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# Vertical distribution of Atlantic bluefin tuna *Thunnus thynnus* and bonito *Sarda sarda* larvae is related to temperature preference

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ABSTRACT: Temperature ranges are important in explaining the worldwide distribution of tuna and bonito larval habitats. Less is known about how the thermal tolerance of these species' larvae restricts their vertical distribution. Here we combined field and laboratory data to explore the role of temperature on the vertical distribution of Atlantic bluefin tuna *Thunnus thynnus* and Atlantic bonito *Sarda sarda* larvae. First, we related the vertical structure of several environmental variables to larval vertical distribution in a recognized tuna spawning area in the Mediterranean. The field data indicated temperature-dependent behavior both in bluefin tuna and albacore larvae, with a clear preference for the higher temperatures found in upper water layers in strong thermal-gradient environments. No Atlantic bonito larvae were caught in the field samples. Second, we confirmed such behavior under controlled conditions, observing fed larvae of bluefin tuna and bonito in experimental columns with temperature gradients similar to those experienced in the NW Mediterranean (22–25.6°C for bluefin, 18–23°C for bonito) and with no temperature gradients (24.4°C for bluefin, 23°C for bonito). The larvae were distributed significantly shallower in the stratified than in the isothermal experimental water columns in both light and dark conditions. These results suggest that the vertical distribution of tuna and bonito larvae is spatially constrained by larval temperature tolerance. In comparing our results to other geographical areas, we found that the vertical habitat of tuna larvae that spawn in regions with strong thermal gradients is smaller than in regions with weaker thermal gradients.

KEY WORDS: Thermocline · Vertical distribution · Temperature-dependent behaviour · Larval fish · Tuna · Bonito



## INTRODUCTION

Variability in water column properties may influence the growth, survival and dispersal of larval fish and thus their distribution and abundance. Larval fish can modify their behavior in response to their environment by moving into more favorable conditions that increase survival. Diel vertical migrations

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illustrate behavior that could balance trade-offs between feeding and predation risk within the water column (Fiksen et al. 2007).

The vertical position of the larvae also influences their horizontal advection from spawning to optimal nursery areas, since currents are vertically stratified in the water column (Leis 2006). Whatever the behavioral response to biological and physical conditions, the overall vertical distribution range will be limited by the organism's physiological tolerance to temperature imposed by either their maximum or minimum thermal constraints. When large changes in water temperatures occur over short vertical distances, as in strong thermoclines, the prevalence of an organism in a depth range may be related to its temperature preferences. This effect may be stronger in larval fish that are very sensitive to temperature variability, particularly in species whose early life stages have a relative narrow thermal tolerance range (Pörtner & Peck 2010, Vollset et al. 2013).

All climate change projections for the Mediterranean Sea indicate substantial warming and an increase in the frequency and intensity of heat waves (Lionello et al. 2012). This semi-enclosed sea offers an analogy to explore the resiliency of top predators in pelagic oceanic systems (Lejeusne et al. 2010). This type of study is of particular importance for the large pelagic top predatory species that migrate annually to their summer spawning grounds in the NW Mediterranean, such as tuna and bonito. These species share the common trait that their larvae inhabit only warm waters at sea temperatures above 20–22°C (Boyce et al. 2008, Reglero et al. 2014a, Muhling et al. 2017).

Thus, high temperatures seem to be a general characteristic of tuna and bonito larval habitats, although the role of temperature as an environmental cue for the vertical distribution of larvae in the water column and its importance in relation to other environmental variables is not yet clear. In the NW Mediterranean, where climate warming is expected to strongly increase the stratification of the water column (Coma et al. 2009), it is important to know how temperature stratification triggers larval distribution.

Bluefin tunas have the most spatially and temporally constrained larval habitats of all tuna species (Reglero et al. 2014a). Atlantic bluefin tuna *Thunnus thynnus*, 1 of the 3 bluefin tuna species (the other 2 being Pacific bluefin tuna *T. orientalis* and southern bluefin tuna *T. maccoyii*), reproduces in the NW Mediterranean Sea. Albacore *T. alalunga* and bullet tuna *Auxis rochei* larvae co-occur with Atlantic bluefin tuna larvae in the NW Mediterranean (Alemany et al. 2010, Reglero et al. 2012). Aside from these species, larvae of the small tropical tunas little tunny *Euthynnus alleteratus* and skipjack tuna *Katsuwonus pelamis* are also caught in this area (Alemany et al. 2010, Torres et al. 2011). These species usually have a wider spawning area and a more extended spawning period than bluefin tunas (Reglero et al. 2014a) and can be found both in tropical areas, where water temperatures show only a slight temperature gradient over the upper 100 m, and in temperate areas, where water temperatures show a strong vertical gradient (Llopiz & Hobday 2015). Atlantic bonito *Sarda sarda* share the Atlantic bluefin tuna spawning grounds in the NW Mediterranean, where water temperatures are above 18°C (Sabatés & Recasens 2001, Torres et al. 2011). Since most tuna species, including Atlantic bluefin tuna and Atlantic bonito, are already strongly piscivorous during their larval stage and can prey on each other (Reglero et al. 2014b, Llopiz & Hobday 2015), it is important to understand the mechanisms that may increase spatial overlap in the water column among these species.

The thermal preferences of the larvae of the 3 bluefin tuna species have been relatively well studied. When reared in captivity, temperatures above 19–20°C are necessary for the larvae to hatch, and larvae have been successfully reared at temperatures above 22°C (Miyashita et al. 2000, Tanaka et al. 2008, Woolley et al. 2009, Wexler et al. 2011, Gordoa & Carreras 2014, Reglero et al. 2014b), suggesting a minimum thermal constraint at 19°C. Although it is generally accepted that bluefin tuna larvae are associated with warm temperatures, it is difficult to disentangle the effect of temperature on the vertical distribution of these species. Southern bluefin tuna larvae in the Indian and Pacific Oceans, and Atlantic bluefin tuna larvae in the Gulf of Mexico, though most abundant in the first 20 m, have been caught down to depths of 50–60 m, a depth range where temperatures are above 20°C during the spawning season (Davis et al. 1990, Boehlert & Mundy 1994, Habtes et al. 2014). More restricted vertical distributions, down to only 20 m, have been described for Pacific bluefin tuna, a pattern that has been related to variables other than temperature, in particular to the existence of pycnoclines (Satoh 2010). Atlantic bluefin tuna, Atlantic bonito and striped bonito *S. orientalis* have been successfully reared in captivity at temperatures above 19°C (McFarlane et al. 2000, Ortega & Mourente 2010, Reglero et al. 2014b, Ortega 2015, Blanco et al. 2017). There are no descriptions of the vertical distribution of these and companion species of Atlantic bluefin tuna in the NW Mediterranean.

