TROPHIC BIOLOGY OF AGE-0 ATLANTIC BLUEFIN TUNA IN THE WESTERN MEDITERRANEAN SEA: NEW INSIGHTS FROM STOMACH CONTENT AND STABLE ISOTOPE ANALYSES

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INTRODUCTION

The western Mediterranean basin is an important breeding area that provides feeding grounds for the offspring of many fish species including the Atlantic bluefin tuna (ABFT). Survival and growth rates of fish larvae and juveniles greatly affect recruitment and thereby resiliency to exploitation by fisheries. Hence, studies on the trophic biology of juvenile stages are essential for the understanding of population dynamics and stock recruitment variations. Feeding patterns and trophic biology of age-0 *Thunnus thynnus* (ABFT) were investigated at two locations off the Iberian Mediterranean coast (Cambrils and Valencia, Figure 1) between 2012 and 2014 using stomach content analysis (SCA), and stable isotope analyses of bulk muscle (BSIA) and individual amino acids (AA-CSIA).

RESULTS

Although the diet of age-0 ABFT was diverse, their major food resources were anchovy (%AI = 91.15 and 63.03 for Cambrils and Valencia, respectively) and clupeids (%AI = 8.62 and 24.08, respectively) (Table 1). Significant interannual and geographic differences were observed in both diet and bulk isotopic compositions (bifactorial permutational multivariate analysis of variance PERMANOVA, $p < 0.01$). SCA and BSIA results suggest a wider trophic niche for ABFT from Valencia, where the proportions of prey items were more homogeneous (H' and SEAc, respectively; Table 1 and Figure 2).

While essential AAs showed lower $\delta^{13}C$ values relative to bulk samples in both ABFT and copepods, non-essential AAs generally had higher $\delta^{13}C$ values than bulk samples. With regard to $\delta^{15}N$, trophic AAs of ABFT tissues were significantly ^{15}N-enriched relative to copepods, while $\delta^{15}N$ values of ABFT source AAs were similar to or lower than those measured in copepods (Figure 3).

The trophic position (TP) values calculated from SCA (TP_{SCA}) showed a narrow range of variation (4.00-4.24). The TP_{BSIA} estimates were more variable (range: 2.79-4.69), those calculated from isotopic data of liver tissue being lower than those obtained from muscle. Unlike the results of TP_{SCA}, the TP_{BSIA} suggested that the fish from Cambrils occupied a higher trophic level than those sampled off Valencia. In contrast, the mean TP_{CSIA} was higher in Valencia than in Cambrils (Table 2).

Table 1. Results of SCA of the juvenile ABFT sampled in Cambrils and Valencia (2012-2014). %AI: alimentary index expressed as percentage (%AI=[(%O × %W)/(∑%W × %O)] × 100) (Kawakami and Vazzoler, 1980), where %O, frequency of occurrence; %W: percentage of weigh. exp (H') trophic niche width (Hill, 1973), H' being the Shannon diversity index (H'=-∑[pi ln(pi)]), where pi is the proportion of prey i.

Cambrils

	%AI			
Preys	**2012**	**2013**	**2014**	**Overall**
Teleosts	**98.18**	**99.99**	**99.94**	**99.65**
Engraulis encrasicolus	81.21	91.55	98.56	91.15
Clupeids	17.68	8.43	1.42	8.62
Sprattus sprattus	0.02	<0.01		<0.01
Trachurus spp.		0.01		<0.01
Cepola macrophthalma	<0.01			<0.01
Teleost larvae	<0.01			<0.01
Myctophids	<0.01			<0.01
Unidentified teleosts	<0.01			<0.01
Molluscs	**1.52**	**0.01**	**0.05**	**0.31**
Illex coindetii	0.98	0.01	0.01	0.21
Sepiolids	0.01			<0.01
Todaropsis eblanae			0.01	<0.01
Unidentified ommastrephid spp	<0.01			<0.01
Gastropods	<0.01			<0.01
Crustaceans	**0.30**		**<0.01**	**0.05**
Squilla spp. (larvae)	0.05		<0.01	0.01
Medorippe lanata (larvae)	<0.01			<0.01
Decapod larvae	0.02			<0.01
Portunids	<0.01			<0.01
Portunid larvae	<0.01			<0.01
Streetsia spp.	<0.01			<0.01
Gammarids	<0.01			<0.01
Percnon gibbesi (larvae)	<0.01			<0.01
Carideans	<0.01		<0.01	<0.01
Brachyscelus sp.	<0.01			<0.01
Unidentified crustaceans	0.01		<0.01	<0.01
Cnidarians	**<0.01**			**<0.01**
Siphonophorans	<0.01			<0.01
Trachaeophytes			**<0.01**	**<0.01**
Zostera sp.			<0.01	<0.01
Exp (H')	2.76	1.67	1.40	1.99

