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Hydrographic patterns conditioning variable trophic pathways and early life dynamics of bullet tuna *Auxis rochei* larvae in the Balearic Sea

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ABSTRACT: This study aims to analyze the influence of trophic interactions on larval growth variability throughout ontogenic development using data on carbon (C) and nitrogen (N) content and isotopic composition combined with a growth study by means of otolith microstructure analysis. To fulfill this objective, *Auxis rochei* larvae were sampled in the Balearic Sea (NW Mediterranean). A principal components analysis distinguished 2 types of surface water masses from a total of 40 stations that showed particular hydrobiological differences — resident Atlantic water (hereafter MW) and fresh Atlantic water (hereafter AW) masses — which showed significant differences in temperature, salinity and mesozooplankton biomass. Size-fractioned zooplankton analysis revealed greater biomass in the >250 µm fraction at AW stations, while the N and C composition of this fraction was higher at MW stations. Bullet tuna from MW were significantly larger and heavier and, consequently, had a higher Fulton's condition factor and higher daily growth rates. The $\delta^{15}N$ and $\delta^{13}C$ values of the zooplankton size fractions did not show significant differences between MW and AW. However, significantly higher $\delta^{13}C$ found in MW larvae suggests differences in the origin of food supplies. Moreover, the significantly higher ^{15}N values in bullet larvae collected in MW indicate a greater trophic specialization, possibly due to differences in the zooplanktonic food web of the water masses. *Auxis rochei* larvae showed a higher trophic level resulting from its higher ^{15}N isotopic enrichment compared with the small zooplankton size fraction. The stable isotope analysis demonstrates its potential in distinguishing variable trophic pathways in early life stages of fish inhabiting open sea marine ecosystems.

KEY WORDS: Bullet tuna · Larvae · Daily growth · Stable isotope · Trophic ecology · C:N ratio · Balearic Sea

INTRODUCTION

Bullet tuna *Auxis rochei* is the most abundant of the small tuna species off the Spanish Mediterranean coasts, where seasonal artisanal fisheries exploit this resource that is particularly abundant in Catalonian and Balearic waters (Sabatés & Recasens 2001, Valeiras & Abad 2007, Torres et al. 2011). Their extraordinary abundance and voracity point them out as likely predators of other young tunas (Bakun & Broad 2003) because the species spawns concurrently with bluefin tuna *Thunnus thynnus* and albacore *T. alalunga* in the Balearic Sea (García et al. 2009, Alemany et al. 2010) and with bonito *Sarda sarda* (Sabatés & Recasens 2001) along the Catalonian coasts.

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Bullet tuna have a rather extended spawning season, showing maximum abundance in the summer months from June to September (Sabatés & Recasens 2001). Consequently, it shows a wider tolerance to different temperature and salinity ranges than bluefin tuna, whose spawning season occurs during June–July (García et al. 2005, Alemany et al. 2010). The species inhabits NW Mediterranean waters yearround, where temperature and salinity regimes vary considerably, and shows a preference for the shallower waters near the shelf break surrounding the Balearic Islands (Alemany et al. 2010).

The Balearic Sea from a hydrographic standpoint is considered a transition zone as a result of the encounter of water masses of Atlantic origin with Mediterranean waters, which causes an intense geostrophic circulation in the area (López-Jurado et al. 1995, Vélez-Belchí & Tintoré 2001). This hydrographic scenario induces prominent mesoscale features, such as frontal structures and gyres that generate a suitable spawning habitat for bluefin (García et al. 2005, Alemany et al. 2010). The Balearic Sea is characterized by its nutrient deficiency, as occurs in the oligotrophic open sea waters of the Mediterranean (Siokou-Frangou et al. 2010). Consequently, zooplankton variability is strongly influenced by mesoscale phenomena, which relate to climate-driven oscillations (Pinot et al. 1995, Fernández de Puelles et al. 2004, Fernández de Puelles et al. 2007). Prior studies have shown that bullet tuna larval growth rates and nutritional condition measured by RNA/DNA ratios (Cortés et al. 2004, Laiz-Carrión et al. 2010) vary as a function of the origin of water masses of the Balearic Sea.

Nitrogen (N) and carbon (C) stable isotope analysis are used to assess the trophic position and C flow to consumers in food webs (Minagawa & Wada 1984, Peterson & Fry 1987, Post 2002). $\delta^{15}N$ provides an estimate of trophic level, and $\delta^{13}C$ assesses the sources of C for an organism when the isotopic nature of the sources are different, since C isotope ratios undergo small changes within the food web (Peterson & Fry 1987, France & Peters 1997). C:N ratios evaluate the nutritional status, as the levels of these elements are a direct consequence of protein synthesis and lipid/fatty acids, respectively (Checkley 1984, von Westernhagen et al. 1998, Coombs et al. 1999, Kloppmann et al. 2002).

Throughout bullet tuna larval development, planktonic diet undergoes changes, from small prey organisms at the onset of feeding, shifting towards a larger prey spectrum as teeth develop (larval size 3 to 5 mm). Ultimately, when larvae reach sizes over 5 mm, their diet shifts towards larger prey such as fish larvae and appendicularians (Uotani et al. 1981, Morote et al. 2008), the latter comprising the main fraction (19%) of Balearic mesozooplankton (Fernández de Puelles et al. 2004).

