

**LARVAL BLUEFIN TUNA (THUNNUS THYNNUS)
TROPHODYNAMICS FROM BALEARIC SEA (WM) AND GULF
OF MEXICO SPAWNING ECOSYSTEMS BY STABLE ISOTOPE**

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SUMMARY

The present study uses stable isotopes of nitrogen and carbon ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) as trophic indicators for Atlantic bluefin tuna larvae (BFT) (6-10 mm SL) in the highly contrasting environmental conditions of the Gulf of Mexico (GOM) and the Balearic Sea (MED). The study analyzes ontogenetic changes in the food sources and trophic levels (TL) of BFT larvae from each spawning habitat. The results discuss differences in the ontogenic dietary shifts observed in the BFT larvae from the GOM and MED as well as trophodynamic differences in relation to the microzooplanktonic baselines used for estimating trophic enrichment. Significant trophic differences between the GOM and MED larvae were observed in relation to $\delta^{15}\text{N}$ signatures in favour of the MED larvae, which may have important implications in their early life growth strategy.

Thunnus thynnus, larval trophic ecology, stable isotopes, Mediterranean, Gulf of Mexico.

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INTRODUCTION

Due to its highly migratory behaviour, Atlantic bluefin tuna (*T. thynnus*) (BFT) is the widest ranging species among teleosts in the pelagic ecosystems of the North Atlantic and its adjacent seas, including the Mediterranean (Fromentin and Fonteneau, 2001). Bluefin populations are separated into an Eastern and Western Atlantic stock (Block *et al.*, 2005; Fromentin and Powers, 2005), each one having distinct spawning grounds located in the Gulf of Mexico (GOM) and in the Mediterranean Sea (MED). While spawning takes place in the GOM from April to June, in the Mediterranean Sea bluefin spawning occurs from June to August (Mather *et al.* 1995; Scheffer 2001; Fromentin and Powers, 2005).

The GOM and the MED larval spawning habitats have well differentiated bio-physical and climatic characteristics (Teo *et al.*, 2007; Muhling *et al.*, 2010, 2013; Alemany *et al.*, 2010; Reglero *et al.*, 2014), but also share some common features. These include warm temperature regimes (21.5-28°C) in open sea areas where chlorophyll production is low and where a series of hydrographic features occur, (frontal systems and eddy formation), that facilitate larval retention (Muhling *et al.*, 2013). Such mesoscale structures can favor the conditions matching the “ocean triad” hypothesis (Bakun, 2006, 2013). Major BFT larval abundances appear to be linked to anticyclonic gyres or eddies in the Balearic Sea, south of the island of Menorca (García *et al.* 2005) and eddies associated with the GOM Loop Current (Lindo-Atichatti *et al.*, 2012).

The heavy isotope of nitrogen ^{15}N is enriched as it is transferred in higher trophic levels (TL), thereby providing an indicator of the trophic positioning of organisms (Minagawa & Wada, 1984; Peterson & Fry, 1987; Post, 2002). In addition, the heavy isotope of carbon can be used for determining the energy sources of larvae, since it varies significantly among primary producers which have different photosynthetic pathways. Unlike δN^{15} , δC^{13} is not strongly affected by trophic transfers (DeNiro & Epstein, 1981; Peterson & Fry, 1987; Post, 2002). The most promising development for analyzing the structure of food webs is based on the quantification of nitrogen and carbon stable isotope analysis, which provides insight in the trophic relationships between organisms.

This study thus intends to further the understanding of the trophodynamics that drive early life stages of BFT. We aimed to understand how larvae take advantage of the trophic resources of their surrounding environment through a comparative approach of contrasting BFT spawning ecosystems. The comparative trophic ecology of GOM and MED bluefin larvae was based on a stable isotope analysis of the larvae in relation to baseline feeding levels defined by two differentiated micro- and mesozooplanktonic size fractions.

