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Ripartizione delle risorse tra piccoli pesci pelagici (*Scomber* spp., *Trachurus* spp.) nel Mare Adriatico: un approccio integrato tramite analisi dei contenuti stomacali e degli isotopi stabili

Resource partitioning among small pelagic fishes (*Scomber* spp., *Trachurus* spp.) in the Adriatic Sea: an integrated approach through stomach contents and stable isotopes analyses

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SUMMARY

RIPARTIZIONE DELLE RISORSE TRA PICCOLI PESCI PELAGICI (SCOMBER SPP., TRACHURUS SPP.) NEL MARE ADRIATICO: UN APPROCCIO INTEGRATO TRAMITE ANALISI DEI CONTENUTI STOMACALI E DEGLI ISOTOPI STABILI (Riassunto in italiano)

Questo elaborato ha come obiettivo lo studio del partizionamento delle risorse trofiche, nel Mare Adriatico, tra quattro specie di pesci pelagici tipiche di questo mare, due appartenenti al genere *Scomber* e due al genere *Trachurus*, attraverso l'analisi dei contenuti stomacali (ACS) e degli isotopi stabili (AIS) di azoto e carbonio.

I campioni di pesci sono stati raccolti durante la campagna oceanografica di survey acustico MEDIAS 2019 nelle GSA 17 e 18, a bordo della nave da ricerca "G. Dallaporta", nei mesi di giugno e luglio 2019, percorrendo da nord a sud i settori occidentali dell'Adriatico. Il prelievo dei campioni biologici è avvenuto mediante una rete volante monobarca utilizzata ad hoc per la ricerca scientifica, alternando le cale in momenti differenti della giornata e a diverse distanze dalla linea di costa. Tramite una sonda CTD, sono stati raccolti i dati sulle variabili oceanografiche, utili per individuare i possibili driver ambientali più influenti sulla dieta delle specie oggetto di questo studio. I pesci sono stati identificati a bordo al termine di ogni cala ed immediatamente congelati a -20 °C, per le successive analisi di laboratorio.

Una volta decongelati, i campioni sono stati analizzati presso i laboratori dell'Università Politecnica delle Marche e del CNR-IRBIM di Ancona. Di ciascun campione è stato prima misurato il peso e la lunghezza totale, poi il peso di organi quali il fegato, lo stomaco e le gonadi, oltre che allo stadio di maturità di quest'ultime. Si sono poi susseguite le ACS, mirate alla caratterizzazione della dieta di ciascun campione, cercando di classificare i contenuti stomacali al livello tassonomico più basso possibile. I contenuti sono stati poi contati, pesati e ne è stata misurata la frequenza di ritrovamento, per calcolare l'indice di importanza relativa (IRI) di ciascuno. Sono stati poi selezionati dei sub-campioni di pesce da tutte le cale e da questi si è prelevato un pezzo di muscolo bianco, il muscolo è stato seccato e preparato per le AIS, avvenute all'Università di Palermo. Queste hanno fornito i valori di $\delta^{13}C$ e $\delta^{15}N$ di ciascun sub-campione, oltre al %TOC e %TN, fondamentali per determinare la posizione delle specie all'interno delle reti trofiche marine.

Dall'integrazione dei risultati delle ACS e delle AIS e dal confronto con lavori condotti sulle stesse specie, in aree limitrofe e/o più distanti, sono emerse varie similitudini ma anche alcune sostanziali differenze. *Scomber colias* è risultata essere una specie con una dieta mirata a prede del compartimento pelagico, con un'evidente predisposizione al consumo di taliacei e di eufausiacei, soprattutto nei settori offshore, popolati dagli esemplari di taglia maggiore di questa specie.

Anche *Scomber scombrus*, campionato solamente in aree costiere, si è dimostrato un predatore dello zooplancton, con particolare interesse per taliacei e prede di livello trofico più alto come larve di decapodi, pesci e cefalopodi. *Trachurus mediterraneus* è risultato essere una specie che attua uno shift del compartimento di predazione con l'aumento di taglia, passando da prede pelagiche di più basso livello trofico come i copepodi a prede del benthos di livello trofico più alto come decapodi bentonici ed anfipodi gammaridei. Gli esemplari di taglia maggiore di tale specie popolano le aree costiere mentre i giovanili quelle a largo della costa. La specie *Trachurus trachurus* ha evidenziato una preferenza per le aree offshore, mentre non si è potuto osservare un evidente *shift* della dieta con l'aumento di taglia (tipico del suo congenerico), in quanto a supporto di questa ipotesi vi erano solo le AIS. Analizzando la ripartizione delle risorse tra specie è emerso che le specie di *Scomber* e di *Trachurus* hanno una dieta pressoché simile ma la sovrapposizione di nicchia trofica viene evitata grazie alla segregazione spaziale tra le due specie, evidenziata anche da altri autori, in aree diverse. Le specie di sgombri si distribuiscono nel Mare Adriatico seguendo un gradiente latitudinale, con *S. colias* maggiormente presente nei settori più meridionali e *S. scombrus* in quelli settentrionali. Nei tracuri, la possibile sovrapposizione di nicchia viene ostacolata tramite una diversa ripartizione spaziale più legata alla profondità, con gli esemplari di *T. trachurus* relegati offshore in aree meridionali di elevate

profondità e quelli di *T. mediterraneus* maggiormente presenti in aree settentrionali a basse profondità. Dalle variabili oceanografiche si è evinto come quest'ultime possano avere un ruolo importante nella ripartizione delle risorse tra le specie. Difatti, parametri quali la salinità e i valori di clorofilla un mese prima del campionamento influenzano maggiormente la dieta dei tracuri mentre, la temperatura e i valori di clorofilla diversi mesi prima del campionamento influenzano quella degli sgombri.