The vertical distribution of bullet tuna has been described as restricted to the top 20 m of the water column, although temperature profiles were not analyzed (Morote et al. 2008). In other areas, skipjack tuna larvae have been caught down to 50 m (Matsumoto 1958, Davis et al. 1990, Habtes et al. 2014) and even at 80 m depth (Boehlert & Mundy 1994, Llopiz et al. 2010), while albacore larvae have been caught down to 40 m (Davis et al. 1990, Habtes et al. 2014) and little tunny down to 100 m depth (Llopiz et al. 2010, Habtes et al. 2014). These different distribution patterns among bluefin and other tuna species indicate the need for studies at the local scale to ensure that vertical distributions are well described.

Analyzing the effect of vertical temperature gradients on vertical larval distribution from field studies alone can be complex due to the many confounding variables besides temperature, such as light absorption, the existence of pycnoclines or the occurrence of prey items. Observing the behavior of larvae in relation to experimental vertical temperature gradients can help to understand the response to temperature gradients in the field (Vollset et al. 2009). The comparison between experimental and field studies will improve our understanding of the larval response of tuna and bonito to temperature gradients. We expect the vertical distribution of larvae of tuna and bonito species, particularly Atlantic bluefin tuna and Atlantic bonito larvae, for which a minimum thermal constraint has been identified in the laboratory, to be limited by their thermal tolerance in the sharp thermal gradients observed in the NW Mediterranean. We hypothesized that the vertical distribution of Atlantic bluefin tuna larvae in the NW Mediterranean, where the thermal structure of the water column during the summer, coinciding with the spawning season, presents sharp vertical gradients (Torres et al. 2014), may be a response to bluefin tuna larvae thermal preferences. Therefore, the depth distribution range for bluefin tuna larvae is dependent on the window of physiologically favorable temperatures.

Our aim in this study was to evaluate whether temperature may be a dominant cue influencing the vertical distribution of tuna and bonito larvae in temperate areas using a combination of field and laboratory experiments. Field data on the vertical distribution of scombrid larvae were obtained from 2 surveys conducted in the Balearic Sea (NW Mediterranean) during summer 2011 and 2012 and then correlated with temperature, salinity and chlorophyll measurements. The behavior of Atlantic bluefin tuna and Atlantic bonito larvae in response to vertical environmental temperature gradients similar to those observed in nature was investigated through the use of experimental thermoclines. Knowledge of the ecology of the larvae of Atlantic bluefin tuna and other co-occuring scombrids, has improved our understanding of the environmental requirements for Atlantic bluefin tuna spawning grounds and stock management (Ingram et al. 2017, Muhling et al. 2017). Improving our understanding of the mechanisms that explain the role of temperature in driving the vertical distribution of large pelagic fish is necessary to assess these animals' vulnerability to climate warming and enhanced stratification and to improve their conservation and management (Horodysky et al. 2016).

## MATERIALS AND METHODS

### Field surveys

Two multidisciplinary research surveys were conducted off the Balearic Islands, western Mediterranean Sea: one in 2011, onboard the fishing vessel 'Tio Gel II' (Grup Balfegó SL), and the other in 2012, onboard the RV 'Ramon Margalef.' Within these cruises, a series of ichthyoplankton samplings were carried out by means of oblique tows using a bongo net of 90 cm mouth diameter with a mesh size of 500 µm, performed over a systematic 10 × 10 nautical mile grid, to localize patches with high densities of tuna larvae (see Alemany et al. 2010 for a detailed description of the systematic grid). Once a high-density bluefin tuna larval patch was identified, which in both years occurred off the Cabrera archipelago, its position was tracked by a Lagrangian iridium buoy fixed in the mixed layer with a sock drogue deployed between 8 and 15 m depth. While following the tuna larval patches, we conducted 14 stratified vertical sampling tows between 20 and 22 June 2011 and 9 tows between 9 and 11 July 2012 (Fig. 1). In 2011, we used a HYDRO-BIOS multi-net, sampling 5 depth strata (0–5, 5–10, 10–15, 15–20, 20–30 m), whereas in 2012 we used a Multiple Opening Closing Net and Environmental Sensing System (MOCNESS), sampling 6 depth strata (0–10, 10–20, 20–30, 30–40, 40–50, 50–60 m). The net mouth openings were 0.25 and 1 $m^2$, respectively, and the mesh size was 333 µm for both nets. Both devices were repeatedly towed at ~2 knots during day and night. All samples were preserved immediately after collection in ~4% borax-buffered formaldehyde, prepared using seawater. Both in 2011 and 2012, vertical profiles of temperature, salinity and fluorescence were determined

