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Climate-induced environmental conditions influencing interannual variability of Mediterranean bluefin (*Thunnus thynnus*) larval growth

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ABSTRACT

Daily growth variability of bluefin (*Thunnus thynnus*) larvae sampled in their Balearic Sea spawning grounds during the 2003–2005 spawning seasons was examined. Multi-factorial ANOVA was applied to study the effects of environmental variables, such as temperature at 10 m depth (T10), microzooplankton dry weight (MDW) and protein/dry weight ratio (PROT/MDW) on larval growth. The 2003 bluefin tuna (BFT) larval cohort showed the fastest growth, recognizable from enhanced otolith and somatic mass increment compared to the 2004–2005 larval cohorts. The 2003 BFT larvae showed greater recent growth than the 2004–2005 BFT cohorts, which decreased in the last stages of development. Growth differences between the 2004 and 2005 larval cohorts were not significant. The environmental conditions between 2003 and 2004–2005 were highly contrasting as a result of the 2003 warming anomaly. Somatic and otolith growth rates (OGR) were significantly related to T10 and MDW, as well as to the PROT/MDW ratios. Nonetheless, the effect of T10 on OGR depended on the relative high (H) or low (L) levels of MDW and PROT/DW. Higher OGR was observed when T10 was high, MDW was low and PROT/DW was high. This environmental scenario conditions were met during 2003, which recorded the highest surface temperature and low planktonic biomass. Somatic growth, expressed as larval DW growth increase (DWGR), showed three-factor significant interactions with T10*MDW*PROT/MDW, in which the two-way interactions of MDW*PROT/MDW showed differences in the function of T10 levels.

Key words: Balearic Sea, bluefin larvae, climate variation, daily growth, microzooplankton, surface temperature

INTRODUCTION

The Atlantic bluefin tuna (BFT) (*Thunnus thynnus*) is the largest scombriform, reaching weights of up to 650 kg and living for up to 20 years (Scott *et al.*, 1993). Due to its extremely high commercial value, it is one of the most heavily fished species in the oceans. The overexploitation of BFT in the Mediterranean has led to its dramatic decline in recent years (Fromentin and Powers, 2005), threatening a population collapse (MacKenzie *et al.*, 2009). In consequence, recommendations for implementing a recovery plan were proposed to the International Commission for the Conservation of Atlantic Tunas, (ICCAT 2005). As a major top predator of the pelagic ecosystems of the North Atlantic and Mediterranean Sea, the species may play key roles in the food web structure and ecosystem dynamics (Bakun and Broad, 2003; Bakun, 2006). Its extinction could cause drastic changes in the functioning and structure of the pelagic ecosystem, resulting in a cascade of effects on the pelagic food web (Pauly *et al.*, 1998; Essington *et al.*, 2002; Scheffer *et al.*, 2005).

Atlantic BFT is highly migratory. During its life cycle, the species undertakes a reproductive migration towards the Mediterranean across the Strait of Gibraltar during the months of May–June (Rey, 1983; Mather *et al.*, 1995; Fromentin and Powers, 2005). BFT enters the Mediterranean Sea, following the inflowing Atlantic surface current towards the Northwestern Mediterranean reaching the Balearic Sea. As far back as the early 1970s, the region proved favourable for BFT spawning (Dicenta and Piccinetti, 1975; Dicenta,

1977). Tsuji et al. (1995) substantiated BFT spawning around the Balearic archipelago during a survey undertaken in 1994 covering the western and eastern Mediterranean waters. Currently, the waters off the Balearic archipelago may represent one of the most important BFT spawning habitats in the Mediterranean, as evidenced by the results of the yearly 2001–2005 TUNIBAL surveys (García et al., 2003; Alemany et al., 2010).

The area may be considered an essential fish habitat for this species as its waters meet adequate environmental conditions for tuna breeding, spawning and larval survival. The Balearic Sea is characterized by the confluence of incoming surface waters from the Atlantic (Modified Atlantic Water masses, MAW) that moves northward and the Surface Mediterranean Water masses (SMW) (López-Jurado et al., 1995; Lopez-Jurado, 2002) that moves southward. These converging water masses have contrasting physical properties. The southern incoming surface MAW is less saline, usually warmer and nutrient-poor, in contrast to the SMW, which is more saline, cooler and richer in nutrients. Several factors may be considered responsible for the higher nutrient concentration in the SMW masses. The Northern Current flows along the Catalonian coasts where a series of enrichment processes occur, for example river run-offs (Salat et al., 2002), shelf-slope frontal systems (Castellón et al., 1990; Estrada, 1996) and a particular wind regime off the Gulf of Lions and the Catalonian coasts (Millot, 1990; Bakun and Agostini, 2001; Salat et al., 2002), all of which provide suitable spawning habitat conditions for a variety of fish species (Sabatés et al., 2007) and concentrate the largest biomass of small pelagic stocks in the NW Mediterranean (Abad et al., 1998) which could provide foraging needs after the end of the BFT spawning season.

The convergence of these water masses of contrasting physical and biological properties produces strong hydrodynamic activity in the Balearic Sea. In consequence, mesoscale structures such as gyres and surface fronts (Vélez-Belchí and Tintoré, 2001) occur. These structures may have a strong influence in determining the distribution of BFT schools and their larval patches (Mariani et al., 2010) and, consequently, their spawning habitat (Platonenko and de la Serna, 1997; García et al., 2003; Royer et al., 2004; Alemany et al., 2006). The mechanical energy of these hydrographic structures may increase the trophic energy available to organisms (Bakun, 2006), as demonstrated in the Balearic Sea open sea ecosystem by Pinot et al. (1995). Consequently, BFT may come across environmental opportunities that favour larval survival in the oligotrophic open sea waters of the Balearic archipelago (Bakun and Broad, 2003).

This study focuses on BFT larval growth and its inter-annual variability in the face of variable natural environmental conditions occurring during the 2003–2005 BFT spawning seasons. This type of information from field-captured larvae is very sparse, bearing in mind the usual scarcity of BFT larval catches by plankton sampling. Thus, most studies from the field have been oriented to report on the time and location of BFT larval catches in the Mediterranean Sea, whereas BFT larval daily growth studies have been normally undertaken with laboratory-reared specimens (Itoh et al., 2000; Miyashita et al., 2001). During July 2003, the Western and Central Mediterranean was affected by an intense heat wave that strongly increased sea surface temperatures (Schär et al., 2004; Sparnocchia et al., 2006; Olita et al., 2007). The effects of this heat wave were worldwide and registered among the three highest values of historical records (Levinson and Waple, 2004). This climatic anomaly offered the opportunity of analyzing early larval growth variability under highly contrasting environmental BFT spawning scenarios. In comparison, the summers of 2004 and 2005, according to the MEDAR/MEDATLAS II Climate data base (http://gcmd.nasa.gov/index.html), recorded mean temperatures slightly colder and warmer than usual, respectively. Distinct environmental conditions of abiotic and biotic nature during the early stages of development of fishes undoubtedly influence larval growth rates.