Bearing in mind the differences in the biophysical nature of the water masses that surround the Balearic archipelago, the goal of this study is to analyze the influence of contrasting hydrographic properties on larval growth variability throughout ontogenic development by way of trophic web interactions using data on the C and N isotopic composition. To fulfill this objective, resident Atlantic water (hereafter MW) and fresh Atlantic water (hereafter AW) bullet tuna larval growth and trophic characteristics are compared. This study aims to elucidate whether these differences may be caused by particular trophic pathways stemming from intrinsic differences that characterize each water mass.

MATERIALS AND METHODS

Field sampling of larvae and zooplankton

Early stage *Auxis rochei* were collected in August 2008 off the island of Mallorca (Balearic archipelago, Western Mediterranean; Fig. 1) onboard the RV 'Odon de Buen'. The area is one of the main spawning grounds for a number of scombrid species inhabiting the Mediterranean Sea (Alemany 1997).

A total of 40 stations, separated either by 5 or 10 nautical miles, were sampled from 31 July to 11 August 2008. Fish larvae were sampled during daytime by standard double oblique hauls using a squared-mouth Bongo frame of 90 cm with a 500 µm mesh net. To calculate the volume filtered, General Oceanics 2030 flowmeters were placed at the center of the net's mouth.

The plankton tow focused on 20 m depth, coinciding with the thermocline boundary (Torres et al. 2011). Once hauled onboard, cod-ends were washed and emptied onto a glass tray where individual larvae were sorted, placed in cryogenic vials and immediately stored in liquid nitrogen, as described by García et al. (2006). In addition, a 20 cm diameter Bongo net was geared above the Bongo 90 to sample different zooplankton fractions by employing 100 and 250 µm mesh nets together with their respective General Oceanics flowmeters.

A total of 262 bullet tuna larvae were sorted onboard, from which 96 were selected for further analysis. The rest of the plankton samples were

preserved in 96% ethanol (Replicate 1) and 4% seawater-buffered formalin (Replicate 2). Larvae preserved in ethanol were used for daily growth analysis. In the laboratory, larvae were thawed to ambient temperature, measured to standard length (SL), dehydrated in a freeze dryer for 24 h and weighed (dry weight, DW) on a precision microbalance for subsequent analysis of N and C isotopes.

Large mesozooplankton (>250 µm) samples were collected, directly concentrating the cod-end from the 250 µm mesh net. Samples from the 100 µm mesh nets were sieved on board in order to separate the small mesozooplankton (100 to 250 µm) fraction. The small mesozooplankton fraction was concentrated onto pre-weighed Whatman (GF/C) filters. In the laboratory, both zooplankton samples were weighed to the nearest 1 µg on an electronic microbalance after freeze drying for 24 h at −20°C for biomass (mg m^{-3}) estimation.

Hydrographic data were collected at each sampling station using a Seabird 19+ CTD profiler cast at a minimum depth of 350 m or at least 5 m above the seabed. The vertical profiles of temperature were used to determine the thermocline location (for more details, see Torres et al. 2011).

A principal component analysis (PCA) was carried out to characterize different stations in relation to environmental features (Fig. 1A). Temperature and salinity were negatively correlated with PC1, which explained 63.4% of the variance. In contrast, large mesozooplankton biomass was negatively correlated with PC2, which explained 23.2% of the variance. The 2 PCA factors explained 86.6% of the variance, extracting 14 positive stations with 96 larvae grouping 2 environmental classes (Fig. 1). The average mesozooplankton values were similar for both groups. To run this PCA, mean temperature (°C), salinity (ppm) and the large mesozooplankton biomass (mg m^{-3}) in the first 5 m were selected after linear correlations between variables were performed. The 2 groups represent Atlantic (AW) and Mediterranean (MW) water, respectively.

Growth analysis

Larvae conserved in ethanol were used for the daily growth analysis. SL of the target species was measured to the nearest 0.1 mm with an Image