MATERIALS AND METHODS

GOM BFT larvae were collected onboard NOAA's RV *Gordon Gunter* in the northern GOM during spring 2012, from April 24 to May 28, as part of an annual larval survey completed by the National Marine Fisheries Service (NMFS) Southeast Area Monitoring and Assessment (SEAMAP) Program (Fig. 1A). The MED BFT larvae were sampled during summer 2013 (June 19 to July 13) in the Balearic Sea, Western Mediterranean (Fig. 1B) onboard the RV *Socib* as part of the Assessment of the Atlantic Bluefin Tuna population breeding in the western Mediterranean project (ATAME). In the GOM, fish larvae were sampled by towing the net between 0 and 10 meters for 10 minutes using a 505 μ m mesh net attached to a standard 1 x 2 meter neuston frame, whereas in the MED, a squared-mouth Bongo frame of 0.9 meter was used for subsurface tows. General Oceanics 2030 flowmeters were placed at the center of the net's mouth to calculate the water volume filtered.

BFT larvae were sorted from plankton samples immediately after retrieval of the sample. Larvae were then preserved frozen at -20 °C onboard. 49, 31 and 30 larvae were selected from East-GOM, West-GOM and MED respectively for stable isotope analysis as described in Laiz-Carrión *et al.*, 2013. Lipid correction for $\delta^{13}\text{C}$ signatures was performed following Logan *et al* 2010. To sample the planktonic component, a 20 cm diameter Bongo net was positioned above the neuston net to sample different zooplankton fractions by employing 55 and 200 μ m mesh nets, each one equipped with a General Oceanics flowmeter. Mesozooplankton (>200 μ m) samples were equally divided into two equal aliquots using a Folsom plankton sample divider. Samples from

the 55 µm mesh nets were sieved on board to separate the microzooplankton (55 to 200 µm) fraction. The samples were stored frozen at -20°C freezer.

Hydrographic data were collected at each sampling station using a Seabird 19+ CTD profiler cast at a minimum depth of 300 m for both GOM and MED.

RESULTS AND DISCUSSION

The average surface temperatures in the E-GOM and W-GOM were significantly higher than the MED (over 2-3°C); and inversely, surface salinity was higher in the MED (Table 1). The E-GOM and W-GOM also showed surface temperature differences, with the W-GOM being slightly warmer. At 100m depth, temperatures were also much warmer in the GOM areas in comparison to the MED, where at 100m depth the water masses were characteristic of deep Mediterranean water masses. The Balearic Sea water masses showed strong density fronts resulting from the encounter of distinct salinity water masses of Mediterranean and Atlantic origin. The Balearic Sea, in general has cooler waters during BFT spawning than the GOM (Alemany *et al.*, 2010) with the exception of the 2003 heat wave, averaging 23-25°C at surface. This cooler spawning regime in the MED water was confirmed by the difference of average temperatures at surface and more specifically at 100m depth (Table 1) recorded during both surveys.

With respect to zooplankton biomass differentiated by size fractions (Table 2) of the micro- and mesozooplankton components, the GOM represented a relatively richer ecosystem than the MED, which is characterized by its strong oligotrophy. The greatest differences in zooplankton biomass were observed in the mesozooplankton fraction ($p > 0.001$), where the average GOM mesozooplankton biomass can surpass 10 times the average MED values. Both spawning regions have kinetic energy being supplied by mesoscale structures that cause eddy and frontal formations (García *et al.*, 2005; Teo *et al.*, 2007; Muhling *et al.*, 2010, 2013; Reglero *et al.*, 2014). In contrast, to the shelf region, the offshore waters of the GOM although considered oligotrophic, are more productive than similar areas in the MED. This is probably due to the nutrient supply from the Mississippi River and other freshwater input, coupled with unique oceanographic conditions (i.e. Loop Current) that influence the distribution and

abundance of pelagic fishes (Richards et al., 1989, 1993 Rooker et al., 2006 Wells and Rooker, 2009).

While no differences were observed between the E-GOM and W-GOM BFT larval standard length (SL) vs dry weight (DW) relationship, the BFT larvae from the MED and GOM (East and West) showed significant differences (Fig. 1). The Med BFT larvae had higher DW by SL (ANCOVA, $F_{2, 116} = 125.5$; $p < 0.001$) than either east or west GOM larvae (ANCOVA, $F_{2, 116} = 130.9$; $p < 0.001$). Such differences seem to indicate differentiated larval growth strategies between spawning ecosystems which presumably favors the growth of larvae in the GOM due to greater feeding availability and the higher temperature regime of the GOM spawning habitat. During the 2003 Mediterranean heat wave, the 2003 BFT cohort grew faster not only in SL but in DW in a notoriously oligotrophic year in comparison to the 2004-2005 BFT cohorts (García *et al.*, 2013). Further daily growth studies will be necessary to corroborate this hypothesis.