Changes of temperature can show strong effects on the vital rates, and consequently on growth and mortality rates (Chambers and Leggett, 1987; Houde, 1987; Heath, 1992). Otolith microstructure analysis has been shown to be a powerful tool in larval growth studies, which demonstrate the environmental influence on growth. Daily increments (DI) deposited in otoliths on a daily basis can show important variations in their width as a function of the surrounding larval habitat conditions. It has been demonstrated that otolith growth and their corresponding increments widths are closely related to environmental variables such as temperature and feeding (Maillet and Checkley, 1990; Clemmesen and Doan, 1996; Dickey and Isely, 1997; Reichert et al., 2000).

Starvation and predation may be another important source of larval mortality (May, 1974; Bailey and Houde, 1989). However, these cannot be considered independently but are rather inter-related, as starvation leads to decreased growth rates (Buckley, 1984), and hence larvae are more exposed to predation (Folkvord and Hunter, 1986; Purcell et al., 1987). On the basis of such experimental and field-based evidence, it is generally accepted that growth rates at

early life stages may impart strong effects on survival at later stages, and therefore influence recruitment variability (Houde, 1987; Anderson, 1988). Specific examples applicable to tunas have shown that faster growing tuna larvae have higher survival rates and thereby higher recruitment success (Tanaka *et al.*, 2006; Wexler *et al.*, 2007).

MATERIAL AND METHODS

Field sampling

A pre-defined grid of 10 × 10 nautical miles of sampling stations was set up to undertake the hydrographic and planktonic sampling within the TUNIBAL project as described in Alemany *et al.* (2010). For this larval growth study, plankton sampling was increased wherever significant numbers of BFT larvae were caught with any systematic plankton tow. Thus, BFT larvae were collected at sites of major BFT larval concentrations found during the 2003–2005 TUNIBAL yearly surveys (Fig. 1). Table 1 shows the dates in which surveys were carried out (June–July), as well as the number of stations where BFT larvae were collected and used for the daily growth analysis.

Bluefin tuna larvae were sampled by means of short (10 min) sub-surface plankton tows (1–5 m depth) carried out with a squared-mouth Bongo frame measuring 90 cm on each side, equipped with a 500-μm mesh and a canvas cod-end where the plankton is concentrated. The plankton sample was transferred to pyrex glass trays where BFT larvae were sorted and counted. BFT were then conserved dry frozen in liquid nitrogen for the shortest time possible.

The physical properties of the water column were measured by means of CTD (SBE 25) casts (see Alemany *et al.*, 2010). Furthermore, microzooplankton, a proxy used for potential larval feeding resources, was sampled by means of a CalVET plankton net, with mesh size of 55 μm, towed vertically from 70 m depth (García *et al.*, 2006). On board, microzooplankton samples were then sieved through 200- and 55-μm filters to separate the larger mesozooplankton from the microzooplankton fraction. The microzooplankton (55–200 μm) fraction was then stored frozen to estimate its dry weight.

Figure. 1. Number of BFT larvae sampled for the growth study represented by proportional circles for each survey with TUNIBAL sampling grid in the background.
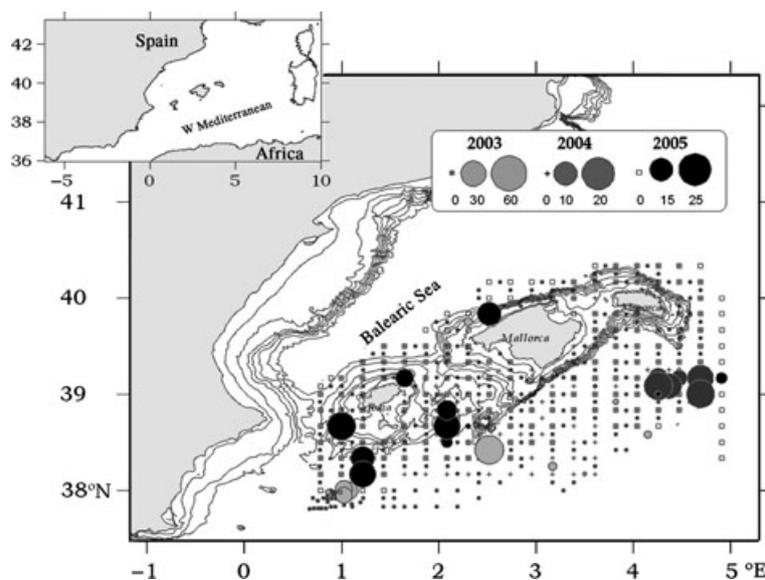
Table 1. Number of stations with positive BFT larval hauls and total BFT larvae analyzed for daily growth in the TUNIBAL survey series of 2003–2005.

Survey	Dates	No. of stations	No. of larvae
TUNIBAL 0703	4–30 July 2003	13	157
TUNIBAL 0604	16 June–12 July 2004	6	99
TUNIBAL 0605	24 June–27 July 27 2005	11	132

Otolith microstructure analysis

Bluefin tuna larvae destined for this growth study were selected from sampling stations where they were most abundant (García *et al.*, 2003, 2004). Randomly selected frozen vials containing larvae (from one to five) were removed from the liquid nitrogen containers for thawing at room temperature. The defrosted larvae were measured for standard length (SL) using the image analysis program IMAGEJ (National Institute of Health, Bethesda, MD, USA).

Larvae were then dry weighed (DW) in a precision balance with a precision of 1 μg. After dry weighing, larvae were re-hydrated with distilled water to facilitate otolith extraction. By means of a small fine scalpel, a cut on the dorsal side of the larva's head was performed to allow the extraction of the otoliths with fine needles. This procedure was done on a slide so that otoliths were moved away from any larval remains. All the extracted otoliths were cleaned with a drop of distilled water and, once dry, fixed onto the slide with nail lacquer. The sagittae could only be differentiated from the lapillus by light microscopy under 1000 × magnitude, due to their small difference in size in pre-flexion larvae, as observed by Wexler *et al.* (2001) in yellowfin tuna larvae (*Thunnus albacares*).

Daily increment formation in the Pacific BFT was validated by Foreman (1996). Age reading criteria is based on the work of Itoh *et al.* (2000) on laboratory-reared BFT larvae which was later applied to field-captured BFT from the Balearic Sea (García *et al.*, 2006).

All daily increment counts (DI) were done at 1000 × along the longest axis of the sagitta using the OTO program, designed by Andersen and Moksness (1988). The program runs on a Macintosh platform connected to an HEI digitizer and a high resolution camera that projects the otolith on a video monitor. This specific software allowed increment widths to be measured. To provide an estimate of recent larval growth, the average width of the last two increments was measured.