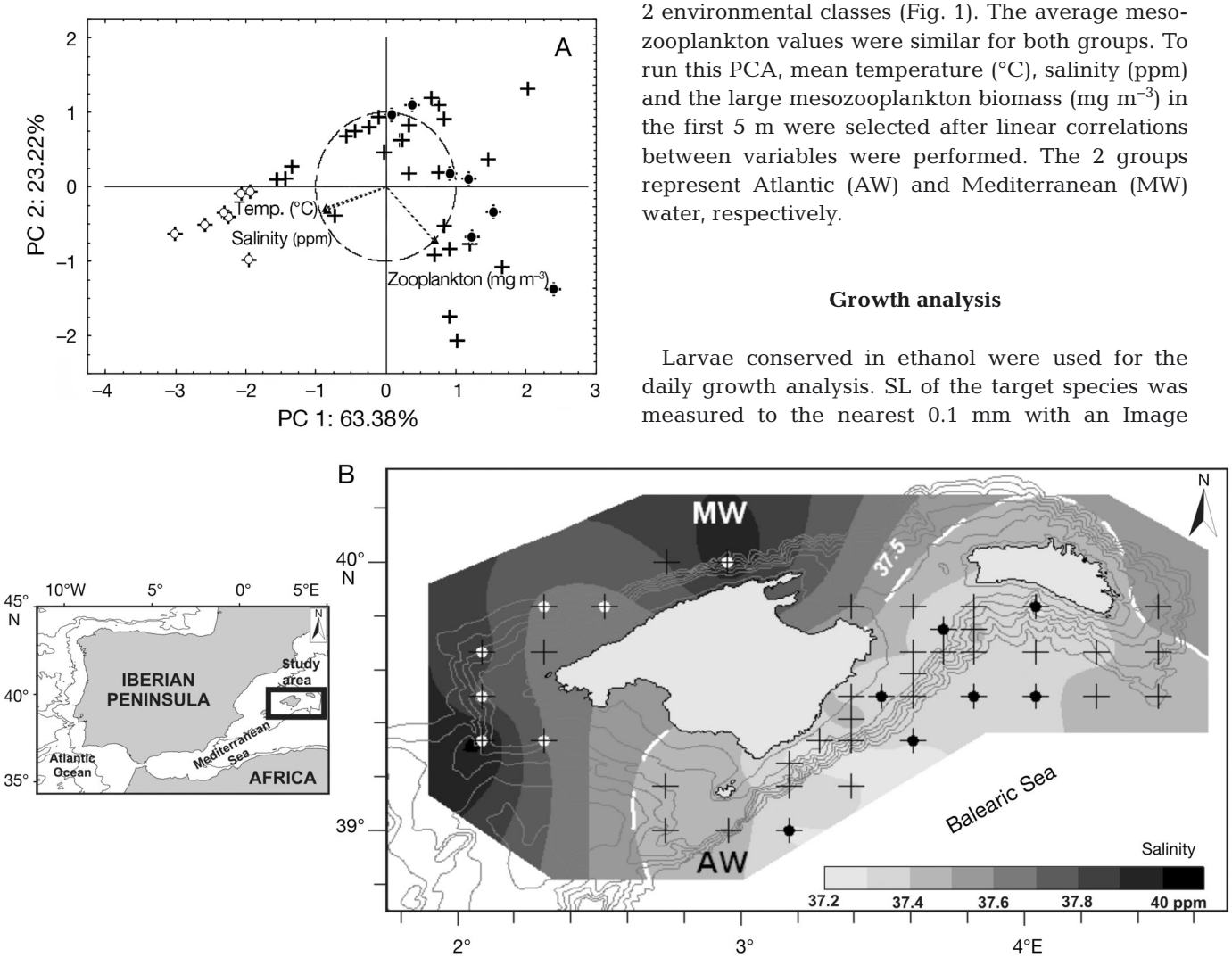

Fig. 1. (A) Principal component analysis (PCA) of the environmental variables of the sampling stations according to 3 factors: temperature, salinity and mesozooplankton biomass (86.6% representation within 2 factors) showing Mediterranean (MW) and Atlantic (AW) stations. (B) Station sampling distribution according to the PCA in the study area. +: station; MW: ○; AW: ●

Analysis System (Image-Pro). Shrinkage, on average, may be around 7%, based on estimates from other genera of fish larvae preserved in ethanol (Fox 1996, García et al. 2004). Nonetheless, it was assumed that shrinkage would not influence the comparison of growth rates among the analyzed specimens because all the samples were preserved following the same methodology. The Fulton larval condition factor ($10^3 \cdot DW \cdot SL^{-3}$) was calculated for each larvae (Suthers 1998, Laiz-Carrión et al. 2011). To extract the sagittae and lapilli otoliths, individual larvae were placed on a glass slide. By means of fine tungsten needles, otoliths were teased out from sacculus and utriculus otic sacs were viewed under a stereoscopic microscope. In the process of extraction, otoliths fall onto the glass slide. Otoliths were then cleansed by submerging them in distilled water and separating all remains of organic matter. Once dry, the otoliths were embedded in Eukitt resin. All age estimates were made with the sagittae at ×1000 magnification. Daily increments were counted, and increment width and radius were measured by means of an Image Analysis System using Image-Pro software. Once age lecture interpretation were met, each otolith was independently read by 2 different readers.

Stable isotope analysis

Bullet tuna larvae conserved in liquid nitrogen from both AW and MW were used for weight and standard length measurements. A total of 96 larvae were analyzed, 49% of which were sampled in MW and 51% in AW. Larvae were dried, weighed (0.05 to 2.3 mg) and packed in tin vials (0.03 ml) before analysis. To avoid size-dependent trophic interactions, a total of 37 bullet tuna larvae within a similar SL range (5.5 to 9 mm) were selected from both MW (41%) and AW (59%) samples. The natural abundance of N ($\delta^{15}N$ and %N) and C ($\delta^{13}C$ and %C) was measured using an isotope-ratio spectrometer (Thermo-Finnigan Delta-plus) coupled to an elemental analyser (FlashEA1112 Thermo-Finnigan) at the Instrumental Analysis Unit of the Universidade da Coruña. Ratios of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ were expressed in conventional delta notation (δ), relative to the international standard, atmospheric air (N_2) and Pee-Dee Belemnite (PDB), respectively, using acetanilide as standard. The analyses precision for $\delta^{15}N$ and $\delta^{13}C$ were 0.15 and 0.13‰, respectively, based on the standard deviation of internal references (repeatability of duplicates). Likewise, the precision for %N and %C determination was 0.16 and 0.11%, respectively. C:N ratios were determined as element ratios.

