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Spatial variations in feeding habits and trophic levels of two small pelagic fish species in the central Mediterranean Sea

P. Rumolo [a], A. Bonanno [b, *, 1], M. Barra [a], E. Fanelli [c], M. Calabrò [b], S. Genovese [b], R. Ferreri [b], S. Mazzola [b], G. Basilone [b]

[a] Istituto per l'Ambiente Marino Costiero, Consiglio Nazionale delle Ricerche, Calata Porta di Massa, 80100, Naples, Italy
[b] Istituto per l'Ambiente Marino Costiero, Consiglio Nazionale delle Ricerche, UOS di Capo Granitola, Via del Mare, 3 Torretta Granitola, 91021, Campobello di Mazara, Italy
[c] ENEA, Marine Environment Research Centre, Santa Teresa Pozzuolo di Lerici, 19100, La Spezia, Italy

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ABSTRACT

Trophic ecology of adults of European sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*) was examined and compared among various regions of central Mediterranean Sea.

Carbon and nitrogen stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) were adopted as a tool to determine changes in feeding behaviour of adults of sardines and anchovies. In the study period (summer) a clear geographical pattern was recognized in the isotopic composition of both species, with an increasing trend northward.

The highest variations in isotopic signal were linked to the geographical positions of the samples and, especially, between pairs of areas: South Sicily/South Campania and Gulf of Gaeta/South Elba. Higher isotope values were found in the anchovies and sardines caught in northern Tyrrhenian Sea, while lower values were mostly estimated in the southern region. Higher carbon and nitrogen isotopes may reflect a more coastal behaviour of both species, being ^{13}C-enriched source from benthic primary producers in addition to phytoplankton. Variations in the nitrogen isotope ratio may reflect not only differences in the trophic level of prey species, but also variations in the baseline level of food webs.

Our results support the hypothesis that feeding behaviour of both species is directly or indirectly influenced by local factors, or by resource partitioning based on zooplankton size. Findings can supply knowledge needed for improving fish stock management and promoting plans able to take into account also local ecosystem analysis.

1. Introduction

The Mediterranean Sea is highly heterogeneous in terms of hydrography, bathymetry and productivity (e.g. Millot and Taupier-Letage, 2005; Bonanno et al., 2014a). Even though it is generally considered an oligotrophic area, the presence of local dynamic events, especially in coastal systems, may influence the composition and spatial distribution of both plankton communities (Micheli, 1999; Mazzocchi et al., 2012) and small pelagic fishes (Tugores et al., 2011; Giannoulaki et al., 2013). Although nekton community can be highly variable in terms of species composition in different sectors of the Mediterranean Sea (Schwartzlose et al., 1999), some pelagic fishes such as European sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*) are common members in many areas of the Mediterranean (mainly in the western and central basins) giving the opportunity to assess the effects of diverse hydrological settings on their feeding behaviour. Moreover, sardines and anchovies are often caught by fishermen in the same hauls allowing researchers to hypothesize that the two species could either be competitors towards the same preys or share their "*habitat food*" (Costalago et al., 2012, 2014; Yamamoto and Katayama, 2012). Generally, the diet of fish species is analysed by means of stomach contents analysis; however, it does not provide information on trophic behaviour of the species in a long time interval, giving only a "snapshot" of the diet in a specific time or space (Tudela and Palomera, 1997; Conway et al., 1998; Pinnegar and Polunin, 1999; Morote et al., 2010).

* Corresponding author.
E-mail address: angelo.bonanno@iamc.cnr.it (A. Bonanno).
[1] URL: http://www.iamc.cnr.it.

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Recently, trophic relationships within pelagic ecosystems were examined through stable isotope analysis of muscular tissues of the fishes (Polunin and Pinnegar, 2008; Fanelli et al., 2011a; Costalago et al., 2012; Fanelli et al., 2014; Cardona et al., 2015). Stable isotope analysis, using nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) ratios, is a common tool to clarify both the relative trophic position and the main sources of food for an organism (Post, 2002). Moreover, contrary to gut contents analysis, this technique does not consider the last food intake but focuses on long-term feeding behaviour. Consumers preferentially retain heavier isotope of their diet, and then, for each trophic level, distribution of isotopic values acts as a time-averaged signature of the organism's assimilated diet. The difference in $\delta^{13}C$ or $\delta^{15}N$ between the predator's tissue and its diet is termed "Trophic Enrichment Factor" (e.g. Peterson and Fry, 1987; Post, 2002), evaluated between 2.5 and 5‰ for $\delta^{15}N$ and <1‰ for $\delta^{13}C$ (De Niro and Epstein, 1978, 1981; Vanderklift and Ponsard, 2003; Caut et al., 2009).

In this study we examined the feeding behaviour and trophic levels of adults of sardines and anchovies from different regions of the Central Mediterranean Sea, from the Strait of Sicily to the Tyrrhenian Sea, using stable isotope analysis of nitrogen and carbon.

The objective of the present study is to assess whether in different areas (i.e. areas with hydrological and coastline morphology differences) anchovies and sardines exhibit (or not) a feeding overlap in adult stage, and if their spatial variations in both feeding habits and trophic level are directly influenced by local factors.

