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Estimating diets of pre-spawning Atlantic bluefin tuna from stomach content and stable isotope analyses

José L. Varela [a], Enrique Rodríguez-Marín [b], Antonio Medina [a,*]

[a] Departamento de Biología, Universidad de Cádiz, 11510 Puerto Real, Andalucía, Spain
[b] Instituto Español de Oceanografía (IEO), Centro Oceanográfico de Santander, 39080 Santander, Cantabria, Spain

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ABSTRACT

Stomach content analysis (SCA) and stable isotope analysis (SIA) coupled with isotopic mixing model analysis were used to estimate diet composition of pre-spawning Atlantic bluefin tuna (ABFT), *Thunnus thynnus*, caught by trap in the Strait of Gibraltar area. SCA provided poor information on diet as most of the stomachs appeared empty or contained only hard remains. Mixing model diet compositions estimated from muscle tissue SIA data did not show clear inter-annual variations and suggested that ABFT fed on prey that occupy high and intermediate level positions of the food web. Otherwise, diet compositions estimated from liver tissue SIA showed greater inter-annual variations and appeared to indicate that ABFT fed on prey located at lower trophic levels. The different dietary compositions inferred from muscle and liver samples were most probably due to the different turnover rates of these organs, which would provide trophic information at two distinct time scales. Our findings suggest that a combination of SCA and SIA is more suitable than using SCA alone to determine temporal and regional variations in ABFT diet composition.

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1. Introduction

The Atlantic bluefin tuna (ABFT), *Thunnus thynnus* (Linnaeus, 1758), is a large migratory fish that occurs throughout the North Atlantic Ocean and the Mediterranean Sea and exhibits physiological and morphological adaptations that enable it to exploit a wide range of pelagic environments (Collette et al., 2001; Fromentin and Powers, 2005; Mather et al., 1995). Since the 1990s ABFT stocks have become heavily depleted because of overfishing. ABFT stock assessment is currently hampered by an incomplete knowledge of the species' life history and ecology, including foraging habits. Based on stomach content analysis (SCA), studies of diet composition have been carried out in ABFT from foraging areas of the Atlantic Ocean and Mediterranean Sea (Bigelow and Schroeder, 1953; Butler et al., 2010; Chase, 2002; Crane, 1936; Dragovich, 1970; Eggleston and Bochenek, 1990; Karakulak, et al., 2009; Krumholz, 1959; Logan et al., 2011; Ortiz de Zárate and Cort, 1986). These studies describe the ABFT as a key apex predator that feeds on a great variety of fish and invertebrates, and so has been regarded as an opportunistic and generalist feeder.

Spanish traps are set in the Atlantic coast at the entrance of the Strait of Gibraltar and have been used since ancient times to catch tuna that swim close to the coastline in their "arrival" and "return" seasonal migrations in and out of the Mediterranean Sea. An eastward or "arrival" run of pre-spawning fish takes place in April and May, and a westward or "return" run of spent fish occurs in July and August (Mather et al., 1995; Rodríguez-Roda, 1964). A prior SCA of specimens captured on eastward migration in the Strait of Gibraltar area indicated that the stomachs are most often empty (Rodríguez-Roda, 1964), which has led to the conclusion that ABFT do not feed during their migration to Mediterranean spawning grounds. However, no further foraging studies have been made that support this assumption.

SCA provides useful but incomplete information of ABFT diets due to fast digestion, uneven digestion rates of different food items, and probable food regurgitation during fishing operations, which may result in a high percentage of empty, or nearly empty, stomachs (Carey et al., 1984; Chase, 2002). Furthermore, as SCA reveals only the composition of recently ingested food, tracking diet throughout a broad geographic and temporal scale requires a large number of samples across space and time. Stable isotope analysis (SIA) has been used to reconstruct ABFT diets as a suitable complement to SCA (Estrada et al., 2005; Logan, 2009; Logan et al., 2011; Sarà and Sarà, 2007). The carbon stable isotope composition ($\delta^{13}C$) is regarded as a dietary source indicator (Pinnegar and Polunin, 2000), while nitrogen isotopic ratios ($\delta^{15}N$) serve as appropriate indicators of consumer trophic position (Jennings et al., 2008; McCutchan et al., 2003; Minagawa and Wada, 1984). Both $\delta^{13}C$ and $\delta^{15}N$ can provide trophic information over weeks or months, depending on the tissue turnover rate (Gannes et al., 1998). In fishes, slow turnover tissues like muscle (Hesslein et al., 1993; MacAvoy et al., 2001) produce information on feeding at mid-time scale (months), whereas tissues with faster metabolic rates such as liver (Guelinckx et al., 2007; Suzuki et al., 2005) give information at a shorter time scale (Logan et al., 2006; MacNeil et al., 2006). Knowing the isotopic signatures of ABFT tissues and

* Corresponding author.
E-mail address: antonio.medina@uca.es (A. Medina).