Valencia

	%AI			
Preys	**2012**	**2013**	**2014**	**Overall**
Teleosts	**85.58**	**96.43**	**80.16**	**91.10**
Engraulis encrasicolus	37.50	66.28	70.18	63.03
Clupeids	46.21	26.30	6.16	24.08
Trachurus spp.		0.97	1.34	0.67
Arctozenus risso		0.03		0.01
Teleost larvae	<0.01	0.02	0.05	0.02
Unidentified teleosts	1.26	0.62	0.71	0.93
Molluscs	**11.99**	**1.82**	**19.56**	**7.44**
Illex coindetii	6.28	4.16	18.30	8.63
Todaropsis eblanae	1.87		2.12	0.65
Unidentified ommastrephid spp	1.79			0.12
Other unidentified cephalopods			0.90	0.08
Gastropods		<0.01		<0.01
Crustaceans	**2.43**	**1.75**	**0.28**	**1.46**
Squilla spp. (larvae)	5.09	1.14	0.06	1.56
Hyperids	<0.01	0.10	<0.01	0.03
Decapod larvae	<0.01	0.02	0.08	0.03
Scyllarus pygmaeus (larvae)		<0.01		<0.01
Carideans			<0.01	<0.01
Portunid larvae	<0.01	<0.01		<0.01
Anomuran larvae	<0.01			<0.01
Unidentified crustaceans		0.38	0.09	0.16
Cnidarians	**<0.01**		**<0.01**	**<0.01**
Siphonophorans	<0.01		<0.01	<0.01
Macrophytes		**<0.01**		**<0.01**
Algae		<0.01		<0.01
Exp (H')	4.86	3.19	4.63	4.56

