



## Stable C and N Isotope Composition of European Anchovy, *Engraulis encrasicolus*, from the Marmara Sea and the Black Sea

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### ARTICLE INFO

Research Article

Received : 27/01/2021

Accepted : 27/02/2021

Keywords:

Stable isotopes

*Engraulis encrasicolus*

Fish

Carbon

Nitrogen

### ABSTRACT

The aim of this study is to determine the stable isotope ratios of anchovy caught in the Black Sea and Marmara Sea. Stable carbon and nitrogen isotope ratios ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) of European anchovy (*Engraulis encrasicolus*) were estimated at four sampling sites (İğneada, İstanbul, Trabzon and Hopa) in the Black Sea and Marmara Sea (Turkey).  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of European anchovy ranged from -22.31 to -19.19 ‰ and from 3.81 to 12.79 ‰, while C/N ratios ranged from 2.01 to 6.21 in muscle tissue of European anchovy, respectively. İğneada station had more depleted  $\delta^{13}\text{C}$  values and more enriched  $\delta^{15}\text{N}$  values than other stations. This difference might be due to the terrestrial input and agricultural activities in this region.

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## Introduction

Stable isotope analysis (SIA) can be used as ecological tracers of the diet (Peterson and Fry, 1987; Abend and Smith, 1997; Dempson et al., 2010; Kloskowski et al., 2019) and can provide information about dietary sources (Lesutiene et al., 2018). While isotope ratios of oxygen, hydrogen, sulphur, and strontium help ecological studies, stable isotopes of carbon and nitrogen are the most widely used one (Bodey et al., 2011). The stable nitrogen isotope ratio ( $\delta^{15}\text{N}$ ) have been used to define trophic levels of organisms and to estimate of the source of nitrogen (Koszelnik et al., 2008; Duarte et al., 2018; Noh et al., 2019), while the stable carbon isotope ratio ( $\delta^{13}\text{C}$ ) have been used to determine organic material sources and fluxes in biological systems (Fry and Sherr, 1984; Peterson and Fry, 1987; Owens, 1988; Einfalt et al., 2020). Stable isotope ratios of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) differ between autotrophs, and these isotopic signs taken by consumers are reflected in their tissues, at whatever trophic level they occur (Fry, 1984; Peterson, 1999; Melville and Connolly, 2003). It is also known that the isotope composition of fish tissue provides information

about the background, feeding, and movement of fish. While consumer's  $\delta^{13}\text{C}$  is enriched by 0.4 ‰ per trophic level,  $\delta^{15}\text{N}$  is increased by 3-4 ‰ per trophic level (Post, 2002; Davias et al., 2014).

The majority of aquaculture production in Turkey is obtained through catching and is supplied from a 79% portion of the marine area (Şahin et al., 2006). Most caught species of fish are European anchovy (*Engraulis encrasicolus* Linnaeus, 1758) in Turkey (TUIK, 2019). The European anchovy is a fast-growing and short-lived species that reaches its sexual maturity approximately at the first age with 9 cm length (Prodanov et al., 1997; Kideys et al., 1999; Samsun et al., 2004). It spawns in ten or more batches between May and September, and its mean individual fecundity was reported as 42000 number eggs (Polat and Ergün, 2008). European anchovy in the Black Sea is monitored as two subspecies. These are *Engraulis encrasicolus ponticus* (Black Sea anchovy) that can grow up to 18-20 cm and *Engraulis encrasicolus maeticus* (Azov anchovy) that can grow up to 15 cm (Erdoğan Sağlam and Sağlam, 2013).

Stable isotope ratios of European anchovy (*Engraulis encrasicolus*) have been studied by many researchers (Kideys et al., 1999; Wan et al., 2010; Rumolo et al., 2018). But there has been no research on this subject in Turkey. The purpose of this study is the isotopic determination of geographical variations between European anchovy samples caught in the Marmara and the Black Sea. To that end, the C and N ratios in European anchovy were determined and compared with studies in different ecosystems.