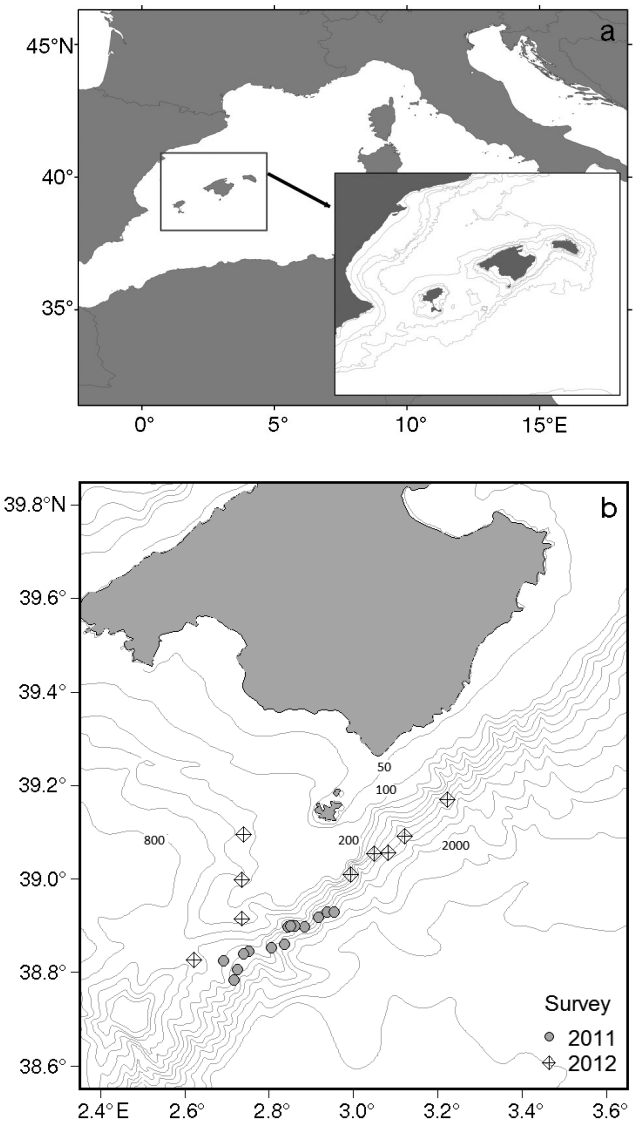


Fig. 1. (a) Study region off the Balearic Islands in the NW Mediterranean Sea, a primary spawning area for tuna species. (b) Stratified vertical sampling locations with tuna larvae sampled in 2011 (14 operations, grey circles) and 2012 (9 operations, crossed rhomboids). Light grey lines represent the bathymetric lines with depth (m). Only those stations where vertical resolution sampling was conducted are shown

using a CTD (SBE 911). Once in the laboratory, plankton samples were sorted for fish larvae, which were individually identified to species level (Alemany 1997). Tuna species were identified, photographed and their standard length (SL, mm) measured using an image analysis system equipped with Image-Pro Plus 6.2 software (Media Cybernetics). Lengths of the preserved larvae were corrected for shrinkage using the algorithm developed for *Thunnus thynnus* by Reglero et al. (2013). The number of larvae caught in each depth range was standardized to number of larvae 100 $m^{-3}$.

### Larval rearing

Rearing of Atlantic bonito and Atlantic bluefin tuna was conducted in the summers of 2013 and 2014, respectively. These are the only bonito and tuna species reproducing in the Mediterranean Sea for which laboratory culture methods have been developed (Reglero et al. 2014b, Ortega 2015). Bonito eggs were obtained from captive broodstocks at the Spanish Institute of Oceanography (IEO) rearing facilities at Mazarrón (Spain), where all experiments were conducted. Batches of fertilized bluefin tuna eggs were obtained from naturally spawning captive adult tuna in farming facilities at El Gorguel (Caladeros del Mediterráneo, Spain) and transported to the experimental facilities at Mazarrón. Bonito and bluefin tuna eggs were incubated separately, and the larvae were fed a planktivorous diet following the protocol described by Reglero et al. (2014b). The experimental treatments were conducted on larvae of (mean ± SD) 8.3 ± 0.83 mm SL for tuna and 7.5 ± 0.96 mm for bonito, corresponding to the flexion and post-flexion developmental stage. Small individuals in the yolk sac and the pre-flexion developmental stage were not included in the analyses due to potential errors associated with limitations on the visual identification of the larvae in the experimental columns.

### Experimental set-up

The experimental set-up was modified from that described by Vollset et al. (2009). It consisted of 6 cylindrical methacrylate experimental columns 102 cm long and 20 cm diameter with the capacity for approximately 30 l of water. The columns were illuminated from behind, uniformly over the whole water column, using fluorescent lamps. A mark was drawn with a permanent pen dividing each column every 17 cm (ca. 5 l in each segment).

During the evening, each column was filled with 25 l of oxygen-saturated marine water, leaving the upper 17 cm of the water column empty. Two column stratification regimes were used, i.e. stratified and isothermal. To create the thermocline, 3 submersible aquarium heaters were placed in each of the columns at depths between 17 and 34 cm from the top of the column to warm the water in the surface up to 23°C for the experiments with Atlantic bonito and to

25.5°C for the experiments with Atlantic bluefin. The room temperature was kept at 19 and 22°C for bonito and bluefin, respectively, by means of an air conditioner, to create a gradient with temperatures ranging from 23 to 18°C for bonito and 25.6 to 22°C for bluefin tuna (Fig. 2). For the isothermal treatment, the air-conditioning of the room was set so that overall the experimental column temperatures were (mean ± SD) 23.1°C ± 0.08°C and 24.4°C ± 0.1°C for bonito and bluefin, respectively (Fig. 2), maintaining the heaters inside the column but not in operation. Differences in the temperature range for both species were set according to their temperature range in captive conditions (Ortega 2015). For bonito, we simultaneously used 3 experimental columns for the thermocline treatment and 3 for the isothermal treatment each day. For bluefin, 6 experimental columns were used daily either for the thermocline or the isothermal treatment. The water in the columns was mixed before the onset of the isothermal trials. No aeration was provided in any of the columns since oxygen levels were always closed to saturation for the duration of the experiments. The vertical temperature distribution in each water column was measured before and after the completion of the experiment using 15 HOBO™ (Onset) data loggers homogenously separated along depth intervals in each water column to ensure that the temperature was stable during the experiment. The HOBOs were removed before the larvae were placed into the water columns. The columns were emptied and cleaned immediately every day after the end of the treatment.

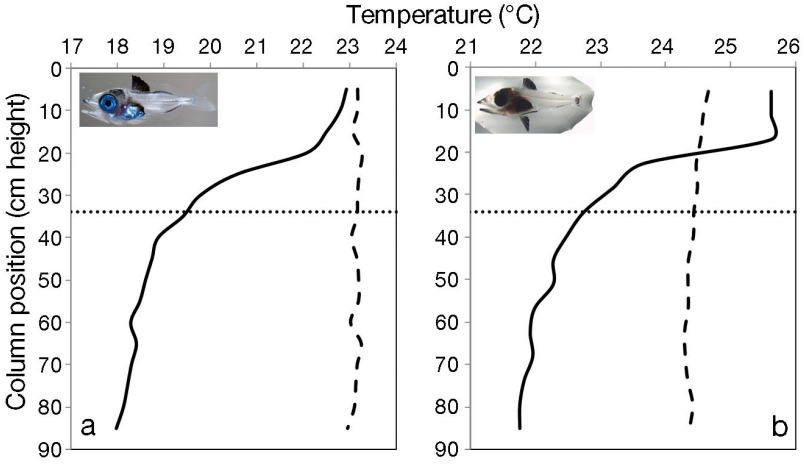


Fig. 2. Average temperatures in the experimental vertical columns for (a) Atlantic bonito *Sarda sarda* and (b) Atlantic bluefin tuna *Thunnus thynnus*. The continuous line represents the temperature regime for the thermocline treatment and the dashed line for the isothermal treatment. The dotted line indicates the separation between the upper and lower sections in the water column. Larvae were counted in the upper part of the water column (upper 34 cm)