The $\delta^{15}\text{N}$ vs $\delta^{13}\text{C}$ relationships of the BFT larvae from the three defined spawning grounds showed clearly segregated $\delta^{15}\text{N}$ signatures (Fig. 2), whereas the $\delta^{13}\text{C}$ values appeared more integrated. The lower signature of $\delta^{15}\text{N}$ of the W-GOM BFT larvae could be a consequence of increased nitrate availability in the ecosystem as a result of nutrient input from freshwater sources such as the Mississippi River. Greater nutrient availability in the ecosystem produces reduction of $\delta^{15}\text{N}$ in the trophic web (Holmes *et al.*, 2002; Montoya *et al.*, 2007).

No significant trend in $\delta^{15}\text{N}$ signatures were observed in regards to SL (Fig. 3A). Highest $\delta^{15}\text{N}$ values corresponded to BFT from the MED, followed by E-GOM and W-GOM larvae. The signatures of $\delta^{13}\text{C}$ of MED BFT were significantly lower than the GOM BFT larvae which did not show significant differences between them. However, the $\delta^{13}\text{C}$ values of the MED BFT larvae did show a significant linear increase with SL ($r = 0.49$; $p < 0.05$; $\delta^{13}\text{C} = -20.5239 + 0.1558 \cdot \text{SL}$), while alternatively, these showed significant linear decrease in the E- and W-GOM BFT larvae ($r = -0.44$; $p < 0.05$; $\delta^{13}\text{C} = -17.1609 - 0.2339 \cdot \text{SL}$ and $r = -0.40$; $p < 0.05$; $\delta^{13}\text{C} = -17.7657 - 0.1293 \cdot \text{SL}$, respectively)(Fig. 3B). The linear increase trend in the MED larvae suggest ontogenic-related diet shifts towards energy sources of continental origin, while the GOM larval energy sources may be more related to neritic processes (Wells and Rooker, 2009).

Within this comparative study, the MED BFT showed significantly greater $\delta^{15}\text{N}$ signatures in comparison to the GOM larvae. A significant difference also occurred between both E-GOM and the W-GOM larval populations where the former group of larvae showed higher $\delta^{15}\text{N}$ signatures. Isotopic fractionation of N and C are sensitive to the differentiated habitat conditions of the general GOM ecosystem. In a much a smaller spatial scale than the GOM system, larval bullet tuna (*Auxis rochei*) stable isotope analysis of N and C have shown differences in their signatures resulting in cohort larval growth differences in relation to the nature of water masses in the Balearic Sea (Laíz-Carrión *et al.*, 2013). The highest trophic enrichment among the three established BFT groupings corresponded to the MED BFT (Fig. 4) which implies a greater trophic specialization and a greater trophic niche in these larvae (Malzahn & Boersma, 2009; Cherel *et al.*, 2010). This results in a major nitrogen efficiency through the food webs (Montoya, 2007).

This comparative study on nitrogen and carbon isotopic fractionation of BFT larvae born in the MED and GOM spawning ecosystems has shown that the environmental scenarios in which these larvae develop show significant differences in temperature regime, nutrient inputs into ecosystems that relate to primary producers and eventually in the biomass of primary consumers. BFT larvae from the MED whose waters are more oligotrophic showed higher trophic enrichment, and thus, higher trophic level (TL) in comparison with the GOM BFT larvae. Moreover, BFT larvae from each ecosystem show during ontogeny opposite dietary shifts in their diet. While MED larvae showed an increasing trend with size of $\delta^{13}\text{C}$ signatures, the GOM larvae showed a decrease with size suggesting changes in the carbon sources from neritic to oceanic origin and vice-versa. These differences stemming from the basic trophic levels of the ecosystem to the BFT larvae may pose important implications in the larval growth strategies and condition of each population, their competition for feeding resources, their exposure to co-occurring apex predator species that could influence larval survival, and thus recruitment success of BFT larvae.

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Table 1. Basic hydrographic data of the selected stations from the East-GOM, West-GOM and Med sampling areas.