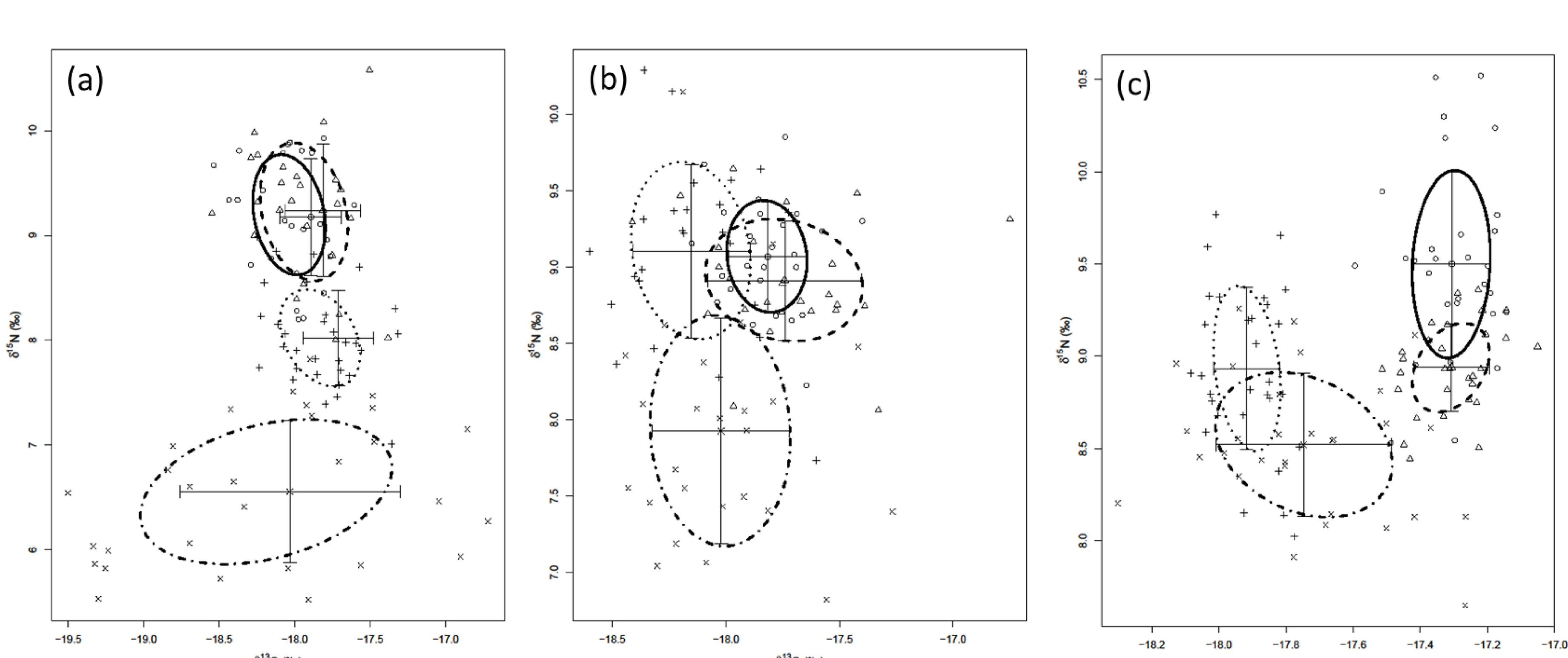

Figure 2. $\delta^{13}C$ and $\delta^{15}N$ bi-plots for age-0 ABFT tissues sampled off Cambrils (O, muscle; Δ, liver) and Valencia (+, muscle; ×, liver) in 2012 (a), 2013 (b) and 2014 (c). The lines enclose the standard ellipse areas (SEAc) estimated for tissue and location: muscle from Cambrils (solid lines), liver from Cambrils (dashed lines), muscle from Valencia (dotted lines), and liver from Valencia (dotdashed lines). SEAc was calculated by analysis of stable isotope Bayesian ellipses using the software package SIBER (Jackson et al. 2011) of SIAR (Parnell et al. 2010).

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MATERIALS AND METHODS

Age-0 (3-4 month old) ABFT (n=324) were caught by trolling line baited with lure during daylight hours in September and October through three consecutive years (2012-2014) in nearshore waters off Cambrils and Valencia (Fig. 1). The stomachs were cut open and their contents removed and blotted dry. The prey items were identified to the lowest possible taxon, grouped in taxonomic categories and weighed to the nearest 0.01 g (Table 1).

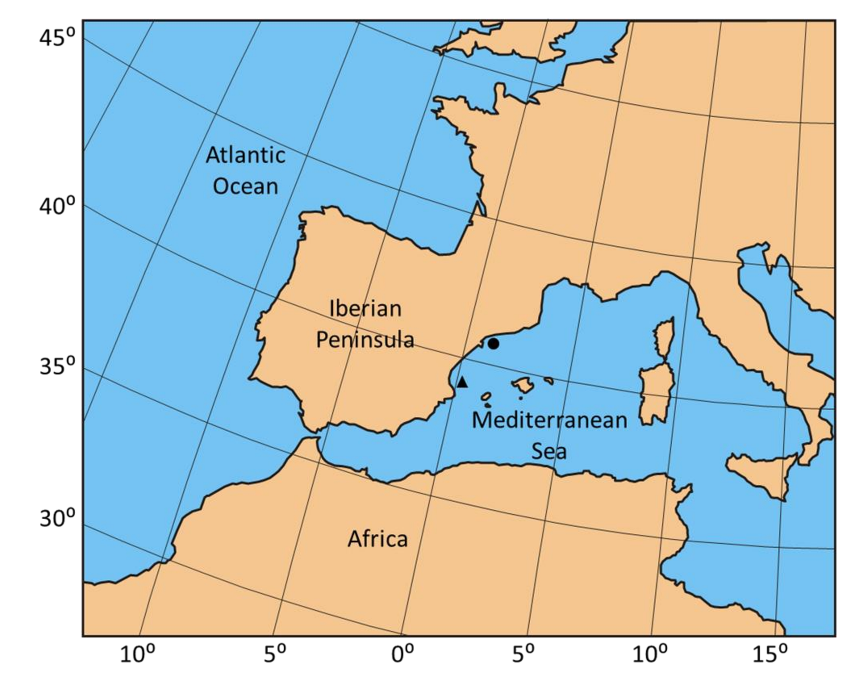

Figure 1. Approximate location of samplings off Cambrils (●) and Valencia (▲).

