothesis.

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