Microzooplankton analysis

Microzooplankton dry weight was determined after drying the samples to a constant weight at 60°C. Samples were weighed to the nearest 0.1 mg. Subsequently, to determine protein content, each sample was homogenized at 0°C in 1 mL of Tris-buffer (0.05 M Tris, 0.1 M NaCl, 0.01 M EDTA, adjusted to pH 8.0 with HCl) by means of ultrasonic pulses (2 × 10 s). Afterwards, 15 μL sodium dodecylsulphate SDS (0.7%) was added to the homogenate. The homogenate was centrifuged at 3800 g for 8 min, at 4°C. An aliquot of 500 μL from the supernatant was taken for protein analysis. Protein content was determined by the method of Lowry *et al.* (1951). The relative proportion of protein content in the total microzooplankton biomass was used as an index of relative quality of microzooplankton, as variations of biomass and biochemical constituents are influenced by the species composition of the sample (Rao and Krupanidhi, 2001; Rao and Kumari, 2002).

Microzooplankton dry weight (MDW) was standardized to mg m^{-3} and the relationship to PROT/MDW was calculated for each station.

Growth and environmental data analysis

Linear and power regressions were tested to model BFT larval growth, as well as to analyze the relative growth of body constituents. ANCOVA was used to test the difference between somatic and otolith growth or allometric relationships between the BFT larval cohorts by year, previously transforming data to natural logarithms to normalize the distribution.

Three-way ANOVA analyses with Tukey's Unequal N HSD test for post-hoc comparisons were carried out to determine how somatic growth expressed by dry weight growth rate (DWGR) and otolith growth rate (OGR) were affected by three environmental factors and their interactions: namely, temperature (T10), microzooplankton dry weight (MDW) and its protein/microzooplankton dry weight ratio (PROT/MDW).

Individual growth rates (DWGR and OGR) of BFT larvae were calculated by the derivative of the corresponding fitted power functions of each sampled year and corrected by their residuals. Environmental data (T10, MDW and PROT/MDW) were assigned to BFT larvae collected at a specific station. Two categorical levels (H for high and L for low) for each of the three environmental factors were considered: surface temperatures above and below 26°C corresponded to High and Low categories, respectively. The High and Low categories of MDW and the PROT/MDW ratio were established at values above and below 1 mg m^{-3} for former and at 0.20 for the latter variable.

Prior to testing the relationship between growth and environmental conditions, age distributions of each cohort were analyzed to find a common age range in which no significant differences were observed using a one-way ANOVA test. Ages ranging from five to 15 daily increments in the 2003 and 2005 BFT larval cohorts proved adequate for testing the influence of environmental conditions on the growth of field-captured BFT specimens.

RESULTS

2003–2005 BFT larval cohorts and their environmental scenario

Basic statistics of the BFT larval tuna used for the BFT larval growth analysis are shown in Table 2. Overall, size frequency distributions of each BFT larval cohort were fairly similar, with length classes of 2.8–8.5 mm (Fig. 2) comprising ontogenic stages from pre-flexion to post-flexion developmental stages. However, the main biological measurements of each annual BFT larval cohort, such as standard length (SL), dry weight (DW), otolith radius (OR) and their estimated age expressed by daily increments counts (DI), showed significant differences (ANOVA, $P < 0.001$). The 2004 BFT larvae were significantly larger in length (SL), whereas the 2003 and 2004 BFT larvae recorded greater larval dry weight (DW). Furthermore, otolith radius (OR) and the corresponding estimated age (DI) were significantly greater in the 2004 BFT larvae. The lowest DI were observed in the 2003 larval cohort in comparison with the 2004–2005 larval cohorts.

The number of BFT larvae used for this study was a function of the amount of larvae caught in each of the larval BFT positive stations. Therefore, the spatial larval distribution of the larvae used for this study corresponds roughly with the detailed spatial distribution of BFT larvae (Alemany *et al.*, 2010). Figures 3, 4 and 5 depict the locations where BFT larvae were sampled during the 2003, 2004 and 2005 yearly BFT larval surveys, together with the spatial representation of surface temperature at 10 m depth (shaded contours) and the superimposed salinity isoline of 37 (signature of MAW), 37.5 (midpoint of transition waters) and 38 (signature of SMW).

In all the yearly larval surveys, BFT larvae were mostly located in the transitional waters of Atlantic

Figure. 2. 2003–2005 BFT larval size frequency distributions analyzed for comparative daily growth study.
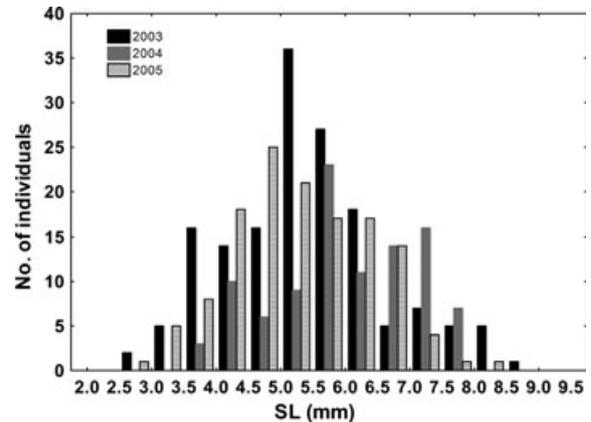
and Mediterranean waters (Alemany *et al.*, 2010). During 2003, BFT larvae were collected in MAW off south Ibiza island and in mixed waters south of the Mallorca channel (Fig. 3). In 2004, BFT larvae were mainly found concentrated in an area over the edge of an anticlonic gyre south of Menorca (Fig. 4), whereas in 2005, BFT larvae were more dispersed over the southern part of Ibiza and Mallorca (Fig. 5). The warmest surface temperatures occurred during the 2003 BFT spawning season and the coolest surface temperatures in 2004.

The principal environmental variables that can affect larval growth, such as temperature (T10), food availability (measured by the relative amount of microzooplankton dry weight, MDW) and the PROT/MDW ratio, showed strong inter-annual differences at stations in which BFT larvae were collected (Table 3, Fig. 6a). All these environmental variables showed significant between-year differences (ANOVA, $P < 0.001$).

Table 2. Basic statistics of 2003–2005 BFT larval cohorts used for otolith microstructure analysis.

Surveys	Variables	Mean	SD	Min	Max	N
2003	Standard length	5.42	1.22	2.83	8.66	157
	Dry weight	0.593	0.566	0.024	2.868	148
	Otolith radius	25.75	13.31	9.9	80.9	157
	Daily increments	7.37	3.26	2	19	157
2004	Standard length	6.03	1.08	3.71	7.83	99
	Dry weight	0.539	0.372	0.054	1.516	99
	Otolith radius	28.40	9.73	13.165	52.08	99
	Daily increments	10.08	2.11	2	19	99
2005	Standard length	5.29	1.07	3	8.34	132
	Dry weight	0.300	0.264	0.017	1.712	132
	Otolith radius	21.70	7.30	11.5	54.5	132
	Daily increments	8.41	1.91	2	19	132

Figure. 3. The 2003 TUNIBAL sampling grid contoured with the distribution of 10 m surface temperature and isohalines of salinity defining Atlantic waters (37.00), transition waters (37.50) and Mediterranean waters (38.00).