To determine the local baseline isotopic composition, single zooplankton samples were collected off Cambrils and Valencia in July 2013 with a Bongo plankton net (BG-40) geared with 333 µm and 250 µm meshes. Five hundred copepod individuals were sorted onboard from each zooplankton sample and rinsed with distilled water prior to storage at -20° C.

Muscle and liver ABFT samples and copepod samples were freeze-dried, powdered and subjected to BSIA as in Varela et al. (2012). Copepod samples and tissue samples from 5 of the ABFT individuals collected at each location were were analysed for AA C and N stable isotope ratios at the University of California Davis Stable Isotope Facility according to the procedure outlined by Yarnes & Herszage (2017).

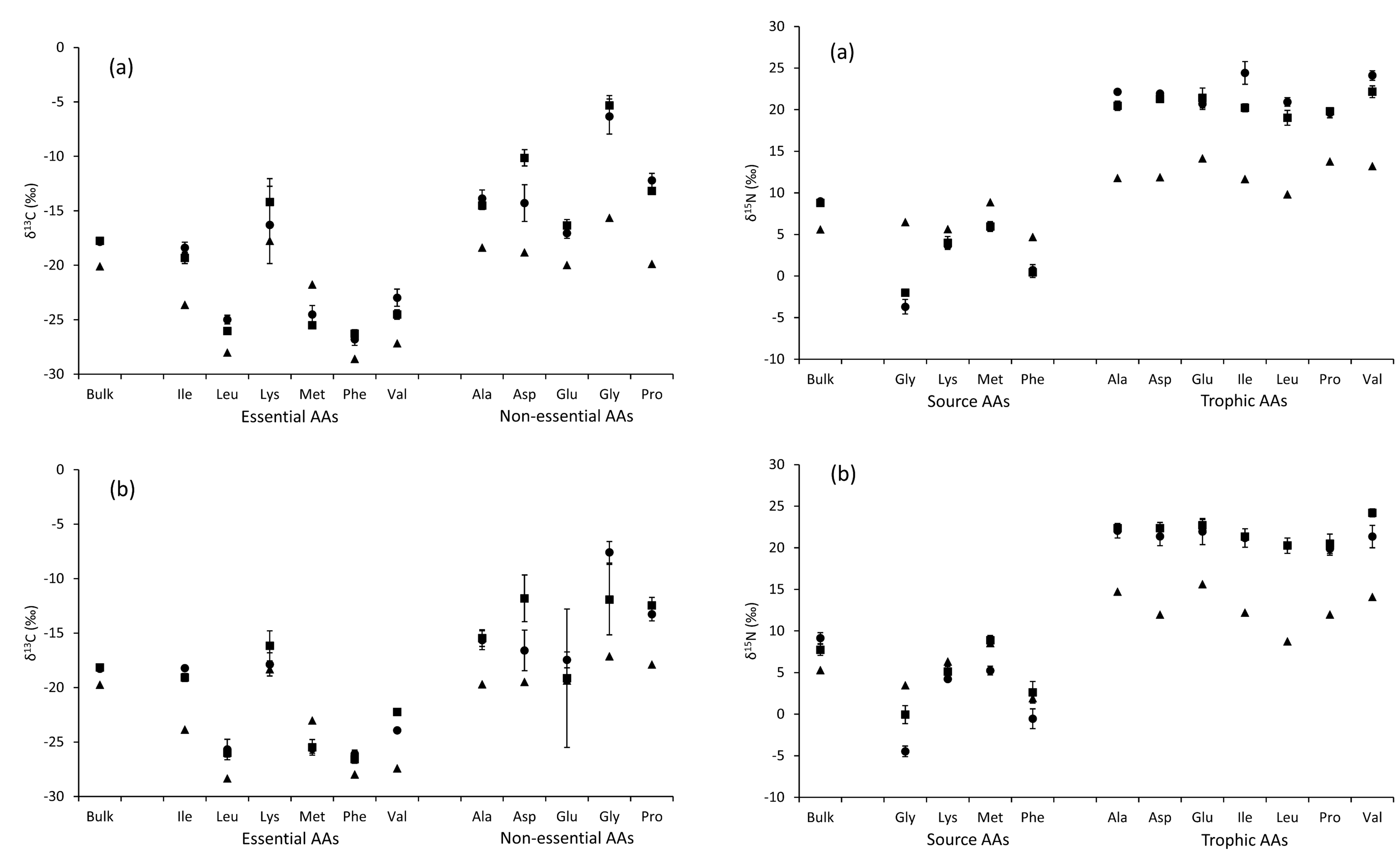

Figure 3. $\delta^{13}C$ and $\delta^{15}N$ values of bulk muscle and individual amino acids (essential and non-essential AAs) of planktonic copepods (triangles), and muscle (circles) and liver (squares) samples of age-0 ABFT collected off Cambrils (a) and Valencia (b) in 2013. Ala, alanine; Asp, aspartic acid; Glu, glutamic acid; Gly, glycine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Val, valine. Bars indicate standard deviation.

Table 2. Trophic positions estimated from stomach content analysis (TP_{SCA}; Christensen and Pauly, 1992), bulk-tissue stable isotope analysis (TP_{BSIA}; Olson et al., 2010), and amino acid compound-specific stable isotope analysis based on $\delta^{15}N$ values of glutamic acid and phenylalanine ($TP_{AA-CSIA}$; Chikaraishi et al., 2009; Bradley et al., 2014) .

	TP_{SCA}		TP_{BSIA} (mean ± SD)				$TP_{AA-CSIA}$ (mean ± SD)	
	Cambrils	**Valencia**	**Cambrils**		**Valencia**		**Cambrils**	**Valencia**
			Muscle	**Liver**	**Muscle**	**Liver**	**Muscle**	**Muscle**