## Material and Method

### Sampling Area

European anchovy samples were collected from four different locations in November, 2019. The sampling locations included: Trabzon (SC, 41°08'21.9"N, 39°24'52.7"E), Hopa (SD, 41°24'51.1"N, 41°24'29.8"E) and İğneada (SA, 41°52'06.5"N, 28°00'48.5"E) in the Black Sea and İstanbul (SB, 41°09'26.0"N, 29°03'22.4"E) in the Marmara Sea (Figure 1). Samples were obtained from fishing vessels using a purse seiner in the Black Sea and the sea of Marmara. Eight fish samples collected from each station were brought to the laboratory in cold storage. All European anchovy samples were kept in a portable refrigerator and stored at -25°C until further analysis.

### Stable Isotope Analysis

White muscle tissue in fish is less variable than other tissues and is the ideal tissue in ecological studies (Pinnegar and Polunin, 1999). A white muscle portion close to the dorsal fin of the fish was removed and dried at 60°C for 48 hours (Buhan et al. 2018). The dried samples were ground to a fine powder (< 6µm) using a mortar and pestle. To remove carbonate from the samples, it was acidified dropwise with 1 molL<sup>-1</sup> HCl until no CO<sub>2</sub> was released. The acidified samples were re-dried at 60°C without rinsing and pulverized again (Wang et al., 2015). For the measurement of stable C and N analysis rates, 0.15 mg of powdered samples were weighed and were placed in tin capsules. Stable isotope ratios were calculated using the equation below (1);

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000 \quad (1)$$

Where R = <sup>13</sup>C/<sup>12</sup>C or <sup>15</sup>N/<sup>14</sup>N ratio. PeeDee Belemnite (VPDB) was used as the standard for carbon, and atmospheric N<sub>2</sub> was used for nitrogen (Fry, 2006). Stable isotope ratios of carbon and nitrogen were measured with a Thermo-Electron Flash EA 2000 elemental analyser (EA) coupled Isotope Ratio Mass Spectrometer (Thermo, Waltham, MA, USA).

C/N ratios could be used to normalize  $\delta^{13}\text{C}$  because lipid content may cause deviations in stable isotope ratios (Post et al., 2007). Since most fish in the Black Sea has high lipid content (Banaru and Harmelin-Vivien, 2009),  $\delta^{13}\text{C}$  values have been rearranged according to the formula calculated by Post et al., (2007) to prevent deviation in stable isotope ratios (2):

$$\delta^{13}\text{C}_{\text{normalised}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \times \text{C/N} \quad (2)$$

According to the results obtained, the effects of  $\delta^{13}\text{C}$  values and lipid concentration on  $\delta^{13}\text{C}$  are normalized.

### Statistical Analysis

Stable isotope ratios of muscle tissue from different locations were statistically compared. All statistical analyses were performed using the platform SPSS 21.0 software. The Kolmogorov-Smirnov test was used in order to check the normality of all data. The non-parametric Kruskal-Wallis H-test was used to determine the differences between stations.