**Treatments**

The larvae were kept in a 1500 l tank without feeding during the night prior to the start of the experiment. Every morning, we switched on the light in the 1500 l tank and the larvae were fed to satiation for 3 h. Afterwards, 60 larvae were removed from the 1500 l tank, and 10 larvae were placed in each of the experimental columns from above while the light was on. The larvae acclimated rapidly to the column, swimming around calmly within a few minutes. After 15 min of acclimation, the larvae in each experimental column were monitored 4 times daily by 2 observers who independently counted the number of larvae in the upper 34 cm of the water column, corresponding to the upper part of the thermocline in the thermocline treatments. To count the larvae, the observers wore dark clothing and during darkness used a lantern with red light at low intensity to avoid disturbing the larvae. The larvae were counted 30 min ($I1_{bft}$ for isothermal, $T1_{bft}$ for thermocline) and 60 min ($I2_{bft}$, $T2_{bft}$) after the onset of the experiment in Atlantic bluefin tuna and 60 min ($I1_{bon}$ for isothermal, $T1_{bon}$ for thermocline) and 120 min ($I2_{bon}$, $T2_{bon}$) after the onset of the experiment in Atlantic bonito while the light was on. The light was completely switched off, the larvae acclimated to darkness during 15 min, and the larvae were monitored again 105 min ($I3_{bft}$, $T3_{bft}$) and 135 min ($I4_{bft}$, $T4_{bft}$) after the onset of the experiment for Atlantic bluefin tuna and after 195 min ($I3_{bon}$, $T3_{bon}$) and 255 min ($I4_{bon}$, $T4_{bon}$) for Atlantic bonito. After the last measurement (135 min for bluefin tuna and 255 min for bonito), the larvae were moved to another tank and kept alive while a subsample was photographed, measured (SL) and frozen.

The experiment with bonito lasted for 8 d, and a total of 470 bonito larvae were used (60 larvae $d^{-1}$, 10 in each experimental column except 1 that was not used due to technical problems). For bluefin tuna, the experiment lasted for 7 d (the thermocline treatment was conducted on experimental days 1, 3, 4, 6 and 7, and the isothermal treatment was conducted on experimental days 2 and 5), and a total of 380 bluefin tuna larvae were used (60 larvae $d^{-1}$, 10 in each experimental column except 4 experimental columns that were not used due to technical problems).

### Data analysis

#### Field data

We used a generalized additive model (GAM), a nonparametric approach that allows non-linear relationships between response and explanatory variables that follows the general equation:

$$N_{larvae} = g^{-1}(\beta_0 + offset(\log(vol)) + \text{factor}(dayornight) + \sum S(environmentalvariable)) \quad (1)$$

where $g$ represents the link function, $\beta_0$ is the model intercept, and $S$ is a smoothing function with no *a priori* assumption of linearity for each explanatory environmental variable. We used a Poisson distribution, suitable for count data, to model the number of larvae ($N_{larvae}$) in relation to temperature, salinity and fluorescence, using a natural-log link function. The volume of water filtered (vol) was included as an offset after natural log transformation to account for the effort used in catching the sample. Time of sampling as day or night was included as a factor in the model to account for daily variability in larval abundance. The variable selection criteria were based on the confidence region for the smoothing effect, the percentage of deviance explained (increasing after adding significant covariates) and the UBRE score (decreasing after adding significant covariates) (Wood 2006, Reglero et al. 2012). GAMs were fitted using the 'mgcv' library in R statistical software (https://cran.r-project.org/web/packages/mgcv).

#### Experimental data

The effect of the thermocline on the vertical distribution of the larvae during light was tested separately for bluefin tuna and bonito using a generalized linear model (GLM). Unlike for the statistical analysis on field data, we were not detecting non-linear effects of the dependent variables on the response variable and therefore used GLMs instead of nonparametric GAMs. The GLM modeled the number of larvae counted in the upper 34 cm of the column including isothermal replicates 1 and 2 ($I1_{bft}$ and $I2_{bft}$ for bluefin tuna and $I1_{bon}$ and $I2_{bon}$ for bonito) and thermocline replicates 1 and 2 ($T1_{bft}$ and $T2_{bft}$; $T1_{bon}$ and $T2_{bon}$) during the light period. The same procedure was used to test the effect of the thermocline on the vertical distribution of the larvae during darkness but including isothermal replicates 3 and 4 ($I3_{bft}$ and $I4_{bft}$; $I3_{bon}$ and $I4_{bon}$) and thermocline replicates 3 and 4 ($T3_{bft}$ and $T4_{bft}$; $T3_{bon}$ and $T4_{bon}$) during the dark period. We then tested differences in the vertical position of the larvae between light and darkness in the thermocline including thermocline replicates during the light period ($T1_{bft}$ and $T2_{bft}$; $T1_{bon}$ and $T2_{bon}$) and during darkness ($T3_{bft}$ and $T4_{bft}$; $T3_{bon}$ and $T4_{bon}$). The same procedure was used to test differences in the vertical position of the larvae during the isothermal treatment between light ($I1_{bft}$ and $I2_{bft}$; $I1_{bon}$ and $I2_{bon}$) and darkness ($I3_{bft}$ and $I4_{bft}$; $I3_{bon}$ and $I4_{bon}$). All comparisons were done separately for bonito and bluefin tuna. We applied a Bonferroni correction to adjust probability values for replicated trials and to avoid type I error (p-adj, defined as the probability divided by the number of tests). All data analyses were conducted using R (www.r-project.org).

## RESULTS

### Field larvae

The vertical distribution of the hydrographical variables, i.e. temperature, salinity and fluorescence, indicates that environmental conditions along the water column were very similar in 2011 and 2012 (Fig. 3). The water column was characterized in both years by a marked thermocline located around 20 m depth (Fig. 3a,b), with no clear halocline (Fig. 3c,d) and chlorophyll increasing towards maximum values at 60 m depth (Fig. 3e,f), though particularly in 2012 chlorophyll values were very low and constant over the water column (Fig. 3f). There was no correlation between the thermocline and the other environmental variables (chlorophyll and salinity).

Larvae of Atlantic bluefin tuna were caught in high abundances in both years, and larvae of albacore and bullet tuna, although less abundant than bluefin, were also caught both years (Table 1). Other tuna species, i.e. 2 larvae of little tunny *Euthynnus alletaratus* (3.1 mm SL) and 1 larva of skipjack *Katsuwonus pelamis* (4.3 mm SL), were caught only in 2012. Atlantic bluefin, albacore and bullet tuna larvae were caught in the first 20 m depth, whereas the little tunny and skipjack larvae were caught in the first 10 m depth.

