Variation in feeding ecology of small pelagic fish (*Trachurus trachurus, Sardina pilchardus* and *Engraulis encrasicolus*) in the western basin and in the central Mediterranean sea using stable isotope analysis

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Summary: The Horse-Mackerel (*Trachurus trachurus*), European Sardine (*Sardina pilchardus*) and European Anchovy (*Engraulis encrasicolus*) are three species of economical and ecological relevance. In this study the stable isotopes of nitrogen and carbon ($\delta^{15}N$ and $\delta^{13}C$) were examined and compared in two region of the Mediterranean sea (the Strait of Sicily and the Tyrrhenian Sea) to provide necessary knowledge on the feeding habits of the species and to assess large-scale spatial variations in its trophic ecology. Significant differences in both $\delta^{15}N$ and $\delta^{13}C$ values were found between samples collected in the Strait of Sicily and in the Tyrrhenian Sea. Correlations between $\delta^{15}N$ and $\delta^{13}C$ values with body length were stronger in the Strait of Sicily than in the Tyrrhenian Sea for *T. trachurus,* while no correlation was observed in the other two species. The results of stable isotope analyzed locally showed that the diet of each species varies in dependence of resource availability, according to geographical and bathymetric differences. A further analysis of samples belonging to different sub-areas of the Tyrrhenian sea highlighted that the species likely adopts different feeding strategies. In particular, in some areas the estimated linear relationships between $\delta^{15}N$ and $\delta^{13}C$ values suggest similar isotopic baselines while in other the species seems to feed in ecosystems with different isotopic baseline.

Introduction: Small pelagic fish occupy the "middle trophic level" and play a significant role in connecting the lower and the upper trophic level (Cury et al.2000) in marine ecosystems. The most important small pelagic fish inhabiting the Mediterranean sea are horse-mackerel (*Trachurus trachurus*), European sardine (*Sardina pilchardus*) and European anchovy (*Engraulis encrasicolus*) These resources have a wide geographical distribution and are commonly found on the continental shelf. In this study we used stable isotopic analysis to allow investigate changes in the feeding strategies or feeding behavior of the species. This method is based on assimilated food and gives information over a relatively long period of time (30-40 days) (Jennings 2002, Post 2002, Boecklen 2011). In particular stable isotope of nitrogen ($\delta^{15}N$) are indicators of trophic positions because consumers are predictably enriched in $\delta^{15}N$ to their food (Post, 2002). Stable isotope ratio of carbon ($\delta^{13}C$) are used as indicators of the feeding area because are useful to tracing the origin of the prey consumed (Michener & Lajtha 2007). Nitrogen is strongly fractionated during dietary assimilation, with a mean trophic increase about 3‰ compared to 1‰ in carbon. To assess large-scale spatial variations in the trophic ecology and provide necessary knowledge on the feeding habits of these species were compared the stable isotopes of nitrogen and carbon ($\delta^{15}N$ and $\delta^{13}C$) in two region of the Mediterranean sea (the Strait of Sicily and the Tyrrhenian Sea). These areas are characterized by different environmental conditions, related to the different climatic and hydrological situations as well as terrigenous input and difference platform. The variability in habitat condition, may produce differences in the physiology of fishes of the same species and consequently also in the isotope intake.

Materials and Methods

Sampling took place on board of a R/V equipped with a midwater pelagic trawl net. The fish samples were immediately frozen at -20 ° C on board. Once in the laboratory at IAMC-CNR for each specimen were recorded total and standard length (1 mm), and total weight (0.01g). For the isotopic analysis of nitrogen and carbon a portion of the dorsal muscle was extracted from each individual and it was placed in the oven at 60 ° C for 24-48 hours. After drying, the muscle was pulverized with a mortar and pestle and weighed to the nearest (0.01 mg). An amount around 0.5 mg of sample for each isotopic measurement was obtained. The isotopic analyses were performed with an elemental analyzer (EA) "ThermoFisher Flash EA 1112" interfaced to the mass spectrometer (IRMS) "Thermo Electron Delta Plus XP. As a standard of analysis was used UREA, with a certificate elemental and isotopic value (TN (%) = 46.65% and TOC (%) = 20%; 0.02 ‰ $\delta^{15}N$ and $\delta^{13}C$ -47.37 ‰). The isotopic values of carbon and nitrogen are expressed in delta notation (δ) in parts per million (‰) according to international standards, respectively "*Vienna Pee Dee Belemnite*" for $\delta^{13}C$ and atmospheric nitrogen (N_2) for $\delta^{15}N$. A minimum of three replicates were analysed for each fish. The relationship between the proportion of total organic carbon (TOC) and total nitrogen (TN%) allowed to evaluate the lipid

content in the samples analyzed. For specimens with C/N ratio > 3.5, the $\delta^{13}C$ values were normalized for lipid concentration according to Post et al., (2007).