Figure. 5. The 2005 TUNIBAL sampling grid contoured with the distribution of 10 m surface temperature and isohalines of salinity defining Atlantic waters (37.00), transition waters (37.50) and Mediterranean waters (38.00).
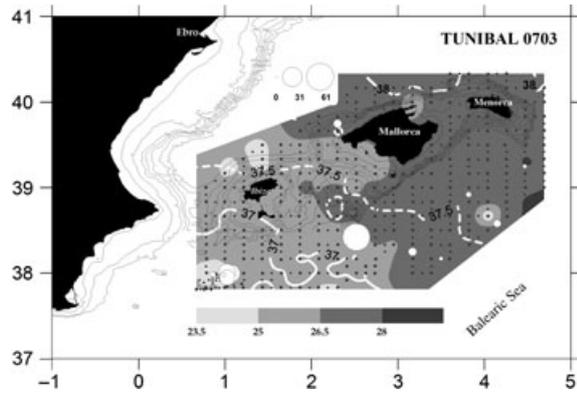
Figure. 4. The 2004 TUNIBAL sampling grid contoured with the distribution of 10 m surface temperature and isohalines of salinity defining Atlantic waters (37.00), transition waters (37.50) and Mediterranean waters (38.00).
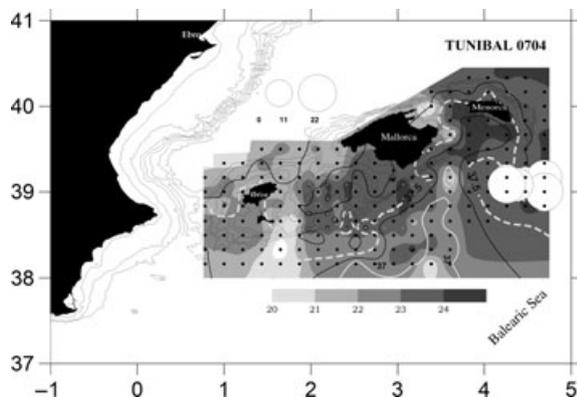
Highly contrasting environmental scenarios were observed between the exceptionally warm 2003 BFT spawning season and that of 2004–2005 (Fig. 6a). BFT larvae were subject to the highest surface temperature regime (T10) during 2003, and inversely the lowest MDW biomass. However, the PROT/MDW ratio was the highest in this period. Conversely, the 2004 larvae experienced the coldest surface temperatures and the highest MDW biomass. The 2005 BFT spawning season showed an intermediate environmental situation.

Inter-annual BFT larval growth variability

Bluefin tuna larval length (SL) at age (DI) at early life stages follow linear relationships (DI) (García *et al.*, 2006). An overall growth potential of each annual

BFT cohort is shown by the results the ANCOVA relationship of SL and DI with DI, where DI is used as covariate (Fig. 6b). The SL versus DI relationship of the 2003 BFT larvae show significant greater larval length at age in comparison with the 2004–2005 larval populations (ANCOVA, $F_{2,\ 384} = 45.7$; $P < 0.001$). On the other hand, the 2004–2005 BFT larval cohorts show identical fits (Fig. 7) showing no statistical differences between them ($P > 0.05$).

A more accurate representation of larval growth in BFT larvae is obtained when examining their growth increment in terms of somatic mass because larvae show a greater tendency during their first stages of development to increase body mass with age rather than body length. Therefore, BFT larval daily growth is best expressed by power functions modelling weight increment with age. In this respect, the 2003 BFT larval cohort showed a significantly greater exponential increase of DW with DI in comparison with the 2004–2005 BFT larvae (ANCOVA, $F_{2,\ 369} = 161.7$; $P < 0.001$) (Fig. 8), which did not show any significant differences between their DW vs DI relationships.

Consistent with the observed greater growth potential of the 2003 larval cohort, their otoliths also grew significantly faster than the 2004–2005 BFT larval populations (Fig. 9) (ANCOVA, $F_{2,\ 384} = 134.5$; $P < 0.001$). No significant differences were observed between the 2004 and 2005 BFT larvae. The greater otolith growth potential of the 2003 BFT larval cohorts is likewise corroborated by significantly wider daily increments being deposited in the otoliths (Fig. 10a).

Table 3. Basic statistics of environmental variables, temperature at 10 m depth (T10), microzooplankton dry weight (M-DW) and protein/microzooplankton ratio (PROT/M-DW ratio).

Surveys	Variables	Mean	SD	Min	Max
2003	Temperature (10 m)	26.038	0.539	25.61	27.85
	MDW (mg m^{-3})	0.707	0.287	0.398	1.418
	PROT/MDW	0.326	0.101	0.134	0.276
2004	Temperature (10 m)	23.87	0.313	23.35	24.33
	MDW (mg m^{-3})	3.535	0.992	1.662	5.014
	PROT/MDW	0.184	0.020	0.145	1.085
2005	Temperature (10 m)	24.96	0.83	23.04	25.70
	MDW (mg m^{-3})	1.790	0.730	0.574	3.555
	PROT/MDW	0.199	0.029	0.125	0.919