Lipid correction was not possible due to the low amount of available sample, which hampered a previous chemical extraction. Nevertheless, a posterior correction of the $\delta^{13}C$ values for lipid content was performed for the different plankton size fractions. The equations proposed by Logan et al. (2008) for marine invertebrates were applied to select the best model predicting the lipid correction. Four equations of the model were applied to estimate a mean value of 0.54‰ (SD = 0.38) and 0.75‰ (SD = 0.23) for small and large mesozooplankton, respectively. These values were used for the $\delta^{13}C$ lipid correction in both zooplankton size fractions. No significant differences in the lipid correction model between water masses were observed ($p = 0.9$). The lipid correction for bullet tuna larvae took into account the parameters of Atlantic bluefin tuna muscle reported by Logan et al. (2008), bearing in mind the species' phylogenic proximity. The mean value for lipid correction was 1.44‰ (SD = 0.34). The estimated correction did not show a statistical difference between the water masses ($p = 0.8$).

Statistical analysis

The differences in SL, DW, %N and %C between the AW and MW larval groups were tested through a 1-way ANOVA after verifying homogeneity of variance, where water mass location (AW and MW) was the main factor and treated as a nominal independent variable. A common size range of larval cohorts ranging from 5.5 to 9 mm showed no significant statistical differences, thus allowing to infer on results of analysis of covariance (ANCOVA) tests. ANCOVA tests were applied to verify differences in the relationship among somatic variables (SL, DW), larval growth variables i.e. age and otolith radius (OR), stable isotopes ($\delta^{15}N$, $\delta^{13}C$), N and C content (%N, %C and C:N) and the Fulton larval condition factor. Logarithmic and arcsine transformations of the data were carried out when necessary to fulfill the conditions of the ANOVA. The differences between groups for $\delta^{15}N$ were analyzed using a crossed 2-way ANOVA with plankton size fractions (small, large mesozooplankton and larvae) and water masses (MW and AW) as main factors. Post hoc comparisons were made using a Tukey's test. All statistical tests were performed using Statistica 7.0 (Statsoft).

RESULTS

Oceanographic and biological factors

The PCA differentiated 2 water masses, where one group was characterized by higher temperature and salinity values (MW), and a second group was characterized by lower temperature and salinity values (AW) (Table 1).

Small mesozooplankton biomass (100 to 250 µm) did not show differences between MW and AW (Table 2). Likewise, neither N and C content nor their respective stable isotope signatures showed significant differences between MW and AW. In contrast, the biomass of the large mesozooplankton (>250µm) was significantly higher in AW, yet the N and C content along with the C:N values were significantly lower. Stable isotopic signatures of C and N of AW and MW did not show any significant differences between groups.

Bullet tuna larval cohorts: morphometric features and growth patterns

The bullet larvae sampled in each of these water masses showed prominent morphometric differences. Bullet larvae from the MW group (7 stations, 48 larvae) had significantly higher SL and DW than bullet larvae from the AW group (7 stations, 51 larvae) (Table 3) and had higher Fulton condition (Table 3). In spite of the morphometric differences in SL and DW, the N and C content of larvae did not show significant differences between water masses.

Table 3. p-values from 1-way ANOVAs of bullet tuna larvae biometric parameters (standard length [SL], dry weight [DW] and Fulton condition factor [CF]) analyzed for both Mediterranean (MW) and Atlantic (AW) water masses. Data are means ± SEM

	AW	MW	p
SL (mm)	5.9 ± 0.32	7.07 ± 0.34	<0.001
DW (mg)	0.28 ± 0.04	0.57 ± 0.08	<0.01
Fulton CF	0.101 ± 0.008	0.137 ± 0.011	<0.01

Likewise, neither the Fulton condition factor nor the C:N ratio were significantly different between the 2 larval cohorts. In the overall larval population, the C:N ratio showed a significant linear decrease with decreasing DW ($r = -0.4571$, $p < 0.005$; Fig. 2). Inversely, a significant linear relationship occured with the Fulton's index vs. DW ($r = 0.869$, $p < 0.001$).

The daily growth of bullet larvae was estimated from alcohol-preserved larvae. Daily growth followed linear fits (Fig. 3). The population growth rate of larvae sampled within MW was significantly higher (0.38 mm d^{-1}) than that of larvae sampled in AW (0.32 mm d^{-1}; $p < 0.01$).