Temperature alone significantly explained most of the variance both in bluefin tuna and albacore (Table S1 in the Supplement at www.int-res.com/articles/suppl/m594p231_supp.pdf, 41% in 2011 both species and 71 and 51% for bluefin tuna and albacore, respectively, in 2012), with increasing larval abundances as temperature increased (Fig. 4). Salinity was also significant, although it explained less

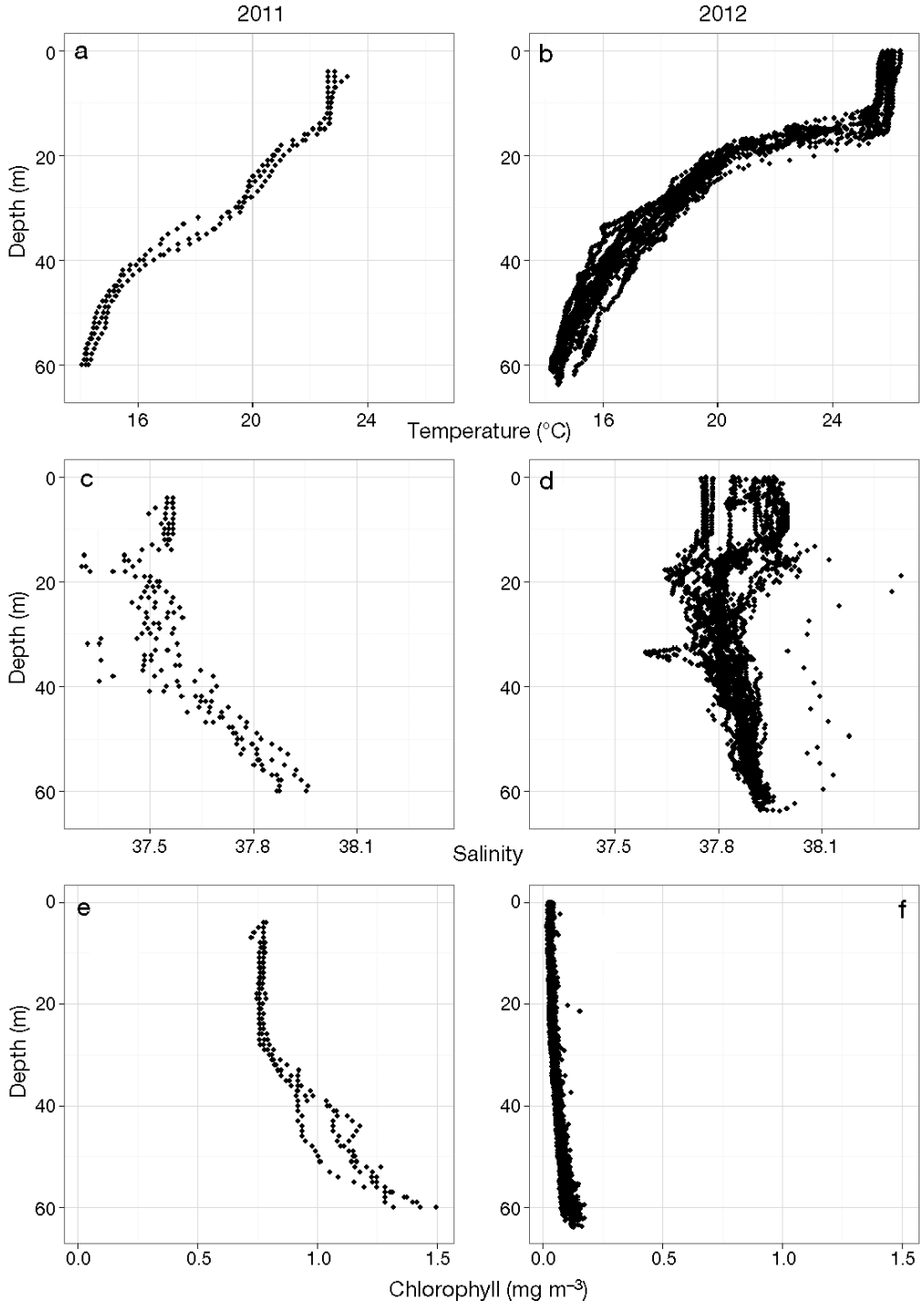


Fig. 3. Vertical profiles of hydrographical variables (temperature, salinity, chlorophyll concentration) measured during the 2011 and 2012 research cruises

variance than temperature (Table S1). The significant relationship with salinity was not related to its vertical profile but indicated that more larvae were caught in stations with slightly less saline waters in the upper layers. No significant differences in the day and night patterns were observed for bluefin tuna and albacore, the most abundant tuna species (Fig. 5; factor [day or night] in Table S1) except in the case of bluefin tuna in 2012 when more larvae were caught at night than during the day; in all other cases, factor (day or night) was not significant (Table S1, Fig. 5). The data for the other species were

Table 1. Total number (N) of larvae of Atlantic bluefin tuna *Thunnus thynnus*, albacore *T. alalunga* and bullet tuna *Auxis rochei*, caught in 2011 and 2012. For each species, mean ± SD standard length (SL) after shrinkage correction is shown

| Species | 2011 | | 2012 | |
|---|---|---|---|---|
| | N | SL (mm) | N | SL (mm) |
| *Thunnus thynnus* | 333 | 4.2 ± 0.6 | 1629 | 4.7 ± 0.8 |
| *Thunnus alalunga* | 13 | 3.9 ± 0.6 | 9 | 4.3 ± 0.9 |
| *Auxis rochei* | 5 | 3.9 ± 0.3 | 1 | 4 |

too limited to compare day vs. night distributions. The average size of the bluefin tuna and albacore larvae was very homogeneous (Table 1), so no ontogenetic variation in the vertical distribution could be analyzed.

### Experiments

Thermal stratified and isothermal conditions were successfully established and documented with vertical temperature profiles (Fig. 2). The number of larvae located above the thermocline in the water column during the thermocline treatment was significantly higher compared to the isothermal treatment, both during light and dark periods in both species (Table S2, p-adj < 0.01, Fig. 6). There was no significant difference in the position of the larvae between light and dark in the thermocline or the isothermal experiment in both species (Table S2, p-adj > 0.01, Fig. 6).