Figure. 6. (a) ANOVA result test for temperature at 10 m depth ($F_{2,385}$ = 381.04 $P<0.01$), microzooplankton DW ($F_{2,385}$ = 522.23, $P < 0.01$), and the PROT/DW ratio ($F_{2,385}$ = 183.04, $P < 0.01$), undertaken in positive BFT hauls; (b) Results of ANCOVA of SL vs DI DW vs DI using DI as covariate. Significant differences among all cohorts is DW vs DI ($P < 0.01$) and between 2003 and 2004–2005 in the SL vs DI.
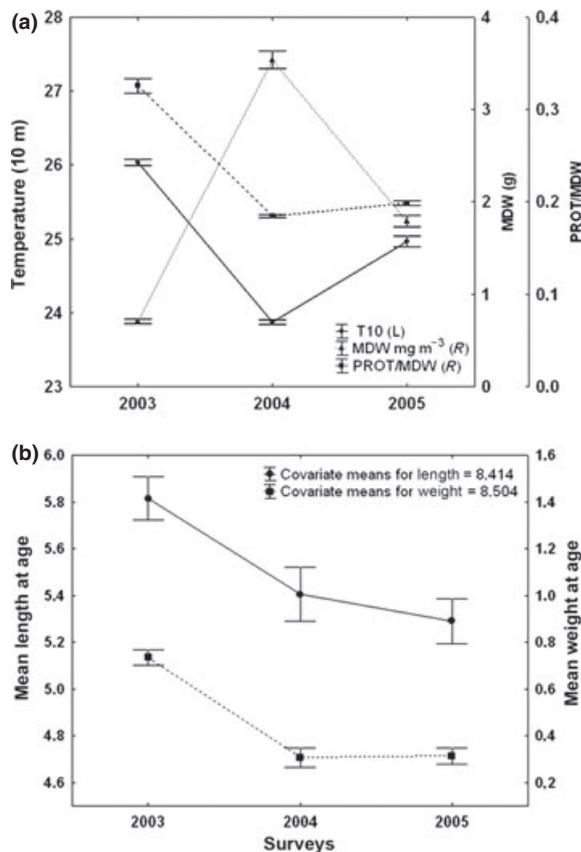
Figure. 7. BFT larval standard length (SL) vs daily increments (DI) relationships of the 2003-2005 BFT larval cohorts.
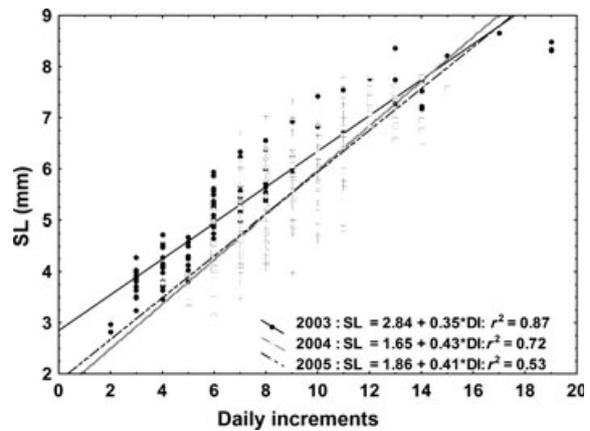
Figure. 8. BFT larval dry weight (DW) vs daily increments (DI) relationships of the 2003–2005 BFT larval cohorts.
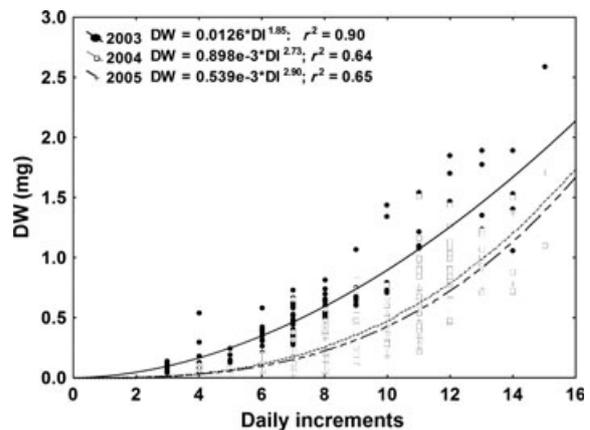
These somatic growth differences between the BFT larval cohorts led to similar differences in the relative growth of SL and DW with OR (Table 4). For a given OR, the 2003 BFT larvae showed significantly smaller SL than the 2004–2005 larvae (ANCOVA, $F_{2, 384}$ = 12.6; $P < 0.001$). The paired comparison

Figure. 9. BFT larval otolith radius (OR) vs daily increments (DI) relationships of the 2003–2005 BFT larval cohorts.

Figure. 10. (a) Mean increment width vs daily increments (DI) of the 2003–2005 BFT larval cohorts; (b) Recent growth as measured by the mean of the last two increment widths of the 2003–2005 BFT larval cohorts. Vertical bars represent standard deviations.
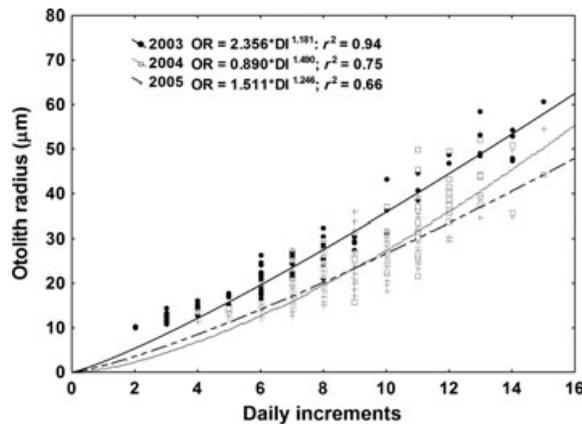
between the latter BFT larval cohorts in consequence did not show any significant differences between them ($P > 0.05$). Inversely, for a given OR, significantly greater DW was observed in the 2003 BFT larvae (ANCOVA, $F_{2,\,369} = 45.2$; $P < 0.001$), whereas no significant differences were observed in the paired comparison between the 2004–2005 larval cohorts ($P > 0.05$).

Consequent with the growth differences observed between the 2003 and 2004–2005 BFT larval cohorts, the SL versus DW relationship between these populations showed significant differences (ANCOVA, $F_{2,\,369} = 80.9$; $P < 0.001$). Resulting from its enhanced growth, the 2003 larvae show greater DW for a given SL (Fig. 11).

Analysis of environmental influence on BFT larval growth

The statistical analysis of the environmental influence on BFT larval growth requires the use of an independent variable for inter-annual comparisons between the specific environmental conditions that affected each BFT larval cohort. Taking into account that variables related to somatic and otolith measurements are age-dependent, age (DI) was selected as an independent variable. Thus, similar age distributions between cohorts were used to analyze the effect of environmental conditions on BFT larval growth. The age

distributions found between 5 and 15 DI counts of the 2003 and 2005 BFT larval cohorts did not show significant differences between them (ANOVA, $P > 0.05$) and therefore these were used for statistical analysis.

The most distinguishable growth differences between the BFT cohorts originate from the somatic growth differences expressed as gain in weight with age (DW versus DI) and from the otolith growth models (OR versus DI) (Figs 8 and 9). Estimates of individual dry weight growth rate (DWGR) of both BFT larval cohorts, expressed as DW (mg d)$^{-1}$, showed

Table 4. Somatic variables (SL and DW) relationship to otolith size (OR).