Le AIS combinate delle quattro specie hanno mostrato come queste abbiano una posizione trofica simile, dati i valori comparabili di $\delta^{15}N$ ottenuti, ma si distribuiscano a distanze differenti dalla costa, in quanto i valori di $\delta^{13}C$ sono poco sovrapposti. Si sono infine combinati i valori isotopici di sgombri e tracuri con quelli di specie del Mediterraneo di livelli trofici differenti, appartenenti allo zooplancton, ai piccoli pelagici, quali sardine e acciughe, e ai grandi predatori. La risultante posizione occupata dalle specie oggetto di questo studio, interposta tra acciughe e sardine e specie di livello trofico più alto come i tunnidi e il tursiope, è la conferma di come queste abbiano un ruolo centrale nel trasferimento di energia nelle reti trofiche marine, dai livelli più bassi a quelli più alti.

Chapter One

1. INTRODUCTION

1.1 Small pelagic fishes

Pelagic fishes are usually associate with the upper section of the water column and sometimes with open ocean instead of coastal ocean environments (Cushing et al., 2019). Pelagic fishes are different in form and function, ranging from small forage fishes, such as anchovies and mackerels, to large predator piscivorous fishes, such as tunas, billfishes, and sharks (Cushing et al., 2019). From an ecological point of view, small pelagic fishes like *Trachurus* spp. and *Scomber* spp. (also listed as middle-sized pelagic fishes), are located in a particular trophic level between the phyto-zooplanktivorous small-pelagic fishes (*e.g.* pilchards and anchovies) and the large piscivorous migrators (*e.g.* tuna and related species) (Bas, 1995). They play a key trophic role, transferring energy from lower to higher levels across the food web. As a consequence, their global biomass is typically smaller than basal small pelagics (pilchards and anchovies) and larger than the top pelagic predators (Rice, 1995). Small pelagic fishes share some common characteristics: several length classes and cohorts inside a population, an elevated trophic plasticity, high gregarity, swimming capability and resistance.

In the Mediterranean and Black Sea, small pelagic species as herrings, sardines and anchovies represented the largest portion (53.8%) of landings, in terms of biomass, for the three-year period 2016-2018 (FAO, 2020). Other small pelagic fishes like *Scomber* spp. and *Trachurus* spp. are not a target for most of the Mediterranean and Black Sea fisheries and represented for the same period less then 4% of landings (FAO, 2020). Few data are available for these species but they can certainly represent a crucial economical resource for small-scale and local fisheries.

1.1.1 Taxonomic classification

The integrated approach through stomach contents and stable isotope analyses of this thesis has been applied to four species of small pelagic fishes: *Scomber colias* (Gmelin, 1789) (Fig. 1.1), *Scomber scombrus* (Linnaeus, 1758) (Fig. 1.2), *Trachurus mediterraneus* (Steindachner, 1868) (Fig. 1.3) and *Trachurus trachurus* (Linnaeus, 1758) (Fig. 1.4).

The first two species are Perciformes that belong to the Scombridae (Rafinesque, 1815) family which consist of 51 widespread species including tunas and bonitos. The last two species are Perciformes belonging to the Carangidae (Rafinesque, 1815) family that has more than 140 species in every Sea of the world, like jacks, amberjacks, pompanos and leerfishes.

Fig. 1.1 *Scomber colias* (source: *Actinopterygians from the North-Eastern Atlantic and the Mediterranean-Volume I (Plates)*, 2013)

Kingdom: Animalia

Phylum: Chordata

Subphylum: Vertebrata

Superclass: Gnathostomata

Class: Actinopterygii

Order: Perciformes

Suborder: Scombroidei

Family: Scombridae

Subfamily: Scombrinae

Genus: *Scomber*

Species: *Scomber colias*

Fig. 1.2 *Scomber scombrus* (source: *Actinopterygians from the North-Eastern Atlantic and the Mediterranean -Volume I (Plates)*, 2013)

Kingdom: Animalia

    Phylum: Chordata

        Subphylum: Vertebrata

            Superclass: Gnathostomata

                Class: Actinopterygii

                    Order: Perciformes

                        Suborder: Scombroidei

                            Family: Scombridae

                                Subfamily: Scombrinae

                                    Genus: *Scomber*

                                        Species: *Scomber scombrus*

Fig. 1.3 *Trachurus mediterraneus* (source: *Actinopterygians from the North-Eastern Atlantic and the Mediterranean -Volume I (Plates)*, 2013)

Kingdom: Animalia

Phylum: Chordata

Subphylum: Vertebrata

Superclass: Gnathostomata

Class: Actinopterygii

Order: Perciformes

Suborder: Percoidei

Family: Carangidae

Genus: *Trachurus*

Species: *Trachurus mediterraneus*

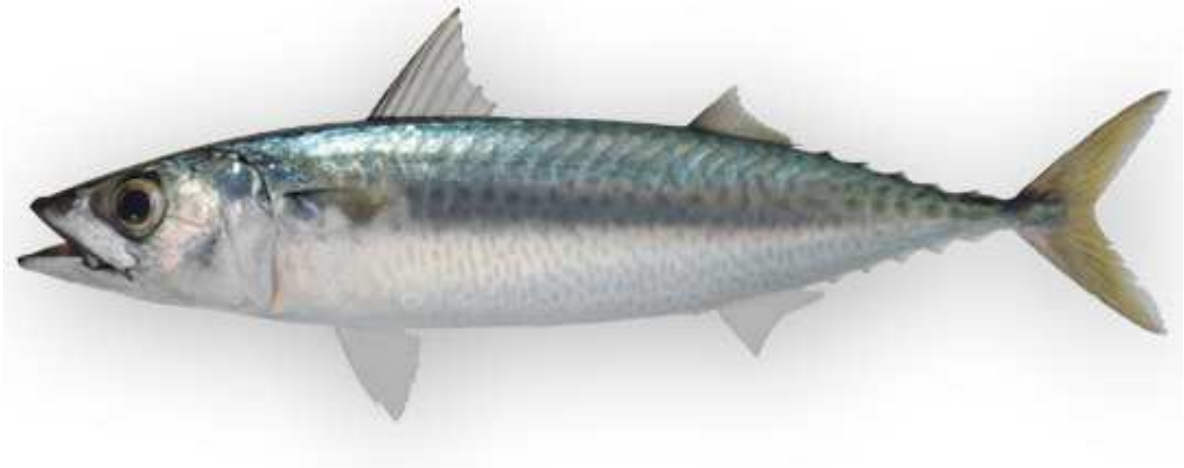Fig. 1.4 *Trachurus trachurus* (source: *Actinopterygians from the North-Eastern Atlantic and the Mediterranean-Volume I (Plates)*, 2013)