## DISCUSSION

Tuna larvae showed a significant preference for the higher water temperatures found above the thermocline. Field data and laboratory experiments on Atlantic bluefin tuna and laboratory experiments on Atlantic bonito confirmed larval preference for warm waters above the thermocline, suggesting that temperature is a significant cue for the vertical distribution of these species during their early life. In the Balearic Islands, tunas spawn during summer when

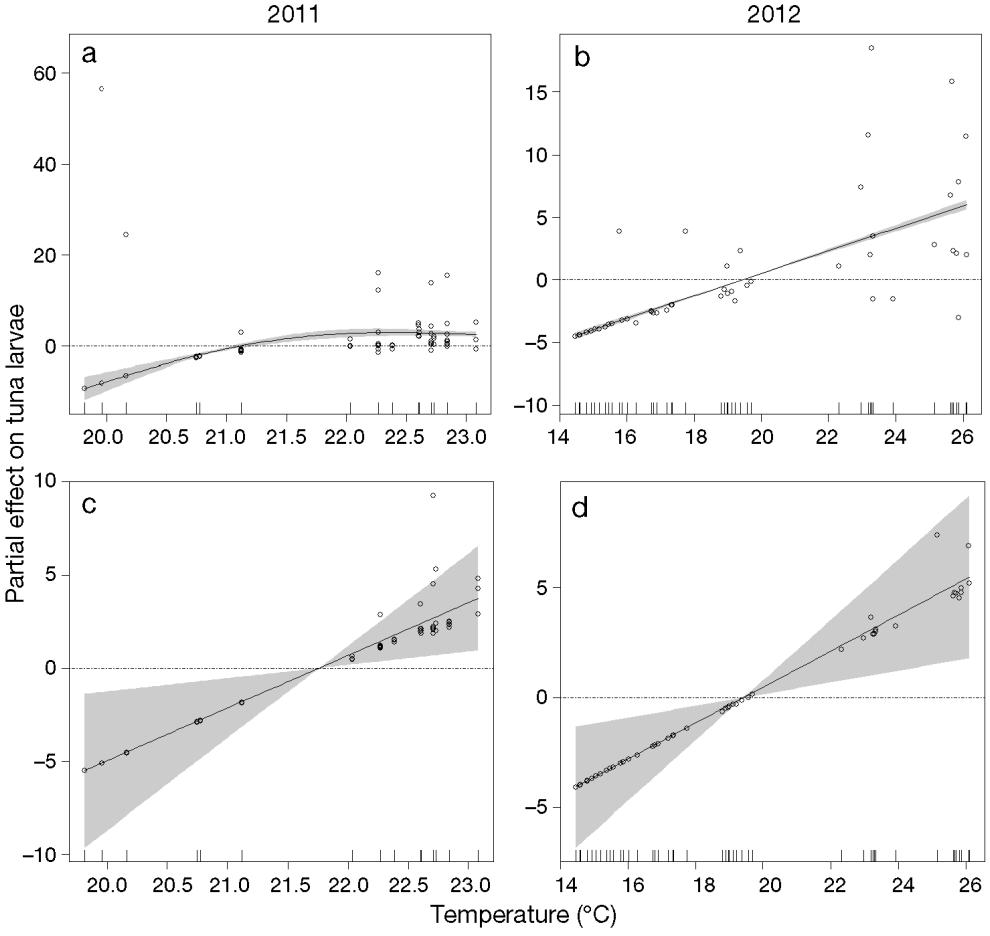


Fig. 4. Model results of the significant partial effect of temperature on larval abundance of (a) bluefin tuna collected from cruises in 2011 and (b) 2012, and (c) albacore in 2011 and (d) 2012; *y*-axes: values below (above) 0 indicate a negative (positive) effect of the variable on the larval abundance. Fitted lines, 95% confidence intervals (grey shaded areas) and partial residuals (dots) are shown. Whiskers on *x*-axes: field observations for that covariate

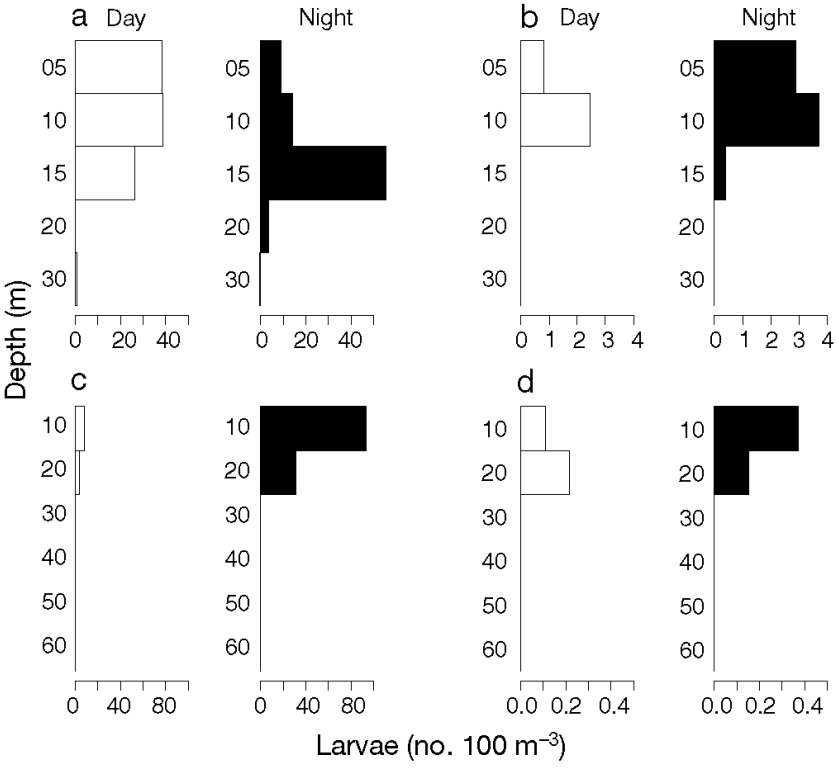


Fig. 5. Vertical distributions of (a,c) *Thunnus thynnus* and (b,d) *T. alalunga* in (a,b) 2011 and (c,d) 2012 during daytime and nighttime. Note that the different axes for depth correspond to the different depth strata sampled in 2011 and 2012

temperatures in the upper layers of the water column reach the minimum required for spawning. We observed that at 20–25 m depth, temperatures decreased below 20°C, the minimum temperature reported to date for the presence of tuna larvae (Reglero et al. 2014a). Atlantic bonito larvae have been reported at sea surface temperatures of 25.4°C (±0.58°C SD) in coastal areas (Sabatés & Recasens 2001) and between 24 and 26°C in the open sea (Torres et al. 2011). The temperature-dependent behavior suggested by the field data for all tuna species is supported by the results obtained from our experiments, where Atlantic bluefin tuna and Atlantic bonito larvae were distributed significantly shallower in the stratified than in the isothermal experimental water columns both during light and dark periods. Therefore, the lowest thermal range for these species sets a natural boundary that confines the optimal vertical habitat for tuna and bonito larvae to the upper part of the water column of the Mediterranean Sea.