Survey	SL versus OR	R^2	DW versus OR	R^2
2003	SL = 1.3147* OR$^{0.4447}$	0.89	DW = 0.00445*OR$^{1.4847}$	0.92
2004	SL = 1.2087* OR$^{0.4848}$	0.90	DW = 0.972e-3*OR$^{1.8645}$	0.90
2005	SL = 0. 9419* OR$^{0.5649}$	0.84	DW = 0.532e-3*OR$^{2.0303}$	0.91

Figure. 11. BFT larval dry weight (DW) vs standard length (SL) relationships of the 2003–2005 BFT larval cohorts.
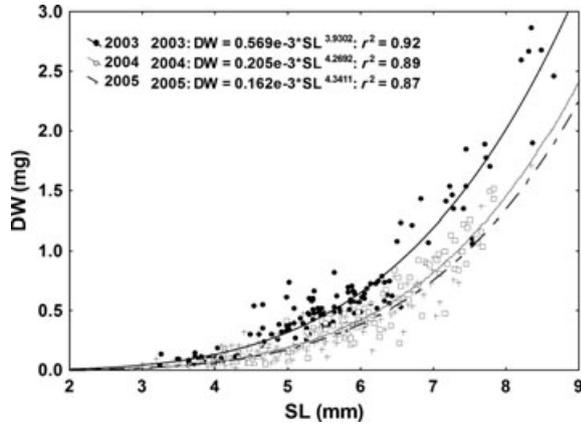
Figure. 12. Linear relationship of individual BFT larval somatic mass (DWGR) and otolith growth rates (OGR) with temperature at 10m depth (T10) of 2003 and 2005 BFT cohorts.

Figure. 13. Linear relationship of individual BFT larval somatic mass (DWGR) and otolith growth rates (OGR) with microzooplankton PROT/DW ratio of 2003 and 2005 BFT cohorts.
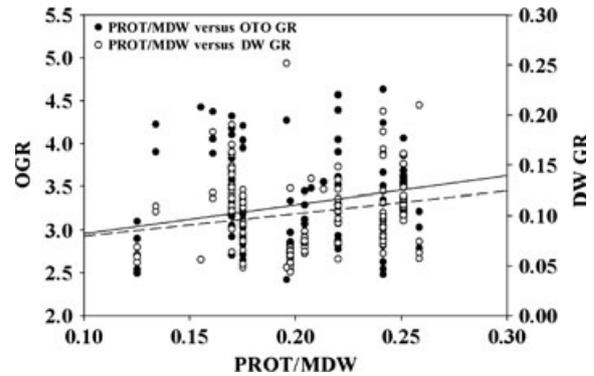
Figure. 14. Linear relationship of individual BFT larval somatic mass (DWGR) and otolith growth rates (OGR) with microzooplankton biomass (MDW) of 2003 and 2005 BFT cohorts.

significant positive linear relationships with T10 ($r^2 = 0.11$; $P < 0.001$) (Fig. 12). Similarly, individual otolith growth rates (OGR) showed significant positive linear relationships with T10 ($r^2 = 0.22$; $P < 0.001$) and the PROT/MDW ratio ($r^2 = 0.36$; $P < 0.001$) (Fig. 13). The relationship of these individual growth rates with MDW was likewise significant but negative (Fig. 14). DWGR was negatively correlated with MDW ($r^2 = 0.20$; $P < 0.001$), as was OGR ($r^2 = 0.30$; $P < 0.001$). Thus, higher DWGR and OGR were observed in the lower range of MDW values.

The combined influence of these environmental variables – T10, PROT/MDW and MDW – on somatic and otolith growth rates (DWGR and OGR, respectively) used as proxies for growth potential was analyzed by means of multifactorial ANOVA. Results of OGR did not provide evidence for a three-way

interaction among the factors (T10*MDW*PROT/MDW). The only significant interactions ($P < 0.01$) were T10*PROT/MDW and T10*MDW, leading us to infer that the T10 effect on OGR is not the same at low and high MDW levels or at low and high PROT/MDW levels: although OGR is greater at high temperatures, this effect is only significant when PROT/MDW is low (Fig. 15). When T10 conditions are high, the levels of MDW do not significantly affect OGR (Fig. 16). Alternatively, MDW levels show a significant effect when T10 levels are low. At high temperatures, OGR were significantly greater when the PROT/MDW levels were low.

Results for somatic growth rates (DWGR) showed significant effects ($P < 0.01$) for the higher-level interaction T10*MDW*PROT/MDW, meaning that the two-way interactions (MDW*PROT/MDW) were different for the two levels of the temperature variable

Figure. 15. Multi-factorial ANOVA of otolith growth rate (OGR) and the interaction of T10 and PROT/MDW ($F_{1,242}$ = 24.6, $P < 0.001$) of 2003 and 2005 BFT cohorts.
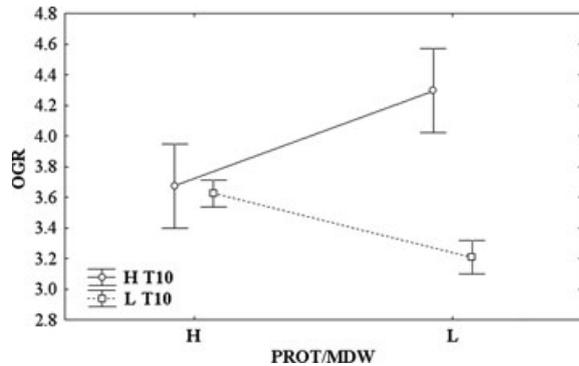
Figure. 16. Multi-factorial ANOVA of otolith growth rate (OGR) and the interaction of T10 and MDW ($F_{1,242}$ = 6.92, $P < 0.001$) of 2003 and 2005 BFT cohorts.
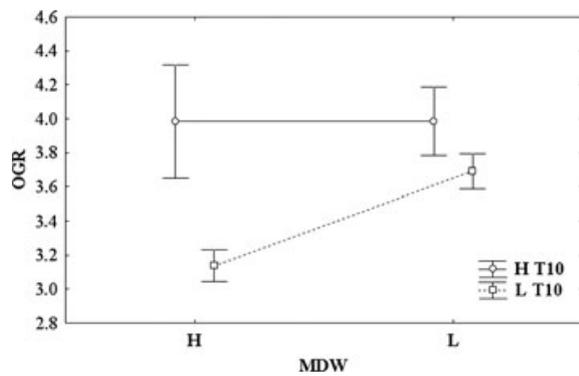
(T10) (Fig. 17). When T10 were at low levels, the two main effects (MDW and PROT/MDW) are significant but there is no interaction between the factors. DWGR is always greater when PROT/MDW levels are high, regardless of the MDW level.

However, when T10 is high, neither main effect is significant but there is an interaction showing that the effect of PROT/MDW depends on the MDW level and vice versa. With low PROT/MDW level, DWGR is greater when MDW levels are at high levels, and lower when MDW is at low levels.