Kingdom: Animalia

Phylum: Chordata

Subphylum: Vertebrata

Superclass: Gnathostomata

Class: Actinopterygii

Order: Perciformes

Suborder: Percoidei

Family: Carangidae

Genus: *Trachurus*

Species: *Trachurus trachurus*

1.1.2 Distributions

The Atlantic chub mackerel (*Scomber colias)* is a widespread sub-tropical fish. It is very common in the warmer areas of the Atlantic Sea, on both hemispheres and coasts but absent in the north-eastern Atlantic Sea. It is also common in the warmest sectors of Mediterranean and Black Sea (Fig. 1.5). Until few years ago, this species was incorrectly named *Scomber japonicus* (Houttuyn, 1782), believing that it was the same species occurring in the Pacific Ocean. A recent study revealed the presence of two well-supported distinct clades corresponding to *S. colias* and *S. japonicus*; morphologic and genetic data are in agreement with the recognition of two different species, *S. colias* in the Atlantic, and *S. japonicus* in the Pacific (Infante et al., 2007).

On the contrary, the Atlantic mackerel (*Scomber scombrus*) is a boreal fish, oceanodromous, inhabits the Atlantic Sea, but only in the northern hemisphere and where mean water temperatures are relatively low (temperate and boreal latitudes). Although less abundant, the species is also present in the Mediterranean and Black Sea, more common in their coldest sectors (Fig. 1.6).
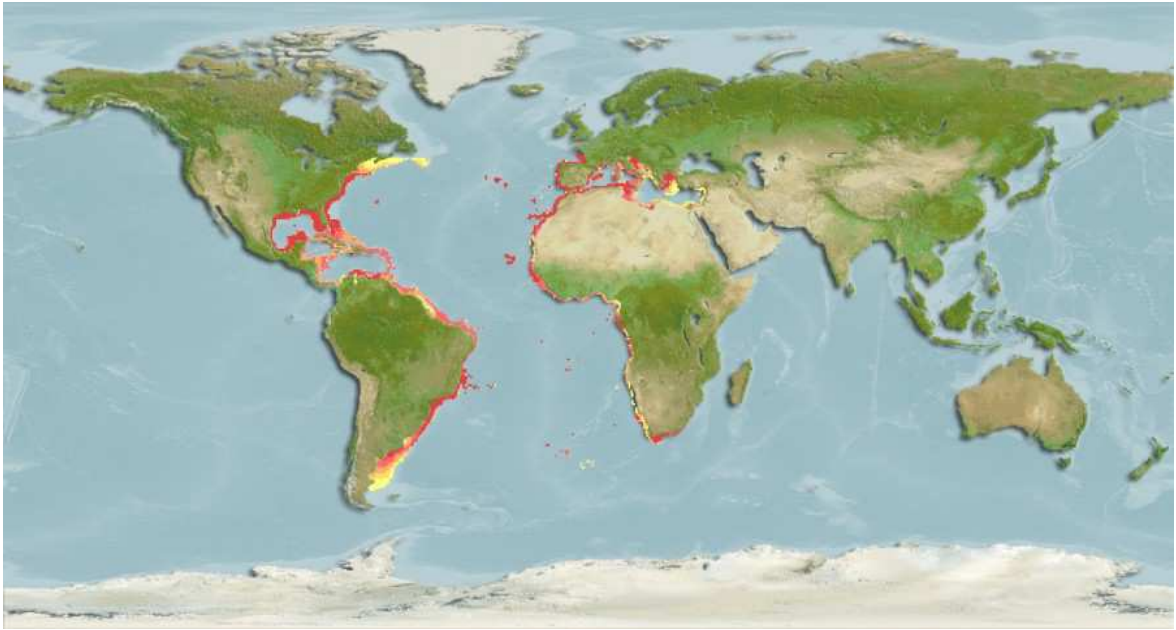
Fig.1.5 Global distribution of *Scomber colias* (source: FishBase)
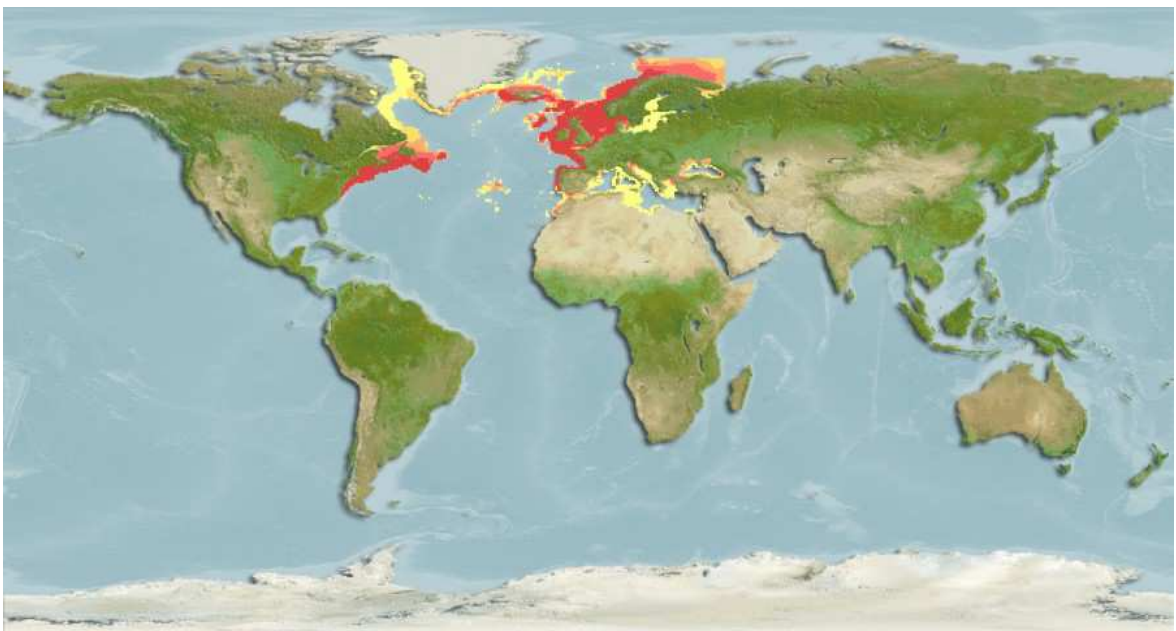
Fig. 1.6 Global distribution of *Scomber scombrus* (source: FishBase)