The temperature-related behavior found in our study for Atlantic bluefin tuna has not been so clearly indicated in previous studies that have used samples taken in other geographic regions or other bluefin tuna species. Similar to our results for Atlantic bluefin tuna in the NW Mediterranean, the depth distribution of larvae of Pacific bluefin tuna in the NW Pacific Ocean ranges from 0 to 20 m, a distribution that has been related to

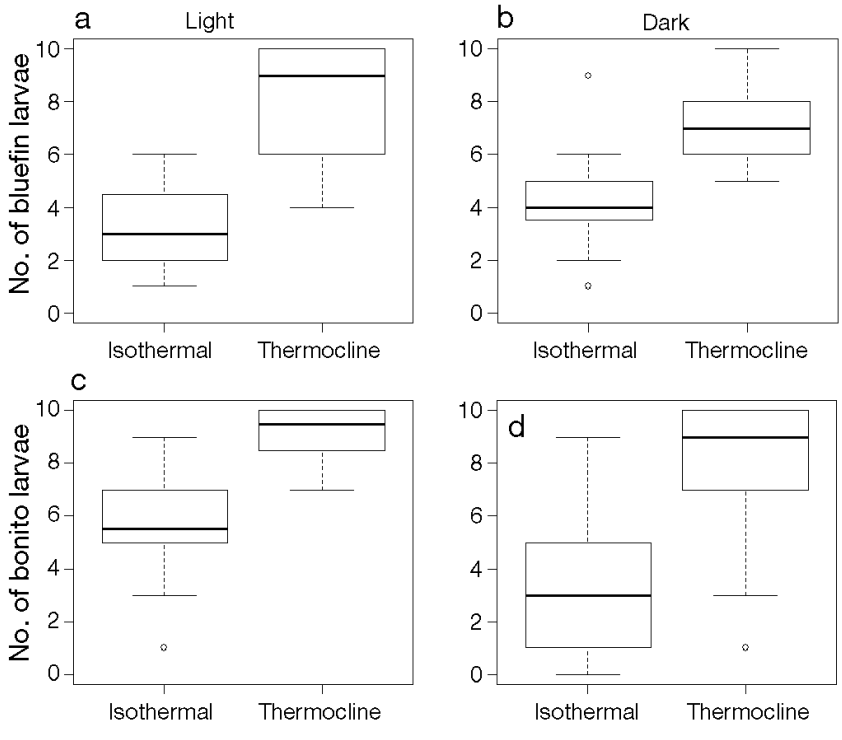


Fig. 6. Number of larvae counted in the upper part of the isothermal and thermocline water experimental columns (top 34 cm of the water column) for (a) light treatment in bluefin tuna, (b) dark treatment in bluefin tuna, (c) light treatment in bonito and (d) dark treatment in bonito larvae. The black line is the median, the top and the bottom of the box represent the 75$^{th}$ and 25$^{th}$ percentiles, respectively, and the whiskers represent the maximum and minimum values. Outliers are shown by open circles

the presence of a pycnocline (Satoh 2010). Atlantic bluefin tuna larvae in the Gulf of Mexico are caught mostly in the first 20 m of the water column, although larvae have been found down to 50 m (Habtes et al. 2014). The fact that Atlantic bluefin tuna larvae are sometimes found deeper than 20 m in the Gulf of Mexico but not in the other areas could be due to the sharper thermal gradients observed in the Mediterranean Sea and the Pacific Ocean than in the Gulf of Mexico (Llopiz & Hobday 2015).

Larvae of all tuna species have been found to be distributed in the mixed layer and usually near the surface, being most abundant in the first 30 m of the water column (Matsumoto 1958, Strasburg 1960, Ueyanagi 1969, Davis et al. 1990, Boehlert & Mundy 1994, Satoh 2010, Habtes et al. 2014). Skipjack has been described as the tuna species with the deepest distribution, with larvae caught at 50 m (Matsumoto 1958, Davis et al. 1990, Habtes et al. 2014), and even down to 80 m (Boehlert & Mundy 1994, Llopiz et al. 2010). Little tunny has been found at 40 m and even 100 m depth in the Gulf of Mexico and waters off the coast of Florida (Llopiz et al. 2010, Habtes et al. 2014). In our study, we only caught 1 larva of skipjack tuna and 2 of little tunny, both in the first meters of the water column. These 2 species of tropical tuna are increasing in abundance in the NW Mediterranean, where they have been periodically spawning in recent years (Saber et al. 2015), and further studies are needed before definitive conclusions can be made regarding their larval vertical distribution. Albacore larvae in our study were clearly distributed in the upper 20 m of the water column. These 3 species, i.e. albacore, skipjack and little tunny, share tropical and temperate larval habitats (Reglero et al. 2014a). Therefore, their vertical distribution may vary from being more widely distributed in areas with slight thermal gradients to being more narrowly distributed and restricted to shallower layers in areas with strong thermal gradients.

There is no common agreement on the diel vertical migration patterns of tuna larvae. Southern bluefin tuna and albacore in the East Indian Ocean show a shallower distribution during daytime than during the night (Davis et al. 1990), but no evidence of daily migrations has been reported for albacore in the Indo-Pacific (Ueyanagi 1969) or for Pacific bluefin tuna (Satoh 2010). On the other hand, ontogenetic patterns in the vertical distribution of larvae of tuna species have never been reported. Pacific bluefin tuna larvae in the laboratory are able to change their density by inflating their swim bladder at night and deflating it during the day. The density of the larvae also increases with age (Takashi et al. 2006). Night inflation of the swim bladder can prevent the larvae from sinking during the night while not swimming, thereby conserving energy (Hunter & Sanchez 1976, Takashi et al. 2006). In Atlantic bluefin tuna and southern bluefin tuna, the swim bladder is completely developed around 10 d after hatching (Woolley et al. 2013, Yúfera et al. 2014).