Table 1. Basic hydrographic data of the selected stations from the Mediterranean (MW) and Atlantic (AW) water masses

	Temperature (°C)			Salinity (‰)		
	Max.	Min.	Mean ± SD	Max.	Min.	Mean ± SD
MW	27.08	26.56	26.89 ± 0.16	38.02	37.67	37.81 ± 0.15
AW	26.26	26.00	26.20 ± 0.15	37.35	37.27	37.30 ± 0.02

Isotopic signature and trophic pathways

The relationship between $\delta^{15}N$ and $\delta^{13}C$ in the size-fractionated categories of zooplankton and bullet tuna larvae shows that these isotopic signatures are statistically differentiated at the latter trophic level (Fig. 4). MW

Table 2. Results of 1-way ANOVAs of biomass, N and C isotopic and elemental composition, and C:N ratios of small and large mesozooplankton of the PCA-selected stations for both Mediterranean (MW) and Atlantic (AW) water masses. Data are means ± SEM. ns: not significant

	Small mesozooplankton			Large mesozooplankton		
	AW	MW	p	AW	MW	p
Biomass (mg m^{-3})	3.2 ± 0.5	2.8 ± 0.4	ns	18.2 ± 4.6	3.4 ± 1.1	<0.001
$\delta^{15}N$ (‰)	2.49 ± 0.07	2.25 ± 0.15	ns	2.58 ± 0.31	2.73 ± 0.27	ns
$\delta^{13}C$ (‰)	−19.67 ± 0.37	−20.20 ± 0.17	ns	−20.95 ± 0.23	−21.03 ± 0.25	ns
%N	5.41 ± 0.27	5.85 ± 0.39	ns	2.67 ± 1.04	7.10 ± 0.76	<0.005
%C	27.06 ± 0.58	26.59 ± 1.61	ns	10.50 ± 4.35	29.18 ± 3.11	<0.005
C:N	5.08 ± 0.27	4.56 ± 0.07	ns	3.75 ± 0.09	4.10 ± 0.05	<0.01

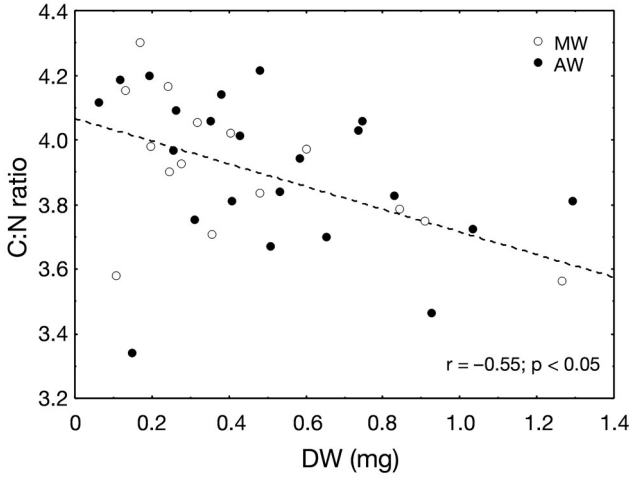

Fig. 2. *Auxis rochei.* Relationship between C:N ratio and dry weight in *A. rochei* larvae in both Mediterranean (MW; ○) and Atlantic (AW; ●) water masses. Least-squares regression line is for both populations

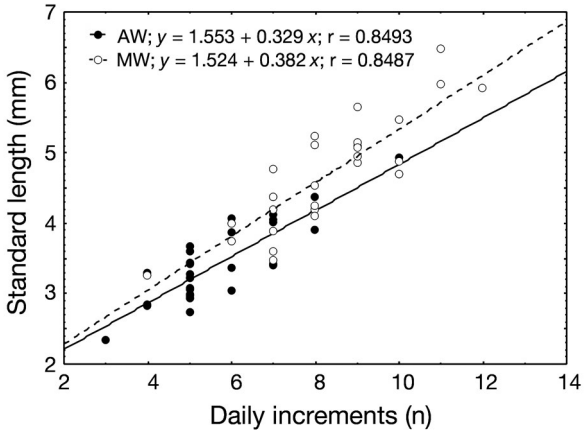

Fig. 3. *Auxis rochei.* Daily larval growth of *A. rochei* larvae in both Mediterranean (MW; ○; $p < 0.001$) and Atlantic (AW; ●; $p < 0.001$) water masses

bullet tuna larvae showed the highest mean $\delta^{15}N$, which was significantly differentiated from the $\delta^{15}N$ values of the AW bullet larvae. Stable isotopes of N and C in the large and small mesozooplankton samples did not show statistical differences between MW and AW. Maximum mean values of $\delta^{13}C$ were found in the mesozooplankton biomass fraction (100 to 250 µm), whereas the lowest $\delta^{15}N$ values belonged to the lower levels of the size-fractionated groups (Fig. 4). The isotopic enrichment from small mesozooplankton to bullet tuna larvae was higher in MW larvae (1.07 ± 0.20‰) than in AW larvae (0.67 ± 0.15‰; Fig. 5).