Trachurus mediterraneus, known as Mediterranean horse mackerel, has the most restricted distribution compared to the other three species. It is widespread in all the Mediterranean basin, less common in the Black Sea. It can be frequently found also on the eastern coast of the Atlantic Sea, but only on the northern hemisphere, from Mauritania to France (Fig. 1.7).

The Atlantic horse mackerel (*Trachurus trachurus*) is distributed all along the eastern coast of the Atlantic Sea from Norway and Iceland to South-Africa (but with lower abundances). This species is also quite common in the Mediterranean and Black Sea (Fig. 1.8).
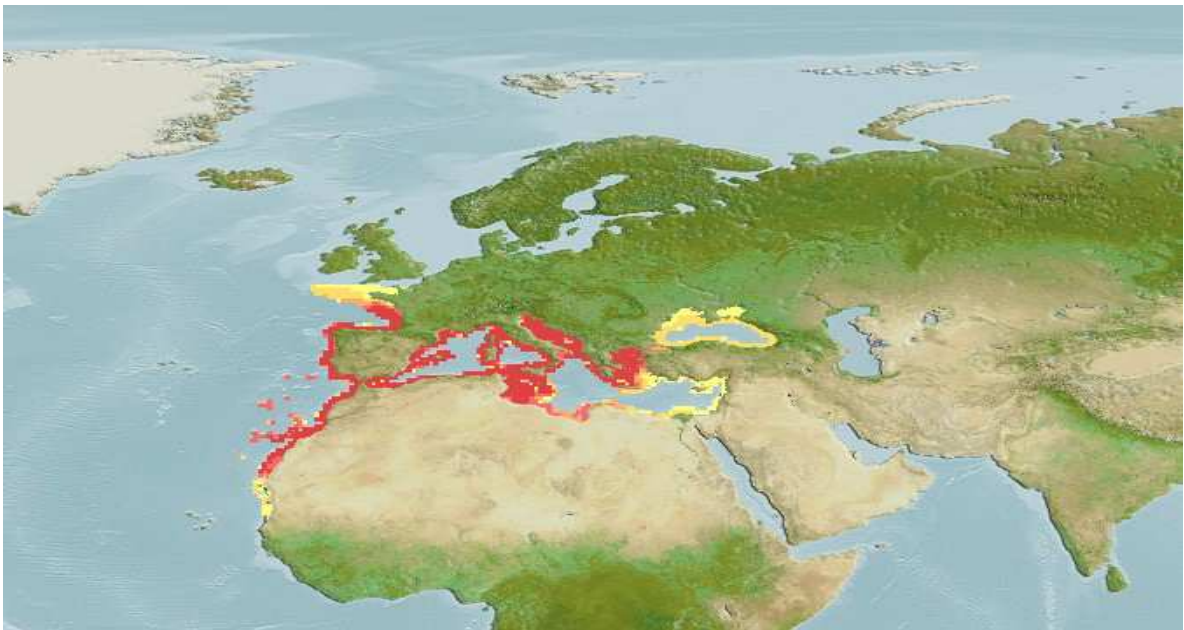
Fig. 1.7 Global distribution of *Trachurus mediterraneus* (source: FishBase)
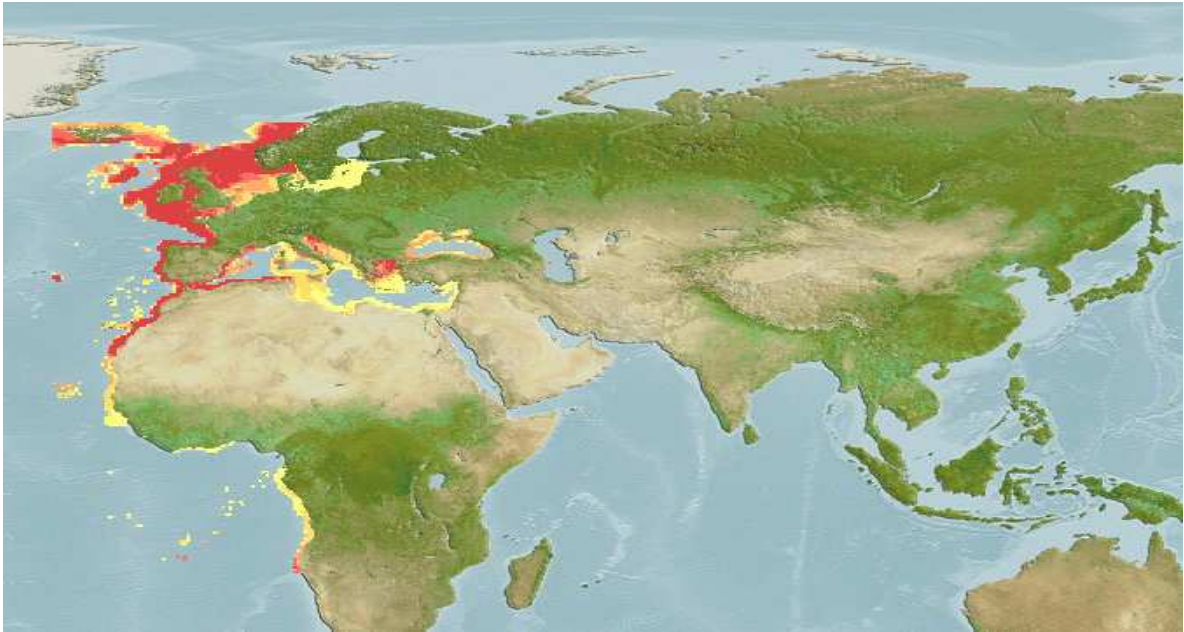
Fig. 1.8 Global distribution of *Trachurus trachurus* (source: FishBase)