5 m depth						
	Temperature °C			Salinity ‰		
	Mean±StdDv	Max.	Min.	Mean±StdDv	Max.	Min.
E-GOM	25.51 ± 0.55	26.13	24.63	36.07 ± 0.23	36.34	35.64
W-GOM	26.65 ± 0.59	27.25	25.95	36.34 ± 0.32	36.63	36.03
MED	22.98 ± 0.68	23.97	21.76	37.72 ± 0.16	38.12	37.51

100 m depth						
	Temperature °C			Salinity ‰		
	Mean±StdDv	Max.	Min.	Mean±StdDv	Max.	Min.
E-GOM	19.99 ± 0.74	20.95	19.16	36.43 ± 0.15	36.58	36.18
W-GOM	20.90 ± 0.39	21.31	20.43	36.50 ± 0.03	36.52	36.45
MED	13.40 ± 0.15	13.64	13.14	38.24 ± 0.09	38.39	38.09

Table 2. One-way ANOVA analysis of both micro- and meso-zooplankton size fraction biomass available in the selected stations for East-GOM, West-GOM and MED scenarios. Post-hoc comparisons were made using a Tukey's test. Different letters indicate a significant difference between ecosystems.

	Zooplankton Biomass (mg m ⁻³)					
	Microzoop. (55-200 µm)			Mesozoop. (>200 µm)		
	Mean±Std.Err.	Max.	Min.	Mean± Std.Err.	Max.	Min.
E-GOM	3.15 ± 0.55	7.81	1.13	44.33 ± 2.47 a	67.84	27.54
W-GOM	3.20 ± 0.90	5.21	0.39	39.21 ± 3.51 a	45.21	30.72
MED	1.71 ± 0.40	3.51	0.71	3.93 ± 2.71 b	8.65	0.19