Although no differences in the somatic variables or N and C content between MW and AW bullet tuna

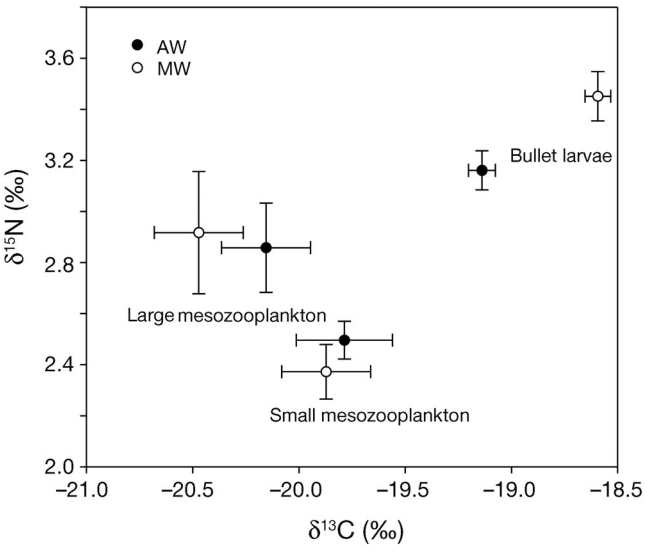

Fig. 4. Mean (±SEM) $\delta^{13}C$ versus $\delta^{15}N$ (‰) values for small and large mesozooplankton size fractions and for *Auxis rochei* larvae in both Mediterranean (○, MW) and Atlantic (●, AW) water masses in the Balearic Sea

larvae were observed, the isotopic signatures of $\delta^{15}N$ and $\delta^{13}C$ were significantly higher ($p < 0.05$ and 0.001, respectively) in the MW bullet tuna larvae. In the overall larval population, $\delta^{15}N$ and $\delta^{13}C$ showed a significant linear relationship (Fig. 6).

DISCUSSION

The circumambient waters of the Balearic archipelago are characterized by the influx of surface Atlantic water encountering Mediterranean water masses of the Northern Current. The dynamics of Balearic Sea circulation are influenced by the presence and amount of the Western Intermediate Waters causing the Northern Current's deflection towards the north of the Mallorca channel, allowing richer Mediterranean waters to occupy the northern part of the archipelago (López-Jurado et al. 1995). This oceanographic scenario seemed to predominate during the survey period, during which the northern part of the archipelago was engulfed by Mediterranean whereas the southern part was occupied by transitional waters characterized by the convergence of AW and MW masses. This is evidenced by the fact that more saline MW masses were located north of the archipelago, mixing with the AW in the south. Furthermore, the MW water masses were clearly differentiated by a significantly warmer surface temperature regime.

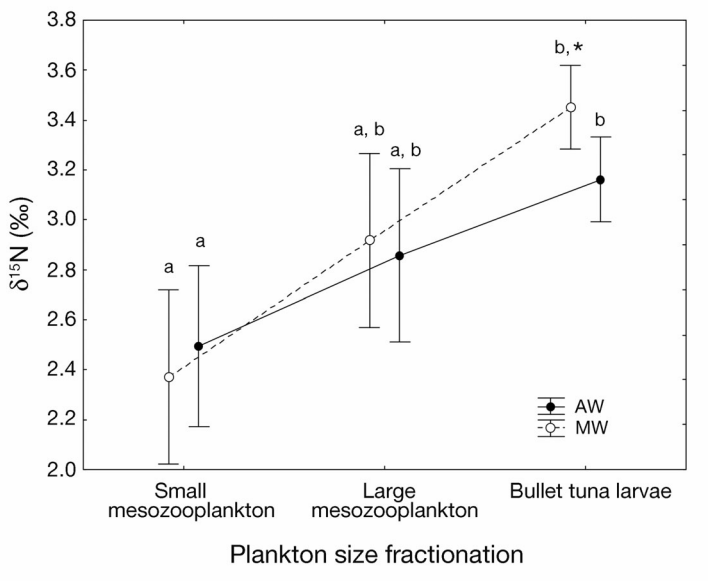

Fig. 5. $\delta^{15}N$ (‰) values in different ecosystem compartments in both Mediterranean (MW; ○) and Atlantic (AW; ●) water masses in the Balearic Sea. Water mass and plankton size fraction (small mesozooplankton [100–250 µm], large mesozooplankton [>250 µm] and *Auxis rochei* larvae) were the main factors for the 2-way ANOVA analysis. Post hoc comparisons were made using a Tukey's test. Different letters indicate significant differences ($p < 0.05$) among plankton size fractions within same water mass. * denotes a significant difference ($p < 0.05$) between water masses for bullet larvae

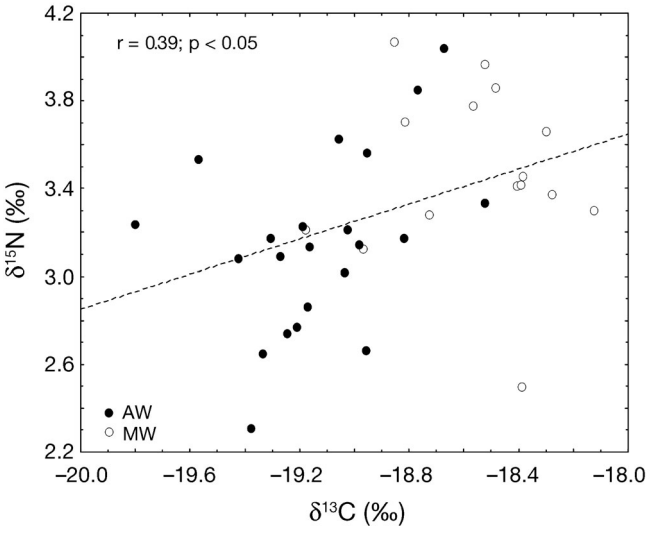

Fig. 6. *Auxis rochei*. Relationship between $\delta^{13}C$ and $\delta^{15}N$ (‰) in *A. rochei* larvae in both Mediterranean (MW; ○) and Atlantic (AW; ●) water masses. The least-squares regression line is shown for overall both populations