1.1.3 Basic morphological distinctions

Mackerels are fishes with a hydrodynamic torpedo-shaped body, a pointed muzzle and a very thin caudal peduncle. They have two well separated and short dorsal fins, five dorsal finlets and five anal finlets. The caudal fin is forked; teeth are small and conical (Collette & Nauen, 1983). Horse mackerels share some of the essential characteristics of Carangidae Family like two dorsal fins close together (the first shorter with hard rays and the second longer with soft rays), a thin caudal peduncle with a markedly forked caudal fin and the presence of keeled and thick lateral scales (Smith-Vaniz, 1986).

Scomber colias (Fig. 1.9) has a steel-blue (sometimes greenish) back with thin and sinuous lines, sometimes yellowish shades are present. It can be easily

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recognized because of its nacreous ventral side with grayish spots and lines and for the presence of a translucent zone between eyes. Its body and its eye are taller and bigger respectively than the Atlantic mackerel ones (Louisy, 2016). *Scomber scombrus* (Fig. 1.10) has a greenish or blue back with sinuous black lines thicker than the chub mackerel ones, the ventral side evenly nacreous without spots and lines (Louisy, 2016). The translucent zone is absent. This species completely lacks the swim bladder.
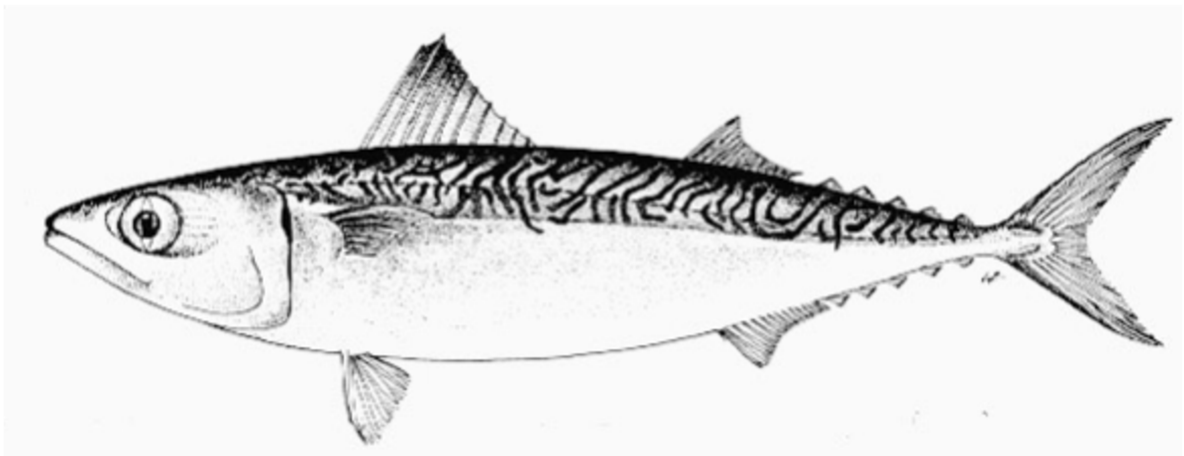
Fig. 1.9 External morphology of *Scomber colias* (source: FAO)
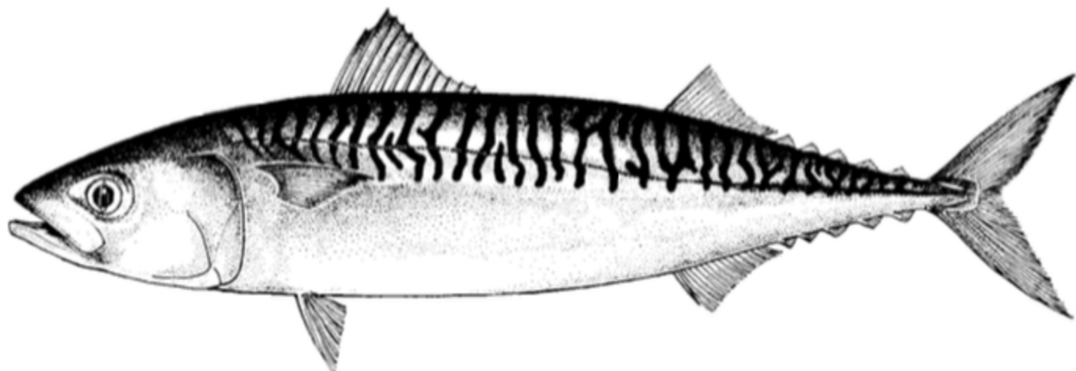
Fig. 1.10 External morphology of *Scomber scombrus* (source: FAO)

Trachurus mediterraneus body is silvery, with a brown yellowish back and golden fins. Opercular spot is almost invisible, the accessory lateral line (along the dorsal profile) ends between first and second dorsal fin. A series of lateral scales occur, taller in the posterior part and smaller than the eye diameter, cover all the lateral line (Louisy, 2016) (Fig. 1.11).