DISCUSSION

Among the three BFT larval growth data sets, the 2003 BFT larvae showed greater SL and DW at age than the 2004–2005 larvae, whereas no significant growth differences were observed between the 2004 and 2005 cohorts. The most obvious growth differences were displayed in the DW versus age relationship; at day 10 the

Figure 17. Multi-factorial ANOVA of somatic growth rate (DWGR) and the interaction of T10, MDW and PROT/MDW ($F_{1,242}$ = 8.7, $P<0.001$) of 2003 and 2005 BFT cohorts.
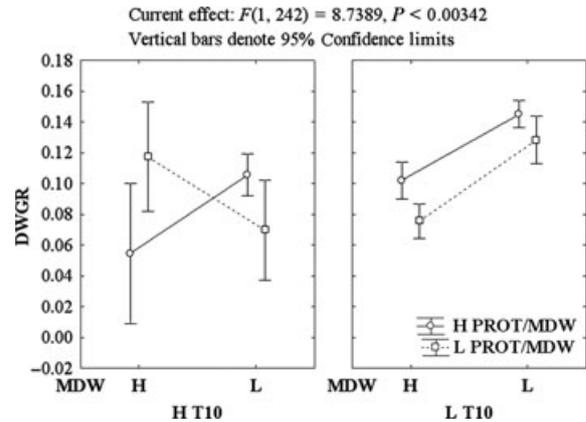
somatic mass (DW) of 2003 cohort was double that of the 2005 cohort (0.896 and 0.427 mg, respectively). This difference in somatic mass is consistent with the results of biochemical analysis of RNA, DNA and protein content of BFT larvae, in which the 2003 larvae showed a significantly higher content with SL of these biochemical constituents (Cortés et al., 2007), indicating a greater capacity for protein synthesis.

Moreover, the SL versus DI relationship indicates that the 2003 larval cohort may have had a larger larval length at hatch. Although the overall population growth rate of the 2003 is apparently lower (0.35 mm day^{-1}) than the 2004–2005 larvae, it is caused by a largely overestimated intercept of the estimated length at hatch (2.84 mm) with respect to the 2004–2005 BFT larval populations (1.65 and 1.86 mm, respectively). However, when considering a common larval length-at-hatch of 1.8 mm, the 2003 BFT larvae showed a higher population growth rate (0.48 mm day^{-1}) than the 2004–2005 BFT larval populations. Nonetheless, the BFT larval morphology, which tends to grow by somatic mass increase with age, demonstrates the faster growth of the 2003 cohort as shown in the comparative DW versus DI relationships (Fig. 8). Supporting evidence for their greater growth potential is based on the significantly higher 2003 RNA/PROT ratio, an index of synthesis efficiency (Cortés et al., 2007) at the early stages of larval development.

Moreover, a greater accretion rate in the early stages of increment deposition (Fig. 10a) occurs in the 2003 BFT larvae, as made evident by their greater recent growth at early ontogenic stages (Fig. 10b). Increment deposition is highly influenced by ambient

temperatures in a variety of fish species (Bradford and Geen, 1992; Otterlei *et al.*, 1999) and the 2003 BFT spawning season was characterized by a prolonged heat wave, particularly important in the Western Mediterranean (Stott *et al.*, 2004; Sparnocchia *et al.*, 2006). This climatic anomaly was responsible for a 2.7°C increase at surface layers averaged over the five consecutive TUNIBAL surveys from 2001 to 2005 (Alemany *et al.*, 2010). Considering that, during the breeding season, BFT mainly occupy surface layers (Teo *et al.*, 2007a,b) the warming event may have also affected spawners.

The effects of the 2003 heat wave not only caused an increase of sea surface temperature, air temperature, a decrease of wind stress and a reduction of upward heat flux in the NW Mediterranean, but also affected the circulation pattern and intensity of the Atlantic Ionian Stream (AIS) and the Atlantic Tunisian Current (ATC) in the Central Mediterranean (Olita *et al.*, 2007). From the perspective of the Northwestern Mediterranean ecosystem, the 2003 heat wave anomaly had an effect on rocky benthic macroinvertebrate species (mainly gorgonians and sponges) (Garrabou *et al.*, 2009) as well as causing an anomalous outburst of mucilage growth, affecting the benthic communities of the Northwestern Mediterranean (Schiaparelli *et al.*, 2007).

In terms of the Balearic Seas' ecosystem, the 2003 heat wave affected planktonic production, as evidenced by the significantly low MDW (Fig. 6a). The 2003 climatic warming anomaly also corresponds with the lowest chlorophyll *a* concentration observed by SeaWIFS satellite (http://reason.gsfc.nasa.gov/Giovanni/) during the 2003–2005 BFT spawning seasons (June–July), which would explain the lowest MDW values observed during 2003, oligotrophic conditions being further enhanced by the great intrusion of nutrient-poor Atlantic waters south of Ibiza and the Mallorca channel (García *et al.*, 2003).

In consequence, such low planktonic production in the area may be held responsible for the low availability of larval feeding resources. According to Catalan *et al.* (2011), the early life stages of tuna mainly feed on copepods, and at lengths of 5–6 mm, BFT larvae tend to feed on cladocerans. Nevertheless, such low food availability may have had an effect on the advanced stages of post-flexion BFT larvae, where the species may switch to a more piscivorous feeding mode (Reglero *et al.*, 2011). At 11–13 DI, all three BFT cohorts showed a decline in the width of the last increments. But in 2003, the decrease of increment widths of BFT larvae is the most abrupt. Taking into account the high temperatures, which could influence the BFT

metabolic rate, the average width of the last two increments was considered a good indicator of recent growth. In the last ontogenic stages, recent growth of the 2003 BFT larval cohort decreased to the values observed in the 2004–2005 BFT larvae, a possible sign of starvation. The decrease of increment widths of the latter cohorts was less prominent (Fig. 10b) than the 2003 cohort. The decline of recent growth of the 2003 BFT larvae suggests that limited feeding conditions may be the result of the impoverished MDW biomass conditions of 2003, which could have led to decreased growth rates at the most advanced developmental stages.

Under normal climatic circumstances, BFT larvae showed a preference for certain sea surface temperature and salinity ranges (Mather *et al.*, 1995; García *et al.*, 2003; Teo *et al.*, 2007a,b; Alemany *et al.*, 2010), BFT larvae tending to occur in the range of 24–25°C. But during the 2003 BFT spawning season, BFT also showed a significant preference for higher temperatures (25–26°C) as indicated by single parameter quotient analysis (van der Lingen *et al.*, 2001; Alemany *et al.*, 2010). This surface temperature increase makes it comparable to the spawning habitat of the Pacific BFT (*Thunnus thynnus orientalis*) reported by Tanaka *et al.* (2006), where, it is worth noting, larval length at hatch is on average 2.83 mm (Miyashita *et al.*, 2001). Moreover, Miyashita *et al.* (2000) found in experimental studies that Pacific BFT could attain better larval development at higher temperatures.

From the early life history perspective of BFT larval ecology, it can be concluded that larval growth variability is influenced by environmental factors of different nature that interact in various ways. From the climatic point of view, temperature can have a great influence by accelerating metabolism at early life stages (Buckley, 1984). Its influence is manifest in faster otolith growth (Fig. 9) and the deposition of wider increments widths (Fig. 10), considered reliable predictors of somatic growth (Dickey and Isely, 1997; Reichert *et al.*, 2000). The somatic growth rates (DWGR) and the otolith growth rates (OGR) have shown significant linear relationships with T10 (see Fig. 12). However, the ways in which T10 interacts with larval growth may vary with other environmental variables related to food availability and food quality.