In general, the Balearic Sea is considered to be oligotrophic (Estrada 1996, López-Jurado et al. 2001) as a result of its nutrient deficiency, which is more accentuated during summer and particularly in waters of Atlantic origin (Fernández de Puelles et al. 2007). Within this nutrient-deficient scenario, the mixing of the 2 water masses causes intense hydrographic circulation that generates important mesoscale features as such as fronts and gyres (Vélez-Belchí & Tintoré 2001) that can drive the nutrient supply into the upper photic layer. Such hydrographic processes can cause a remarkable spatial and temporal heterogeneity in the planktonic community and, consequently, on the planktonic food web (Fernández de Puelles 1996, Alemany et al. 2006). The $\delta^{15}N$ isotopic signature of the mesozooplankton observed in this study is within the range of those reported by Koppelmann et al. (2009) for a similar mesozooplankton size fraction in the Eastern Mediterranean and by Fanelli et al. (2011) for different mesozooplanktonic species from the Catalonian coasts. Under normal nutrient availability conditions, the light isotopes are preferably mobilized by metabolic processes, but under nutrient-deficient circumstances plankton growth is based on nutrients remineralized *in situ*. Thus, a lower proportion of heavy isotopes in plankton should be expected under oligotrophic circumstances.

Furthermore, in nutrient-poor waters, a microbial food web which is actually remineralizing organic matter cannot be disregarded (Bode et al. 2007). Thus, plankton growth can partly depend on remineralized nutrients contributing to a lower proportion of the heavy isotopes, which could partly explain the low $\delta^{15}N$ isotopic signature of the AW bullet tuna larvae.

This oceanographic background, characterized by its oligotrophy and intense hydrodynamics, supports the spawning habitat of various tuna or tuna-like species, of which the most abundant is *Auxis rochei* (Alemany et al. 2006, 2010, García et al. 2009). The spawning of this species is concurrent with bluefin *Thunnus thynnus* and albacore *T. alalunga* spawning, and thus density-dependent factors may determine mortality at early life stages, since bullet tuna larvae show spatial and temporal overlaps throughout their early life stages with both tunnid species (Alemany et al. 2010, Torres et al. 2011). Furthermore, bullet tuna larvae grow much faster than bluefin and albacore larvae, whose growth rates varied from 0.33 to 0.35 mm d^{-1} in a larval size range below 10 mm (García et al. 2006). Within a similar size range, bullet tuna larvae can grow as much as 0.45 mm d^{-1} (A.G. unpubl. data from the 2003 TUNIBAL survey). Thus, it is not unlikely that early juve-

nile bullet tuna prey on other tuna species, as hypothesized by Bakun & Broad (2003). Bullet tuna larvae show the first signs of piscivory at an early stage, upon attaining a size just beyond 5 mm (Uotani et al. 1981, Morote et al. 2008). This piscivorous behavior has also been observed in albacore larvae (Catalán et al. 2007), but not in bluefin larvae (Catalán et al. 2011).

From the standpoint of larval growth, food availability as well as ambient temperature exert a great influence on early larval growth and condition enhancement (Buckley 1984, Ferron & Leggett 1994, Malzahn et al. 2003, Hardy & Litvak 2004). Inasmuch as the large mesozooplankton biomass was 6-fold greater in the AW mass, %N and %C were significantly lower in AW than in MW (see Fig. 7). The low N content in AW is clearly indicative of its nutrient depletion, in accord with its oligotrophic features. This fact implies that the planktonic community comprising AW may have lowered trophic quality as a result of low N available for plankton. Moreover, in a nutrient-depleted state where organic matter is being remineralized through a microbial food web, it could be reasonably assumed that plankton growth is based on remineralized nutrients, thereby causing a lower concentration of the heavy isotopes (Bode et al. 2007). Another plausible reason that may result from the observed differences between the water masses may stem from their specific planktonic compositions, in which blooms of aqueous species of plankton (e.g. cnidarian blooms) may be enhanced. Such types of blooms are reported to be commonplace in this area (García et al. 2002, Sabatés et al. 2010).

With respect to larval condition, previous studies have used the C:N ratio as an indicator of condition factor in fish larvae (von Westernhagen et al. 1998, Coombs et al. 1999, Kloppmann et al. 2002). The relationship between C:N ratio and larval dry weight (Fig. 2) can provide an approximation to metabolic growth rates. Laiz-Carrión et al. (2011) observed a clear relationship of the evolution of C:N ratios with sardine larval age following a similar decreasing pattern of daily growth rates with age.