2. Study areas

Two consecutive oceanographic surveys were carried out, from June to July 2011, in the central Mediterranean Sea (Fig. 1) on board the R/V "G. Dallaporta": the "Ancheva 2011" survey took place in the Strait of Sicily (hereafter named SS), while the "Evatir 2011" survey in the Tyrrhenian Sea (Fig. 1), divided in three sub-areas, South Campania (SC), Gulf of Gaeta (GG) and South Elba (SE). Each area is characterized by different hydrographical conditions, bathymetry and productivity. In particular, in the Strait of Sicily (SS in Fig. 1) the upper layer circulation is mainly controlled by the motion of the Atlantic Ionian Stream (AIS – Robinson et al., 1999), which induces a permanent coastal upwelling along the southern coast of Sicily (Bonanno et al., 2014b) and makes this area highly dynamic from an oceanographic point of view. The South Campania (SC in Fig. 1), characterized by a narrow continental shelf and the presence of very small rivers, is considered a low productivity area; the upper water layer circulation is mainly northward directed even though semi-permanent/recurrent cyclonic structures are observed in coastal areas (Iacono et al., 2013).

The Gulf of Gaeta (GG in Fig. 1) is a partially enclosed area, characterized by low water dynamics and a wide continental shelf. In this area two medium size rivers (Garigliano and Volturno) flow into the gulf, representing the most important (and strongly localized) source of nutrients (Rinaldi, 2012).

The South Elba area (SE in Fig. 1) is characterized by a wide continental shelf. Here the surface circulation is mainly driven by the Middle Tyrrhenian Current (MTC) approaching the coast near Rome, and is strongly influenced by Tiber river, one of the biggest Tyrrhenian rivers (Rinaldi, 2012).

3. Materials and methods

3.1. Samples collection

In each area specimens of anchovy and sardine were caught by a midwater pelagic trawl net. Overall, a total of 171 adult specimens of anchovies and 192 of sardines (Table 1) were selected from the hauls carried out in the four areas and immediately frozen on board at −20 °C.

For the aims of the present study, only adult fishes were selected and analysed. In agreement with the procedure proposed by Costalago et al. (2012), we evaluated as length threshold the minimum length at first maturity observed during the cruises in the Tyrrhenian sea and in the Strait of Sicily. In particular,

- for the Tyrrhenian sea (areas SC, GG and SE) the minimum length at first maturity was 8.5 cm for anchovy and 12 cm for sardine;
- for the Strait of Sicily (area SS) the minimum length at first maturity was 10 cm for anchovy and 12 cm for sardine.

Fish specimens longer than the above lengths were considered adults.

Between 3 and 6 stations in each area were chosen to collect zooplankton samples by means of a standard WP2 net with a 200 μm mesh size. Our choice was to collect mainly mesozooplankton (i.e., zooplankton between 0.2 and 2.0 mm), considering that adult sardines and anchovies feed mostly on mesozooplankton species (e.g., James, 1988; Plounevez and Champalbert, 1999; Raab et al., 2011; Van der Lingen et al., 2006). In each station samples were collected from the bottom to the surface, and then immediately frozen on board (−20 °C).

Water samples for Particulate Organic Matter (POM) analysis were also collected at the same stations with Niskin bottles (Fig. 1). The water samples, collected at different depths (every 25 m from bottom to surface), were filtered on board with Dispensing Pressure Vessels (10 L XX67 00P 10 Millipore Corporation) on glass filters GFF (0.7 μm nominal size). For the aims of the present study, the mean value of POM among the different depths was evaluated in each sampling station.

3.2. Stable isotope analysis

A portion of white muscle was extracted from each fish individual, oven-dried (60 °C for 24 h), powdered and weighted (0.5 mg) into tin capsules.

A qualitative analysis of zooplankton was performed in laboratory and individuals were identified to the lowest taxonomical level under a binocular (WILD Heerbrugg 6x-50x). The sorted individuals (i.e., most abundant species in each sample analysed) were washed, dried and weighted for isotopic analysis.

When possible, a minimum of three replicates was analysed for each species and site, as commonly accepted in studies like this (see Fanelli et al., 2009, 2011b). Several individuals were pooled to obtain sufficient mass (minimum weight = 0.5 mg) for the isotope measurement with the used instrumentation (see details below).

Zooplankton samples were not acidified to remove carbonate, since acidification generally reduces sample biomass leading to too little matter available for isotope analysis (Bode et al., 2004). Moreover, we preferred to avoid zooplankton samples acidification both to maintain standardization among samples (e.g. in Valls et al., 2014) and since some authors revealed negligible differences between acidified and not acidified samples (Bode et al., 2003; Bunn et al., 1995; Letessier et al., 2012; Grey et al., 2001; Jenning et al., 1997; Pomerlau et al., 2014).

Filters of POM were oven-dried at 60 °C, acidified with fumed HCl, and re-dried at 60 °C for 24 h. Then, the filters were stored in desiccators, cut into two parts and packed into tin capsules before the analysis.

Stable isotope measurements were carried out by ThermoFisher Flash EA 1112 elemental analyzer coupled to a Thermo Electron