Our results regarding larval responses to temperature are strengthened by our use of both field and laboratory studies and the comparison of the results from both. Our design of the experimental thermocline used to test the effect of temperature gradients on the vertical distribution of tuna and bonito larvae is based on designs used in previous studies on cod and herring (Vollset et al. 2009, Catalán et al. 2011). The experimental work was designed so that only gradients of temperature were modified while all other variables (internal and external) were controlled. The thermal range that we used in the laboratory was adapted to that experienced by each species in the field and included the thermal tolerances for the 2 species. One substantial modification in relation to work by Vollset et al. (2009) was that the water columns were illuminated from behind instead of from above so that light levels were uniform in the water column. Due to obvious technical reasons, thermal gradients are by necessity steeper in the laboratory than in the field. Therefore, we have not compared the speed or actual distance covered by the larvae in the laboratory and the field. Our main result is that larvae control their vertical position to stay in the warmer upper water layers both in the experimental water column and in the pelagic environment. The larvae in the experimental columns were located significantly shallower in the stratified than in the isothermal experimental water columns, a result that matches results obtained from the field.

Temperature may act as a physiological barrier for the vertical distribution of larvae when gradients in the field are sharp, whereas larvae move to deeper depths in areas where temperatures are homogeneously distributed, although always within the thermal tolerance range of the species. The vertical position within the depth range limited by the physiological tolerance of the larvae to temperature involves trade-offs that could be explained by a thermoregulatory strategy whereby metabolism is reduced at cold temperatures and growth rates are increased at high temperatures (Urtizberea 2009). In our experiments, all larvae were fed before the onset of the experiment, so results were independent of stomach fullness (Vollset et al. 2013). However, the

feeding state could also have an effect on the vertical temperature preference of larvae in the dark. Atlantic bluefin tuna and bonito larvae are visual predators that only feed during daylight and digest their food very fast (Blanco et al. 2017). Once the larvae empty their stomachs they could move down in the water column to colder temperatures during darkness to lower their metabolism until feeding recommences at daylight. However, we did not find any clear evidence of diel vertical migration in the field data. In the experimental columns, no significant differences were found in average larval positioning during periods of light (30–60 and 60–120 min after the beginning of the experiment in bluefin tuna and bonito, respectively) and darkness (105–135 and 195–255 min after the beginning of the experiment, respectively). On the other hand, laboratory experiments on larval Pacific bluefin tuna, Atlantic bluefin tuna and yellowfin tuna have shown increasing growth rates with increasing temperature (tested up to 28°C) when food was provided ad libitum (Kimura et al. 2010, Wexler et al. 2011, Reglero et al. 2018). The same pattern is observed when analyzing data from different tuna species together (Reglero et al. 2011). Therefore, by staying in the upper warmer layers, tuna larvae may grow faster compared to those located in the colder water temperatures, increasing survival if it relates to faster growth.

A global increase in temperature and major stratification of the water column is expected in the Mediterranean Sea with future climate changes (Coma et al. 2009). The highest temperatures in the Mediterranean Sea measured during periods of spawning have not exceeded 28°C, although higher temperatures, up to 30°C, could be reached in the future if climate change follows expected trends. We have identified the minimum thermal constraint that influences the vertical range of tuna and bonito larvae, but less is known regarding maximum thermal constraints. In general, tuna species may have a higher tolerance for warmer waters and a lower tolerance for colder waters (Boyce et al. 2008). The occurrence of tuna larvae in water temperatures up to 30°C worldwide (Reglero et al. 2014a) suggests that these species may adapt to temperatures higher than those observed at present in the Mediterranean Sea.

Larval fish behavioral responses observed under experimental conditions cannot be directly extrapolated to the field, but it is worth noting that they match well with the findings regarding tuna and bonito larval response to thermal gradients in their natural larval habitat. We found no correlation between the thermocline and the other environmental variables. In the Balearic Sea, variations in salinity are linked to the water mass defining frontal structures due to the confluence of recent Atlantic water and resident Atlantic water (Balbín et al. 2014). However, our results showed that variability in salinity over the water column was not important. We used chlorophyll as an indicator for food abundance. We found no correlation between food abundance and the thermocline since most potential prey are located near the deep chlorophyll maximum, which occurs in this region and season between 50 and 70 m depth, far below the thermocline (Torres et al. 2014). However, chlorophyll is not a good proxy for food availability for tuna larvae (Llopiz & Hobday 2015), and therefore prey fields should be further investigated to include micro- and mesozooplankton.

We could not analyze the ontogenetic effect in the vertical distribution of the larvae, either in the field or in the laboratory, since the average length of the larvae caught in the field was very similar across species in both years. Smaller larvae could not be used in the experiments because they are difficult to detect visually, whereas large larvae were stressed when placed in the experimental water columns. The homogeneity of larval sizes in the field is attributable to our sampling strategy, since to ensure representativeness of samples, both night and day, we carried out an intensive sampling on single high-density larval patches, resulting from the spawning activity of adult schools in a given location over a short period of time, and hence most larvae were within restricted age/length ranges. Moreover, we expected little change to occur in the average body length over the sampling period, since the larvae could only be tracked over a few days each year. Aside from Atlantic bluefin tuna larvae, we found few larvae of other tuna species, and no Atlantic bonito larvae were caught. In general, few bonito larvae are caught in ichthyoplankton surveys in the region (Sabatés & Recasens 2001, Torres et al. 2011), despite the fact that *Sarda sarda* is one of the most abundant scombrid species in the Mediterranean Sea, with a spawning season occurring mainly in later spring and early summer (Macías et al. 2005), a period which coincides with our surveys. Most likely, the low abundance of bonito larvae in the NW Mediterranean is in part due to their fast development, since bonito larvae reach the post-flexion stage only 8 d after hatching, at 21°C (Reglero et al. 2014b, 2015) and hence are only available for plankton net samplings during a very short time period each year.

Describing the distribution of critical habitats for the larval stage within the water column in relation to

water temperature can provide essential information for introducing larval behavior into dispersal models and for understanding predator–prey interactions and the coexistence of fish species. Our results show that the vertical habitat of tuna and bonito larvae that spawn in temperate regions with strong thermal gradients is reduced to the first meters of the water column. Therefore, we expect that interactions between tuna species and their prey may be stronger in the first meters of the water column in the Mediterranean than in tropical areas with wider mixed layers due to physiological limitations forced by temperature. The dispersal of larvae towards nursery areas is expected to be strongly affected by processes at the surface of the water column in temperate areas.

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