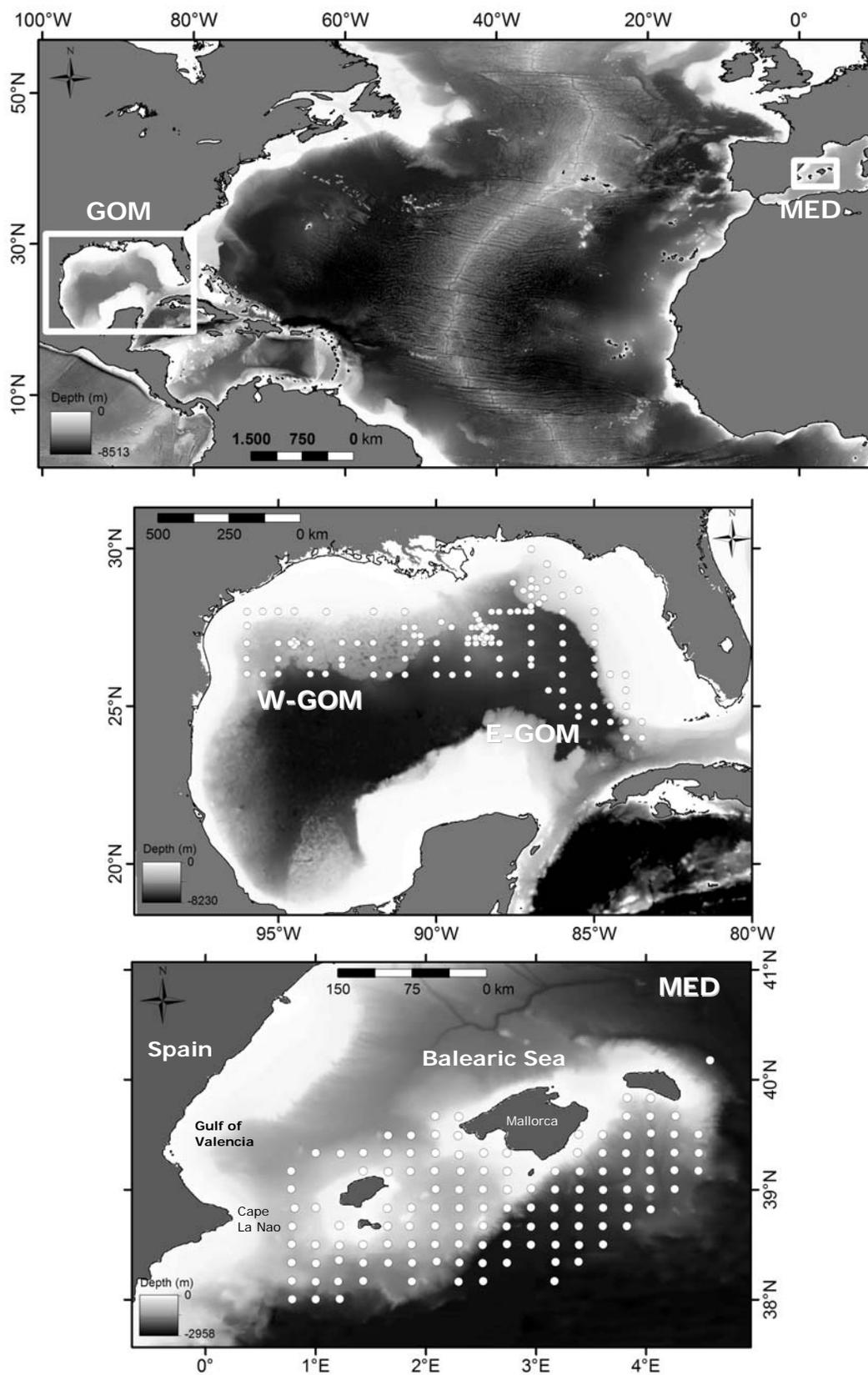


Figure 1.- Geographical location of the study areas including station map of Both East-GOM and West-GOM (A) and Med (B) BFT study area and showing the stations sampling distribution. (bathymetric image generated from ETOPO database).

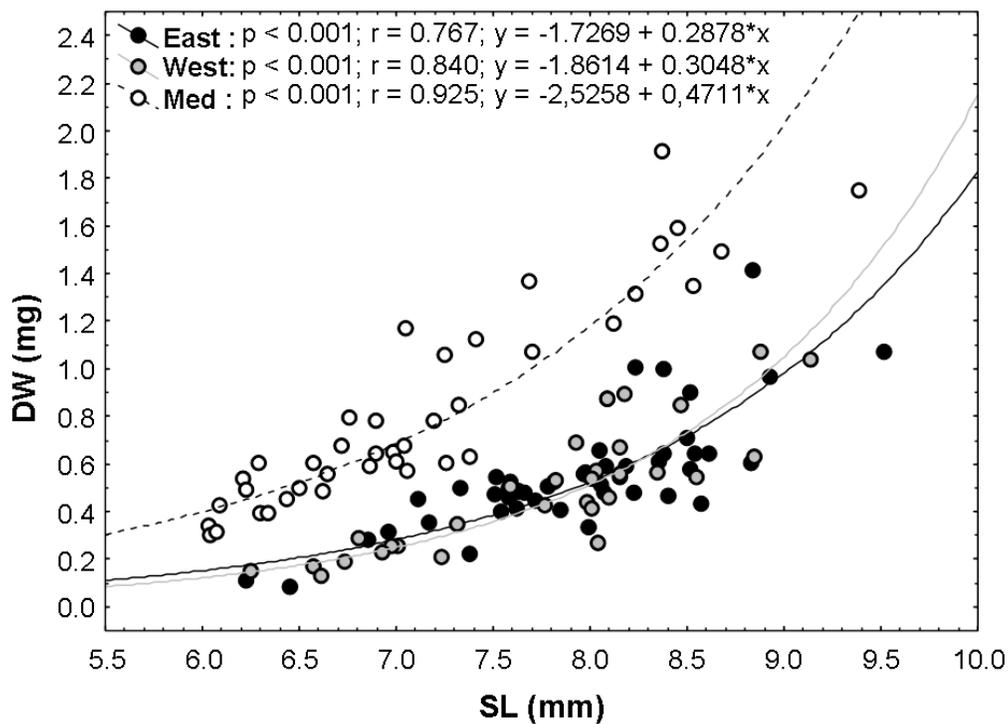


Figure 2.- BFT larval dry weight (DW) vs standard length (SL) relationships for East (black dot), West (grey dot) and Med (white dot) larval cohorts.