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their most common prey, dietary proportions can be estimated by applying mixing models (Erhardt, 2009; Parnell et al., 2010; Phillips and Gregg, 2001, 2003; Semmens and Moore, 2008). Stable isotope mixing models are based on the potential contribution of different isotopic sources (i.e., diet components) to a mixture (i.e., consumer), and require previous knowledge of diet-tissue discrimination factors (Hussey et al., 2010).

In this study, SCA, SIA and stable isotope mixing model analysis were performed to estimate the diet composition of ABFT prior to spawning in the Mediterranean Sea, using muscle and liver tissues as distinct time-scale diet markers.

2. Material and methods

2.1. SCA

ABFT (n = 189), ranging between 143 and 262 cm in straight fork length (FL), were sampled by trap off Barbate (Gulf of Cádiz, southern Spain) in 2009 (May, 11–21), 2010 (May, 4–13) and 2011 (June, 15) as they moved to the Mediterranean Sea to spawn (Fig. 1). The stomachs were removed and stored at −20 °C until analysis. In the laboratory, every stomach was thawed and cut open, and all the contents washed through a 1-mm mesh size sieve. Identification of taxa was carried out to the lowest possible taxonomic level. The wet weight of prey items was recorded to the nearest 0.1 g, and the fish size (FL) measured to the nearest 0.1 cm. Hard part remains (fish otoliths, cephalopod beaks and crab claws) were used for identification of fully digested prey using specific taxonomic keys (Campana, 2004; Clarke, 1986; Härkönnen, 1986; Tuset et al., 2008).

2.2. SIA

White muscle and liver tissue samples were collected from 116 ABFT. Muscle samples (or the whole organism when the prey size was small) from 2 to 9 specimens of each of 13 potential prey species were also taken to predict ABFT diet composition using SIA followed by stable isotope mixing model analysis. The list of prey chosen for SIA was primarily based on the identification of hard remains in the stomach contents, and was completed with known common ABFT prey in the Iberian Atlantic (Logan et al., 2011; Ortiz de Zárate and Cort, 1986). It consisted of 9 fish species (*Trachurus trachurus*, *Trachurus mediterraneus*, *Trachurus picturatus*, *Micromesistius poutassou*, *Sardina pilchardus*, *Engraulis encrasicolus*, *Myctophum punctatum*, *Scomber scombrus* and *Scomber colias*), 3 crustaceans (*Polybius henslowii*, *Meganyctiphanes norvegica* and *Pasiphaea sivado*) and 1 cephalopod (*Illex coindetii*). Prey were collected from the Gulf of Cádiz (Fig. 1) during a research cruise carried out in March, 2009, and stored at −20 °C until use.

In the laboratory, ABFT and prey samples were thawed, rinsed thoroughly with distilled water, transferred into glass vials and freeze-dried for 48 h. Then, they were ground to powder and then separated into two subsamples, one of which was used for $\delta^{15}N$ analysis, while the other was treated three times with chloroform-methanol for lipid extraction prior to $\delta^{13}C$ analysis. Total lipid contents were calculated as described by Varela et al. (2011, 2012). Aliquots (~1 mg) of bulk and lipid-extracted samples were placed into tin capsules. The relative abundances of ^{13}C and ^{15}N (respectively, $\delta^{13}C$ and $\delta^{15}N$) were measured by continuous gas flow system using a Thermo Finnigan Elementary Analyzer Flash EA 1112 coupled to a Finnigan MAT Delta Plus mass spectrometer and expressed as parts per thousand (‰) relative to standards (Peterson and Fry, 1987).

2.3. Mixing model

A Bayesian mixing model (multiple sources, dual-isotope linear mixing-model SIAR; Parnell et al., 2010) was used to estimate the relative proportion of multiple prey species. Mixing model analyses require accurate estimates of isotopic discrimination factors between sources (prey) and mixture (consumer) tissues to reliably ascertain quantitative diet estimates (Bond and Diamond, 2011). Here we applied the prey-muscle discrimination factors previously estimated for ABFT ($\Delta^{13}C$ (‰) = −0.16 ± 0.64, $\Delta^{15}N$ (‰) = 1.64 ± 0.20; Varela et al., 2011). The prey-liver discrimination factors used were $\Delta^{13}C$ (‰) = 0.42 ± 0.34 and $\Delta^{15}N$ (‰) = 0.68 ± 0.42, based on an experimental trial carried out on farmed ABFT (unpublished data).

Fig. 1. Atlantic bluefin tuna (ABFT) were collected by trap off Barbate (Gulf of Cadiz, southern Spain) (●).

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