Trachurus trachurus (Fig. 1.12) has a silver body with a darker back (grey to blueish), fins are greyish or colorless and the opercular spot is normally more visible than in *T. mediterraneus*. The accessory lateral line is longer, ending soon after the second dorsal fin. In the Atlantic horse mackerel lateral scales are taller in the anterior part and greater or equal to the eye diameter (Louisy, 2016).
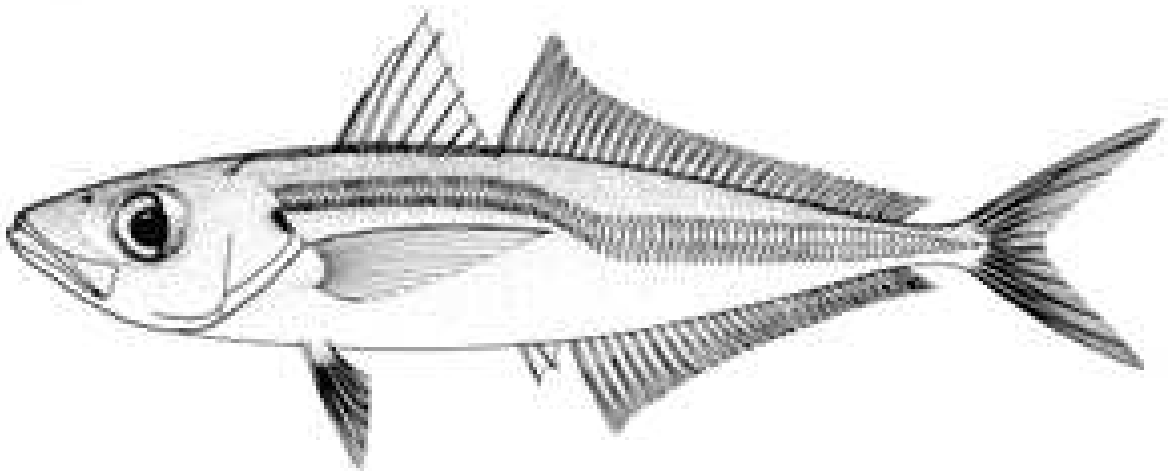
Fig. 1.11 External morphology of *Trachurus mediterraneus* (source: FAO)
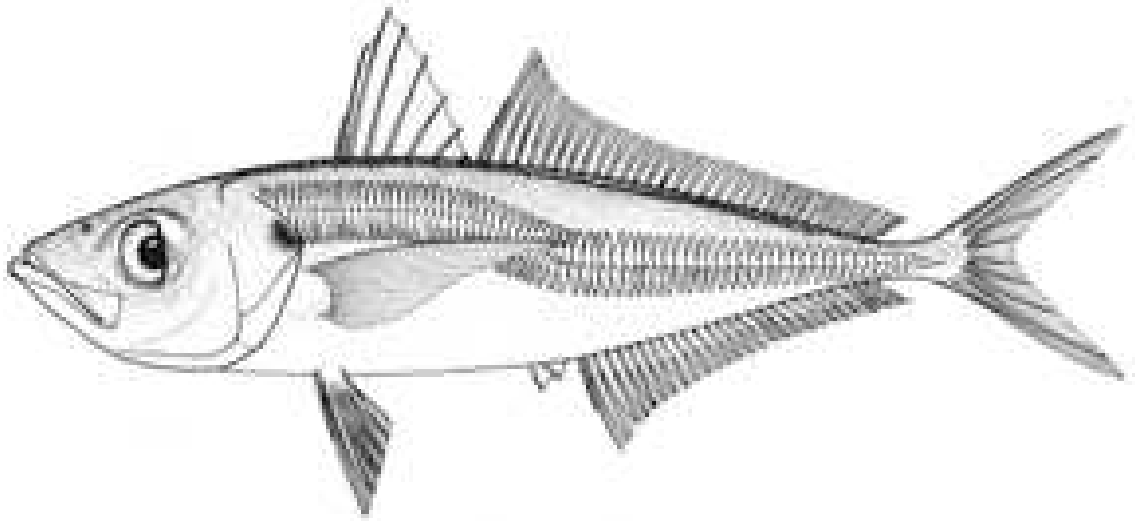
Fig. 1.12 External morphology of *Trachurus trachurus* (source: FAO)

1.1.4 Biology and ecology

Scomber colias is a pelagic, migratory, schooling fish species that privileges warm waters and usually lives between 0 and 300 meters depth (Louisy, 2016). It is a cosmopolitan species with a single panmictic population in the Mediterranean Sea and adjacent Atlantic Ocean waters (Zardoya et al., 2004). It can reach up to 55 cm length and 7 years of longevity (Velasco et al., 2011). In the Adriatic Sea mean Total Lengths (TL) ranges between 22 and 25 cm and 50% of the Atlantic chub mackerel population reaches sexual maturity at a fork length of 18.3 cm (Cikeš Keč & Zorica, 2012). *S. colias* is gonochoric, multiple batch spawner with indeterminate fecundity (Techetach et al., 2020). In the Adriatic Sea, the spawning season ranged from April until September, with peaks in June. During this period Atlantic chub mackerel migrates towards the

coastlines and channel areas for spawning (Cikeš Keč & Zorica, 2012). After spawning, adults and their offsprings, migrate offshore to the deeper and colder waters. In other areas the spawning season occurs in different periods, for example in Moroccan Mediterranean waters peaks of spawning occur in winter (Techetach et al., 2020). Driven by feeding activity, Atlantic chub mackerel is well adapted to daily vertical migrations to follow its most frequent preys, these are mostly represented by crustaceans, like hyperiids, copepods, euphausiids, mysids and decapods' larvae, thaliaceans, small fishes and fish larvae (Cardona et al., 2012; Castro, 1995; Čikeš Keč et al., 2012; Sever et al., 2006).