The BFT spawning habitat in the Balearic Sea is considered nutrient-deficient, and particularly so during summer and in waters of Atlantic origin (Fernández de Puelles *et al.*, 2007). Within this nutrient-deficient scenario, the mixing of the two water masses causes intense hydrographic circulation, producing important mesoscale features such as fronts and

gyres (Vélez-Belchí and Tintoré, 2001) that can drive the nutrient supply into the upper photic layer, leading to a remarkable spatial and temporal heterogeneity of the planktonic community (Fernández de Puelles, 1996; Alemany et al., 2006).

As regards BFT larval survival, the availability and quality of feeding resources can have a decisive role. The three-factor ANOVA of T10, PROT/MDW and MDW on OGR did not show evidence of a three-way interaction with these environmental variables. However, the interaction of T10*PROT/MDW and T10*MDW was significant, indicating an increase of OGR at high levels of temperature when PROT/MDW values are low. Moreover, when T10 conditions were at high levels, MDW did not significantly affect OGR. Such ambient conditions were met by the 2003 BFT larvae (Figs 15 and 16).

With respect to the DWGR, the three-way interaction (T10*MDW*PROT/MDW) showed significant effects, such that the two-way interactions (MDW*PROT/MDW) showed differences for the two levels of T10 (Fig. 17). At low T10, the between-effects were significant but there was no interaction between factors. However, at high T10, although the main effect was not significant, the effect of PROT/MDW showed a dependence on the MDW level, such that DWGR was greater when PROT/MDW levels were low, matching the environmental scenario experienced by the 2003 BFT larvae.

Thus, we can infer that although it is accepted that feeding availability as well as ambient temperature exerts a great influence on early larval growth and condition enhancement (Buckley, 1984; Ferron and Leggett, 1994; Malzahn et al., 2003; Hardy and Litvak, 2004), another factor that cannot be overlooked is food quality, expressed by the ratio PROT/DW. This assertion seems logical bearing in mind that BFT larvae showed a clear preference for the clear and nutrient-poor waters of Atlantic origin in which most plankton in those waters would correspond to microzooplankton (Alemany et al., 2010) where small calanoid copepods can be an important source of food for BFT larvae (Catalan et al., 2011). Such a hypothesis may be supported by the fact that 2003 recorded the lowest abundance of chlorophyll a and microzooplankton. Nevertheless, we cannot overlook that low prey density may also relate to the preference of BFT spawners for water clarity (Teo et al., 2007a,b) in which predator–prey encounters may be decreased and where predator avoidance by BFT larvae may be strategically favourable (Bakun and Broad, 2003; Bakun, 2006).

Density-dependence factors must be considered in the context of BFT larval survival. The Balearic Sea embraces the spawning habitat of various tuna or tuna-like species in which the most abundant are the bullet tuna (Auxis rochei) and albacore (Thunnus albacares) (Alemany et al., 2006, 2010). These species, among other top predator species, are concurrent spawners, and thus their offspring may affect BFT larval survival via density-dependence factors. The fact that BFT spawning is carried out preferentially in waters of Atlantic origin (Alemany et al., 2010) reduces the spatial overlap of BFT larvae with other co-occurring species. Whereas bullet tuna larvae tend to have more coastal distributions, albacore larvae are most abundant in August during the species' peak spawning season. Furthermore, bullet tuna larvae have shown a preference for the shallower waters of the Balearic Sea compared with BFT larvae (Alemany et al., 2010). In conclusion, as BFT shows a spawning preference for the warmer temperature regime of waters of Atlantic origin, it is normal that larval growth rates show a relationship with temperature. But in conjunction with the temperature regime, the Atlantic waters are characterized by nutrient deficiency and, thereby, low planktonic production. These water mass properties may also contribute to the differences observed in the potential feeding qualities of the waters as measured by the relative planktonic composition.

Lastly, among the possible consequences of having fast-growing larvae in a cohort are the higher survival rates that these larvae may have at later stages (Houde, 1987; Anderson, 1988; Hare and Cowen, 1997). Tanaka et al. (2006) found that back-calculated standard lengths of post-flexion Pacific BFT (T. orientalis) larvae were larger-at-age than pre-flexion and flexion larvae, thereby inferring that faster-growing larvae were able to survive to the post-flexion stage and to recruitment stages. Similarly, early larval growth variability of yellowfin (T. albacares) was assumed to be among one of the main causes of recruitment fluctuation (Wexler et al., 2007). Although mortality rates are stage-specific, with species that undergo high growth rates as tunas, the probability of mortality decreases as their length increases (Hare and Cowen, 1997). With respect to the eastern BFT population, their recruitment to the fishery occurs after 3 yr. ICCAT data on BFT recruitment estimated from the Gulf of Biscay fishery show that the 2003 year-class has shown the greatest recruitment since 1997. From this perspective, it may be considered useful to verify the growth differences between the past and future recruitment classes.

In the actual context of global change, although it may seem that a climatic warming anomaly may have

produced advantages for a depleted eastern BFT population, this analysis should be made from an integrated ecosystem perspective. The 2003 heat wave has been reported to have affected benthic communities in the Northwestern Mediterranean ecosystem (Schiaparelli *et al.*, 2007; Garrabou *et al.*, 2009), with effects even on the phytoplankton of the English Channel, which recorded exceptionally high abundance peaks of some indicative dinoflagellate species (Gómez and Souissi, 2008). Furthermore, there are some recent cues indicating that climate-induced changes may have contributed to shifts in the small pelagic species ecosystem. The increase of round sardinella (*Sardinella aurita*) during the past decade, especially during 2003 and 2004, indicated a positive relationship with temperature anomalies (Sabatés *et al.*, 2006). Data from historical annual acoustic surveys carried out off the Spanish Mediterranean coast confirm changes in the small pelagic species complex (Giraldez A., unpublished data). Since the turn of the century, the small pelagic resources has increased its species diversity, with some recent intrusions of medium-sized pelagics such as chub mackerel (*Scomber japonicus*) and the increase of the three Mediterranean horse mackerel species (*Trachurus trachurus*, *Trachurus mediterraneus*, *Trachurus picturatus*), together with a significant declining trend of sardine (*Sardina pilchardus*).

It seems rather evident that the Mediterranean Sea is experiencing a steady increase of temperature and, due to its highly diverse climatic regimes, is likely to undergo more frequent extreme climatic anomalies (Lejeusne *et al.*, 2010). As shown in this study, these climatic anomalies may have effects not only on the Mediterranean biota but also on the physiological rates of keystone species of the pelagic domain, such as BFT.

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