2012	4.06	4.14	4.47 ± 0.38	4.27 ± 0.39	3.88 ± 0.31	2.79 ± 0.42		
2013	4.00	4.05	4.39 ± 0.25	4.06 ± 0.24	4.63 ± 0.39	3.64 ± 0.46	3.63 ± 0.17	4.03 ± 0.16
2014	4.00	4.24	4.69 ± 0.34	4.08 ± 0.15	4.51 ± 0.30	4.01 ± 0.24		
Overall	4.02	4.13	4.53 ± 0.35	4.13 ± 0.28	4.32 ± 0.47	3.48 ± 0.64		

DISCUSSION AND CONCLUSIONS

SCA shows that age-0 ABFT mainly prey on anchovy and clupeids off the Iberian Mediterranean shelf, underpinning the key role of these small pelagics in the food web.

In accordance with SCA, BSIA of white muscle and liver suggests variations in diet composition between locations and years, but could also reflect shifts in $\delta^{13}C$ and $\delta^{15}N$ values at the base of the food web.

SCA and BSIA results indicate a wider trophic niche for ABFT from Valencia, where the proportions of prey items were more homogeneous.

Estimates of the juvenile ABFT TP based on SCA and BSIA lay around 4. In contrast, the results derived from $\delta^{15}N$ values of muscle glutamic acid and phenylalanine slightly underestimated the ABFT TP.

$\delta^{13}C$ values of essential AAs may be good tracers of carbon sources, though clear patterns relative to primary consumers were not found. Patterns of $\delta^{15}N$ values of trophic relative to source AAs reflected differential fractionation rates, indicating the appropriateness of their use as TP indicators.

Integration of the different available techniques, including stomach content and isotopic analyses, increases accuracy and robustness in studies focused on diet reconstruction and trophic relationships in marine food webs. However, further experimental work is needed for more reliable conclusions, especially regarding the application of AA compound-specific stable isotope analysis (AA-CSIA).