Conversely the Atlantic chub mackerel, there is a plenty of literature for the Atlantic mackerel, *Scomber scombrus,* in the Mediterranean. This pelagic species that lives between 0 and 250 m depth, prefers cold waters and forms large shoals off-shore that overwinter in deeper waters and migrates over great distances for reproductive and trophic needs, approaching coastal areas (Lockwood, 1988). This behavior is common in the Atlantic Sea, while it was not observed in the Adriatic Sea. Contrary to *S. colias*, this species shows genetic differentiation along the east - west axis of the Mediterranean Sea basin (Zardoya et al., 2004). The occurrence of a single panmictic population in the northern-central Adriatic Sea has been proved (Papetti et al., 2013), with a different pattern from what observed for *S. scombrus* in the southern Adriatic

Sea and the Atlantic Sea (Zardoya et al., 2004). This single population is composed of relatively small individuals, up to 40 cm TL, with a maximum of 3 years of longevity (Meneghesso et al., 2013). These maximum size and age are considerably smaller than those observed in the Atlantic Ocean, where the species can grow up to 50 cm and attain 20 years of age. The Adriatic population reaches the sexual maturity during the first year of life, with a minimum size of 20 cm (Meneghesso et al., 2013) . Atlantic mackerels from other areas attain sexual maturity later, between 2 and 3 years at sizes of 26 – 30 cm TL (Lockwood, 1988). Breeding of the single northern Adriatic population has been shown to occur in winter (mainly from January to March) (Meneghesso et al., 2013) with a migration that appear limited within the Adriatic Sea: adults migrate and converge from the northern to the central Adriatic to reach a common spawning ground (Papetti et al., 2013). The maximum fecundity estimated from the largest females collected in the Adriatic Sea was 190 000 eggs, similar to the lowest values obtained for Atlantic individuals at comparable length (Meneghesso et al., 2013).

The most intensive period for mackerel feeding occurs from April to August (Trenkel et al., 2014). Preys of the Atlantic mackerel undertake diel vertical migrations, however diel and vertical feeding patterns of mackerel remains poorly known (Jansen et al., 2019). A list of *S. scombrus* preys in the

Mediterranean and Adriatic Sea can hardly be done, due to an almost absent scientific documentation. Main Atlantic preys seem to be similar to the chub mackerel ones: a wide variety of zooplankton taxa (copepods, euphausiids, hyperiids, Decapoda's larvae, thaliaceans), fish eggs, larvae and small fishes (Bachiller & Irigoien, 2015; Cardona et al., 2012; Olaso et al., 2005; Trenkel et al., 2014).

Trachurus mediterraneus is a semi-pelagic, migratory, schooling fish species (Smith-Vaniz, 1986). Adults are located in shelf areas, from 10 to 250 m up to 400 m, are found usually near the bottom and sometimes in surface waters (Louisy, 2016; Smith-Vaniz, 1986). In the Adriatic Sea, adults are common above sandy and muddy bottoms up to 80-200 m (Jardas et al., 2004). Juveniles prefer surface layers in association with jellyfishes or other small pelagic fishes schools (Louisy, 2016). In the Mediterranean, this species reaches a maximum size of 40–60 cm and age of 10–12 years (Ragonese et al., 2005). This serial spawner has a relatively long reproductive phase that occurs in spring-summer, from May to August (Viette et al., 1997). Sexual maturity is reached at 2 years, with an average size that ranges between 16 and 19 cm (Šantić et al., 2011; Viette et al., 1997).

Trachurus trachurus also forms large schools but that can occupy a larger range of depths, reaching up to 500 m depth (especially in winter), with a strong

demersal behavior during daylight hours (Trenkel et al., 2014). In the Mediterranean however, even if it can reach those depths, it prefers coastal areas in summer (Louisy, 2016) with sandy substrate, where large schools can be found (Ragonese et al., 2005). In the Adriatic, the range of depths inhabited by adults and juveniles is the same described for the Mediterranean chub mackerel by Jardas *et al.* (2004) and Louisy (2016). Despite its wider distribution (compared to *Trachurus mediterraneus)*, low levels of genetic differentiation and stable genetic structure occur between Atlantic and Mediterranean populations (Abaunza et al., 2008). This species attains a maximum size of 40-50 cm and can reach more than 30 years of longevity (Abaunza et al., 2003). The species is a batch (multiple) spawner with an extended spawning season (up to 8 months) that varies between regions (Abaunza et al., 2003). In the Adriatic Sea spawning occurs from November to May (Šantić et al., 2013) and sexual maturity is attained by specimens of 16-19 cm (Šantić et al., 2011). In the Atlantic Sea, size and age of first maturity are respectively around 21 cm and 2-4 years (Abaunza et al., 2003) with increasing values with latitude (Abaunza et al., 2008).

For the aforementioned *Trachurus* species, diel migrations have been hypothesized, because variations in the composition of diet reflects typical vertical migrations of zooplankton (Jardas et al., 2004). Diets of both species

can be treated together as early stages of the two species occupy the same habitat, with the consequent exploitation of similar food resources and the consumption of similar types of prey (Šantić et al., 2013). Identified preys are extremely similar also in adults: planktonic copepods and euphausiids, above all. Other preys are represented by Cladocera, Mysidacea, Amphipoda, Decapoda, Mollusca, Polychaeta and several teleosts (Bayhan et al., 2013; Jardas et al., 2004; Paola Rumolo et al., 2017; Šantić et al., 2003, 2005; Trenkel et al., 2014). Almost all authors define both *Trachurus* species as planktivorous, however many benthic and suprabenthic preys have also been highlighted in recent studies (Paola Rumolo et al., 2017).

1.1.5 Fisheries

All four species are rich in protein and own high levels of "good fats" with high proportion of n-3 PUFAs, mainly DHA (Celik, 2008; El Oudiani et al., 2019). As a consequence, they should always be recommended in people diet, also considering their extremely affordable market price.