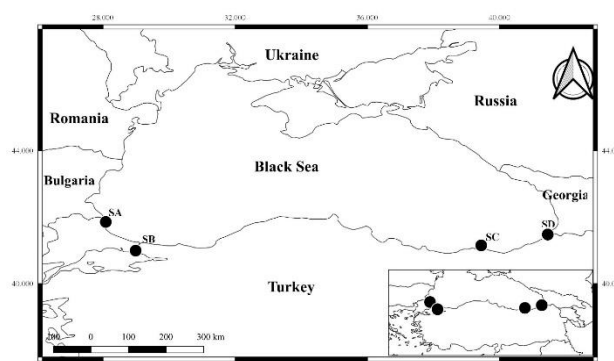


Figure 1. Sampling station of European anchovy in the Marmara and the Black Sea

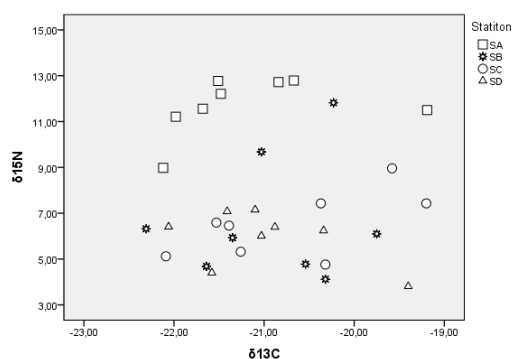


Figure 2. Carbon and nitrogen stable isotope biplots of European anchovy from SA (İğneada), SB (İstanbul), SC (Trabzon), and SD (Hopa)

## Result

Total organic carbon (TOC %), total nitrogen (TN %), and stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) were evaluated in the muscle tissue of European anchovy caught the Marmara and Black Sea. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of European anchovy showed significant differences in the location (Figure 2).

Findings show that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values ranged from -22.31 to -19.19‰ and from 3.81 to 12.79‰, respectively, while C/N ratios ranged from 2.01 to 6.21 in muscle tissue of European anchovy. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of fish varied from -22.12‰ to -19.19‰ and from 8.98‰ to 12.79‰ for SA, from -22.31‰ to -19.75‰ and from 4.12‰ to 11.82‰ for SB, from -22.09‰ to -19.20‰ and from 4.76‰ to 8.96‰ for SC, and from -22.06‰ to -19.40‰ and from 3.81‰ to 7.15‰ for SD, respectively.

Table 1. Stable isotope values (mean  $\pm$  SD) of anchovy in different area.

Location	Species	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Sagami Bay <sup>1</sup>	<i>Engraulis japonicus</i>	84	-17.4 $\pm$ 2.0	11.2 $\pm$ 2.0
Kashima Nada <sup>1</sup>	<i>Engraulis japonicus</i>	4	-17.9 $\pm$ 1.6	10.1 $\pm$ 1.4
Suruga Bay <sup>1</sup>	<i>Engraulis japonicus</i>	5	-17.4 $\pm$ 0.2	11.0 $\pm$ 0.2
the Bay of Biscay <sup>2</sup>	<i>Engraulis encrasicolus</i>		-18.4 $\pm$ 0.4	9.8 $\pm$ 0.6
Cabras Lagoon <sup>3</sup>	<i>Engraulis encrasicolus</i>			16.4 $\pm$ 18.8
Gironde estuary <sup>4</sup>	<i>Engraulis encrasicolus</i>			9.1 $\pm$ 0.6
Mediterranean Sea <sup>5</sup>	<i>Engraulis encrasicolus</i>	171	from -18.66 to -16.18	5.97-11.38
İğneada <sup>6</sup>	<i>Engraulis encrasicolus</i>	8	-21.18 $\pm$ 1.53	11.72 $\pm$ 1.27
İstanbul <sup>6</sup>	<i>Engraulis encrasicolus</i>	8	-20.91 $\pm$ 1.52	6.68 $\pm$ 2.69
Trabzon <sup>6</sup>	<i>Engraulis encrasicolus</i>	8	-20.72 $\pm$ 1.01	6.51 $\pm$ 1.42
Hopa <sup>6</sup>	<i>Engraulis encrasicolus</i>	8	-20.98 $\pm$ 0.82	5.94 $\pm$ 1.21

<sup>1</sup>Miyachi et al. (2013), <sup>2</sup>Chouvelon et al. (2014), <sup>3</sup>Como et al. (2018), <sup>4</sup>Pasquaud et al. (2008), <sup>5</sup>Rumolo et al. (2016), <sup>6</sup>This study