Results and Discussion

Although no relationship with body length was recorded between $\delta^{15}N$ and $\delta^{13}C$ values for anchovy and sardine, a significant correlation was observed in horse mackerel for both areas, stronger in the Strait of Sicily ($\delta^{15}N$; $r^2 = 0.48$; $\delta^{13}C$ $r^2 = 0.52$) than in the Tyrrhenian Sea ($\delta^{15}N$; $r^2 = 0.32$; $\delta^{13}C$ $r^2 = 0.21$).

Table 1: $\delta^{15}N$ and $\delta^{13}C$ average with standard deviations

	Study area	*$\delta^{15}N$*	*st.dev.*	*$\delta^{13}C$*	*st.dev.*
European anchovy	*Strait of Sicily*	7.79 ‰	0.71	-18.11‰	0.77
	Tyrrhenian sea	9.01‰	1.32	-17.31‰	0.46
European sardine	*Strait of Sicily*	6.90‰	0.32	-18.67‰	0.29
	Tyrrhenian sea	8.92‰	1.18	-17.73‰	0.47
Horse mackerel	*Strait of Sicily*	8.87‰	0.56	-18.53‰	0.80
	Tyrrhenian sea	9.37‰	1.16	– 18.62‰	1.33

The analysis of data in relation to its geographical position showed significant differences (Mann-Witney U test, $p<0.005$) in both $\delta^{15}N$ and $\delta^{13}C$ values between samples collected in the Strait of Sicily and in the Tyrrhenian Sea for the two of the three species (Table 1),
namely the Horse Mackerel did not displayed consistent variations ($p>0.01$). The ANOVA performed between the sub-areas (Figure 1) for anchovy and sardine showed significant differences both in $\delta^{15}N$ (respectively $F=81.86$, $p<0.01$ for anchovy and $F=155.33$, $p<0.01$ for sardine) that in $\delta^{13}C$ (respectively $F=74.4$, $p<0.01$ for anchovy and $F=3.21$, $p<0.01$ for sardine). In Horse Mackerel where a direct relationship between isotope ratios with the size has been observed, an analysis of co-variance (ANCOVA) using the software R was carried out to test the statistical significance of such a relationship. The ANCOVA results, taking the $\delta^{15}N$ as the dependent variable and the length as the independent variable, showed significant differences between the various sub-areas ($F=85.06$, $p<0.01$). All results suggest that the diet of each species may varies locally, according to geographical and bathymetric differences in resource availability. These species likely to adopts different feeding strategies belonging to different sub-areas of the Tyrrhenian sea. Furthermore, in some areas the estimated linear relationships between $\delta^{15}N$ and $\delta^{13}C$ values suggest similar isotopic baselines while in other species seems to feed in habitat with different isotopic baseline.

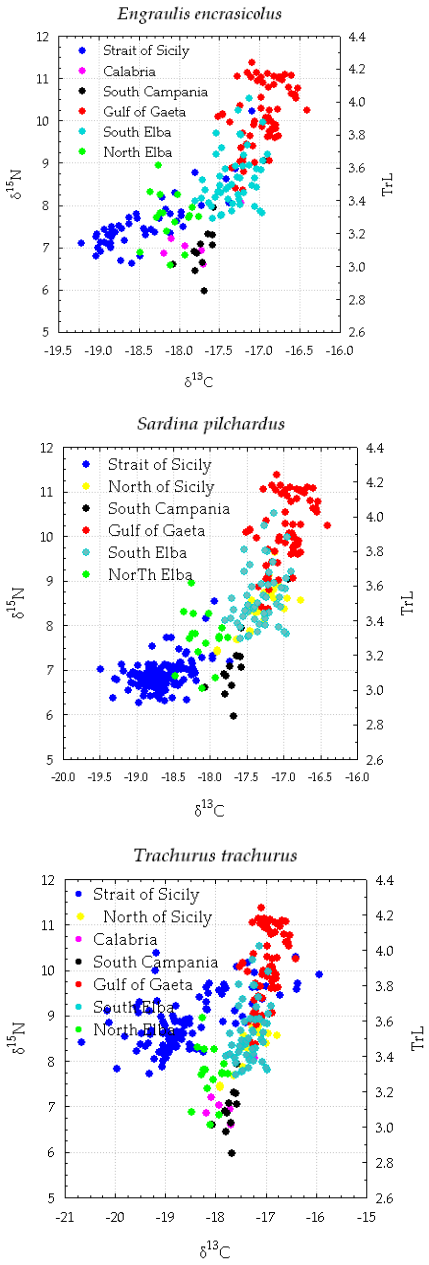

Figure 1: relationships between $\delta^{15}N$ and $\delta^{13}C$ with the trophic level (TrL) for each species: (a) E. e. ; (b) S. p. and (c) T. t .

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