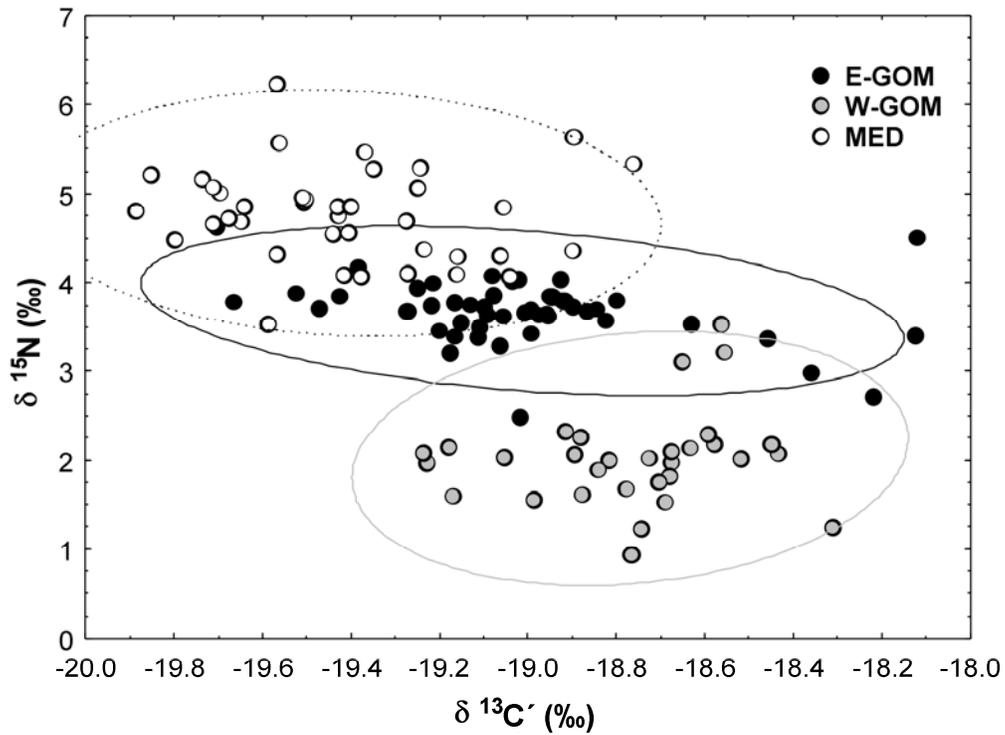
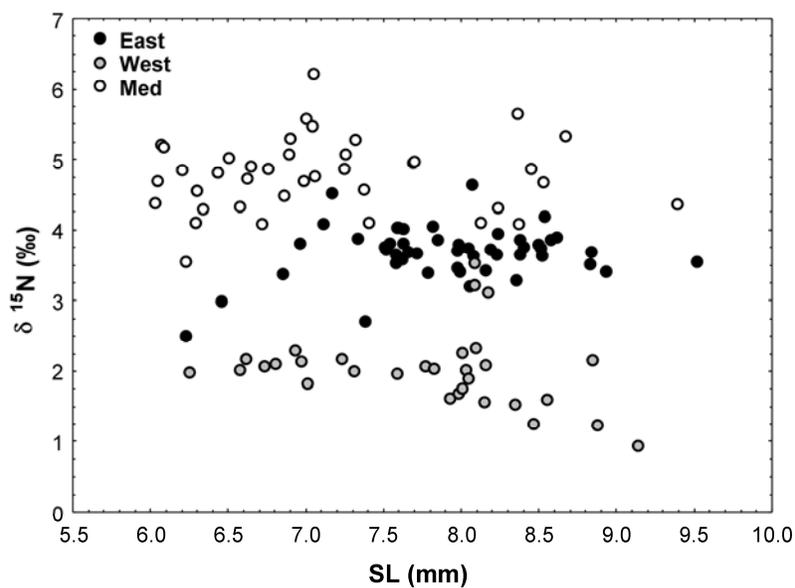


Figure 3.- Relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) in BFT larvae in East (black dot), West (grey dot) and Med (white dot) ecosystems.