Mean isotopic values of sampled fish in Trabzon (SC) and in Hopa (SD) areas were similar both in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . In İstanbul (SB) area, mean  $\delta^{15}\text{N}$  was similar to SC and SD values, while mean  $\delta^{13}\text{C}$  showed a higher value. In İğneada (SA) location, mean  $\delta^{15}\text{N}$  was more enriched than all sites, while mean  $\delta^{13}\text{C}$  was more depleted than other sites (Figure 2). The C/N signatures of fish varied from 4.07 to 2.71 for SA, from 2.92 to 2.01 for SB, from 6.21 to 3.10 for SC, and from 3.74 to 2.98 for SD, respectively. It was observed that the difference was statistically significant in SB and SD stations for  $\delta^{13}\text{C}$  values and only in the SA station for  $\delta^{15}\text{N}$  values ( $P < 0.05$ ). In addition, C/N values at SC and SB stations were found to be statistically significant ( $P < 0.05$ ).

## Discussion and Conclusions

In this study, the isotopic changes of the European anchovy caught in the Black Sea and the Marmara Sea in different ecosystems were monitored. It is found that stable isotope signatures of carbon and nitrogen for anchovy were lower than the reported ones in previous studies (Table 1).

$\delta^{13}\text{C}$  values ranged from -22.31 to -19.19 ‰. The average  $\delta^{13}\text{C}$  value was -20.94‰. The results were found to be more depleted than research in the literature (Miyachi et al., 2013; Chouvelon et al., 2014; Rumolo et al., 2016). Banaru and Harmelin-Vivien (2009) also determined  $\delta^{13}\text{C}$  ratios of anchovy as low in the northern region on the Romanian coast, and they attributed this to the fact that the anchovy is less selective and may be more affected by terrestrial inputs. Chouvelon et al. (2014) reported that significantly lower  $\delta^{13}\text{C}$  values in anchovy might result from a lower trophic diet or more offshore feeding habits. Another study reported that the  $\delta^{13}\text{C}$  difference between anchovies was due to the fact that the Natal anchovy may have lived in the sea longer than Cape anchovy before it was caught (Horton and van der Lingen, 2019).

Our  $\delta^{15}\text{N}$  values ranged from 3.81 to 12.79‰ in this study. The average  $\delta^{15}\text{N}$  value was 7.71‰. The results (except for the SA station) were found to be more depleted than previous studies. The values at the SA station were in line with the results of Miyachi et al. (2013). Moreover, Anchovy feeds on plankton, such as Calanus genus Copepoda, Cirripedia, and Mollusca larvae (Bingel and Gücü, 2010). The differences in  $\delta^{15}\text{N}$  detected in Anchovy can result from different food sources (Miyachi et al., 2015). The diet of Anchovy is variable, and some anchovies can feed in high trophic positions (Pizarro et al., 2019). It has been stated in many studies that urban

development and agricultural activities that cause an increase in denitrification may cause  $\delta^{15}\text{N}$  enrichment (Wada et al., 1987; Altabet et al., 1995; Davias et al., 2014). In a study conducted on the east coast of South Africa, isotopic signs of Natal anchovy and Cape anchovy were compared. According to the results, it has been reported that the higher  $\delta^{15}\text{N}$  value of Cape anchovy than the Natal anchovy is due to the differences in its feeding types and sizes, and may indicate that it feeds at a slightly higher trophic level (Horton and van der Lingen, 2019). In addition to the average C/N ratio was 3.82. This result was similar to the research by Rumolo et al. (Rumolo et al., 2018) in the Central Mediterranean sea.

As a result, C and N isotopic signs of European anchovy specimens caught in the Black Sea and the Sea of Marmara were evaluated in this study. It is thought that European anchovy samples caught at İğneada station have more depleted  $\delta^{13}\text{C}$  values than other stations, which may be due to the effects of terrestrial inputs in the region. In addition, it is estimated that  $\delta^{15}\text{N}$  is enriched in this station more than others due to feed properties and marine effects of agricultural activities. In addition, it is concluded that it is beneficial to use more stable isotopes in the studies in order to obtain clearer results in this region.

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