Increases in C and N content from mesozooplankton to bullet larvae were observed in both MW and AW (see Fig. 7), suggesting an efficient energy transfer from putative prey (mesozooplankton) to their potential predators. Moreover, the lower energetic level for the large mesozooplankton size fraction observed in AW could indicate another trophic mechanism to explain the energetic enrichment of bullet larvae. Under this point of view, the piscivory or cannibalism hypothesis proposed before for tuna larvae in these oligotrophic waters by Reglero et al. (2011) tends to support our results. Under this hypothesis, a higher nitrogen isotopic signature should be expected for the piscivorous/cannibal bullet tuna larvae, while no differences between large mesozooplankton and bullet larvae were observed, positioning both ecosystem fractions at the same trophic level. Further research, involving nitrogen stable-isotope discrimination factors between larvae and their prey together with stomach content analysis, is required to further elucidate the trophic ecology of bullet tuna.

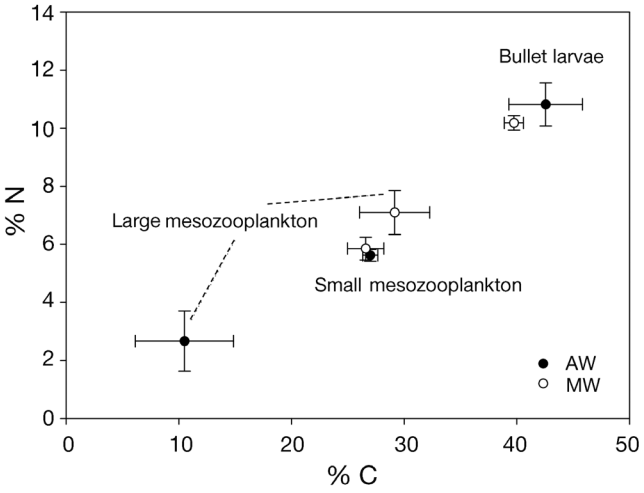

Fig. 7. *Auxis rochei.* Mean (±SE) carbon and nitrogen content for small (100–250 µm) and large (>250 µm) mesozooplankton size fractions and for *A. rochei* larvae in both Mediterranean (MW; ○) and Atlantic (AW; ●) water masses in the Balearic Sea

From the results of this study, it seems, from the trophic enrichment viewpoint, that the small mesozooplankton fraction (100–250 µm) is the most suitable for foraging bullet tuna larvae (see Figs. 4 & 5). This fraction includes a wide suite of zooplankton species and sizes, from nauplius stages, copepodites and cladocerans to appendicularians, which comprise the zooplanktophagous diet of the early stages of larval tunas (Uotani et al. 1981, Catalán et al. 2007, 2011, Morote et al. 2008). Thus, it seems likely that since no differences in mesozooplankton size fractionation or energetic sources were observed (Fig. 4) (Fanelli et al. 2009), the faster growth of MW bullet tuna larvae is possibly related to a greater trophic specialization in MW bullet tuna as demonstrated by the higher $\delta^{15}N$ value. The temperature difference, although rather small, between the water masses cannot be disregarded as another growth-enhancing

factor by way of accelerating metabolic processes (Buckley 1984, Ferron & Leggett 1994, Malzahn et al. 2003, Hardy & Litvak 2004).

Furthermore, bullet tuna larval growth may be enhanced in areas closer to shore, where the species is more abundant and mesozooplankton biomass is higher (Reglero et al. 2009, Alemany et al. 2010), and where appendicularians, the main dietary item at developed larval stages (Uotani et al. 1981, Morote et al. 2008, Llopiz et al. 2010), constitute a substantial fraction of the zooplankton (Fernández de Puelles et al. 2004). Such trophic availability could explain the preference of *Auxis rochei* for shelf waters reported by Alemany et al. (2010).

In summary, a PCA analysis clearly distinguished 2 distinct water masses that show differences in temperature, salinity and mesozooplankton biomass in the >250 µm fraction. Water masses of higher temperature and salinity were categorized as being of Mediterranean origin (MW); in contrast, those with lower temperature and salinity were assigned to waters of Atlantic origin (AW). The mesozooplankton of the latter water masses had significantly higher biomass, yet significantly lower N and C content than the MW. Consequently, the C:N ratio of mesozooplankton was significantly greater in the MW. Despite the difference in N and C content, their associated stable isotopes showed no significant differences. Regarding the smaller mesozooplankton fraction, no significant differences were observed in biomass, the C and N composition or the isotopic signature of both water masses.

Bullet tuna larvae showed significantly faster growth in MW (Fig. 2) which corresponded to their significantly higher values of $\delta^{15}N$ versus $\delta^{13}C$ within the non-differentiated post-flexion size range (Fig. 6). Moreover, the higher $\delta^{15}N$ values in MW larvae indicates a greater trophic specialization than in larvae from the AW, whose $\delta^{15}N$ values were only slightly greater than those of the large mesozooplankton. It is reasonable to assume that AW larvae may feed on prey at lower trophic levels than the large mesozooplankton size fraction. From the viewpoint of trophic enrichment of $\delta^{15}N$ in the food web, it seems that bullet tuna larvae essentially prey upon the small mesozooplankton fraction. Nonetheless, the energy sources do not seem to differ between the larval cohorts, as evidenced by the $\delta^{15}N$ and $\delta^{13}C$ relationship (Fig. 4). In conclusion, it is reasonable to infer that the biological properties of water masses can define particular trophic pathways that have an effect on the growth and condition observed in the larval cohorts of *Auxis rochei* at early life stages.

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