A



B

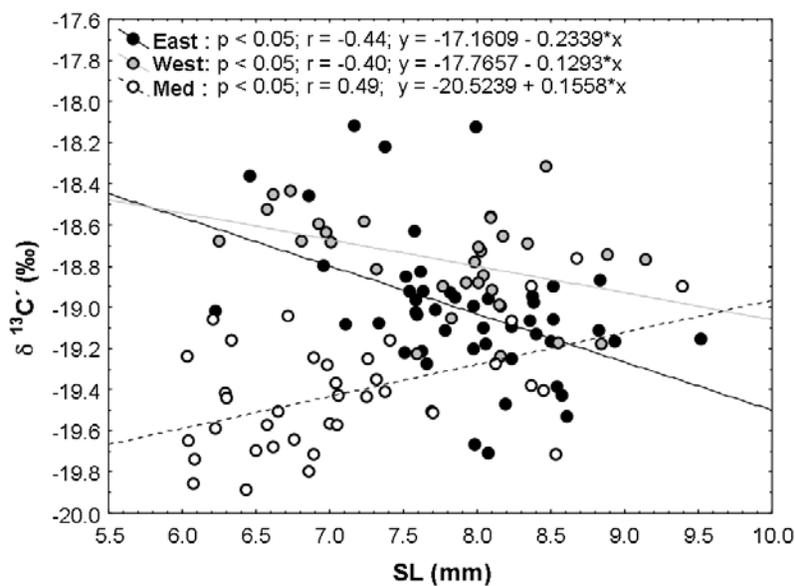


Figure 4.- (A) Nitrogen and (B) carbon-stable isotope ratios and SL relationships of BFT larvae in East (black dot), West (grey dot) and Med (white dot) ecosystems.

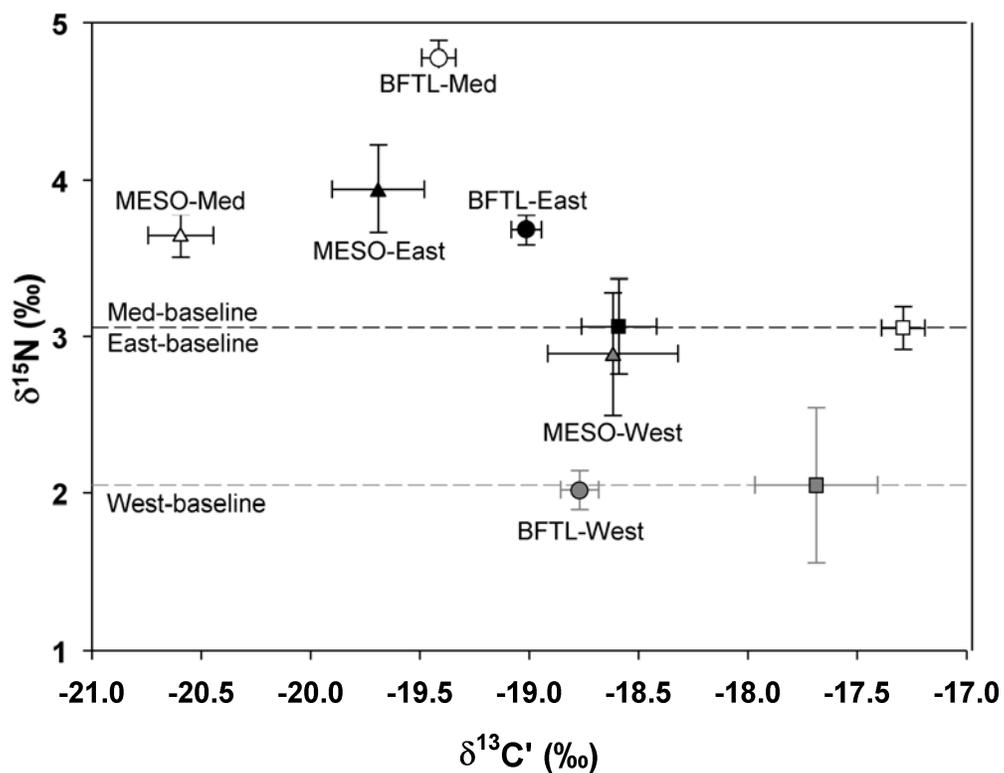


Figure 5.- Mean (\pm SE) $\delta^{13}C$ versus $\delta^{15}N$ (‰) values for microzooplankton (squares), mesozooplankton (triangles) and *T. thynnus* larvae (circles) in East (black), West (grey) and Med (white) ecosystems. Microzooplankton has been used as baseline as primary consumers.