The importance of *Scomber* spp. and *Trachurus* spp. fisheries in the Mediterranean and Black Sea is secondary compared to the uppermost pelagic fishing target: herrings, anchovies and sardines in fact represent 53.8% of the total amount of landings (44.3% considering only the Mediterranean Sea)

(FAO, 2020). In the Mediterranean and Black Sea, horse mackerels represent only 2.1% of total landings, followed by the Atlantic chub mackerel with 1.1% and the Atlantic mackerel with less than 1% (FAO, 2020). Few data exist for horse mackerels as they are not apparently intensively fished, except in Turkey and maybe some southwest Mediterranean countries (FAO, 2011). Yet last reports indicated Mediterranean horse mackerel (*Trachurus mediterraneus*) having high rate of overexploitation in both the Mediterranean and the Black Sea (FAO, 2018). Catches showed an abrupt decline in the early 1990s (from around 100 000 tonnes to around 20 000 tonnes) and since then the species showed such trend up to 2018 (FAO, 2020). Furthermore, *Trachurus* spp. biomass appears to be variable (Fig. 1.13), presumably responding to environmental fluctuations and migratory attitude, making their assessment more difficult. Similar considerations on landings fluctuations and catches data apply to mackerels (*Scomber scombrus* and *Scomber colias*). However, *Scomber colias* is one of the most important commercial fish in Turkey and north-eastern Mediterranean Sea (Celik, 2008), while *Scomber scombrus* seems to be highly landed in the Mediterranean and Black Sea cold-water sectors.
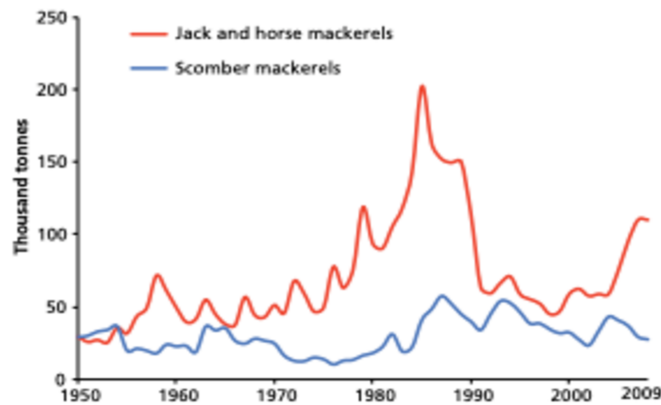
Fig. 1.13 Mediterranean and Black Sea decadal catches fluctuations of mackerels and horse mackerels (source: FAO, 2011)

The Adriatic Sea is one of the world's sea characterized by the highest fishing pressure and here mackerels are targets of multiple fisheries (IREPA, 2011). Chub mackerel is a commercially important fish that is traditionally exploited in the Adriatic Sea by purse seiners (Čikeš Keč et al., 2012). The population of *S. scombrus* from the north and central Adriatic Sea shows clear signs of overexploitation representing ca. 90% of the total scombrid landings (Meneghesso et al., 2013). Indeed a clear decline in catches in the last two decades has been highlighted (Azzurro et al., 2011; Barausse et al., 2011). Horse mackerels are an important object of pelagic and demersal fishing in the Adriatic (Šantić et al., 2003), however, landings data are very scarce. Mackerels are harvested mainly with purse-seine, pelagic and mid-water trawls and poorly with gill and trammel nets; horse mackerels are easily fished with

purse-seine and mid-water trawls, but it may occur also in bottom trawls and nets.

Fisheries management on these species is practically absent. As far as concerns the north Adriatic *S. scombrus* population, despite its particular biology, management actions including the protection of spawning grounds and spawners (*i.e.* large-sized individuals) are still absent, but urgently needed (Meneghesso et al., 2013; Papetti et al., 2013). The only management measure that should protect these species is from an European Community Regulation (Reg. EC 1967/2006) that establishes a minimum capture of individual of 15 cm and 18 cm TL, for *Trachurus* spp. and *Scomber* spp., respectively. These measures are clearly below the first maturity size for both species, thus immature specimens can legally be fished. The same Regulation includes a minimum mesh size for trawl nets, that must be of 50 mm if square or 40 mm if rhomboidal and a minimum mesh size for purse seines of 14 mm. For the Italian seas, a national regulation called "fermo pesca" (L. 19 July 1988, n. 278) is established. This foresees an annual stop of ca. one month for several types of professional fishing. This period varies according to the areas, but occurs always during summertime, from the second half of July to the first week of September. Thus, *S. scombrus* and *T. trachurus* reproduction is not covered at all, while it is only partially protected for the other two species.

1.2 The integration of stomach contents and stable isotopes analyses for trophic ecology studies

Resource partitioning, trophic relationships and prey-predator relationships in marine environments are increasingly studied through an integrated approach using the more recent stable isotopes analysis (SIA) and the traditional stomach contents analysis (SCA).

From a chemical point of view an isotope (from the Greek "same shape") is an atom that, differs from another one of the same element, because of the different number of neutrons. Some isotopes can be considered "stable", when they do not emit radioactivity, thus they can be useful and harmless in trophic ecology studies. As an example, carbon isotopes come in three forms. By far the most common isotope of carbon is carbon-^{12} (^{12}C), which contains six neutrons in addition to its six protons. The next heaviest carbon isotope, carbon-^{13} (^{13}C), has seven neutrons. Both ^{12}C and ^{13}C are called stable isotopes since they do not decay into other forms or elements over time. The rare carbon-^{14} (^{14}C) isotope contains eight neutrons in its nucleus. Unlike ^{12}C and ^{13}C, this isotope is unstable, or radioactive. For a better knowledge of marine trophic webs' structures, the most used are nitrogen δ^{15}N (^{15}N/^{14}N) and carbon δ^{13}C (^{13}C/^{12}C) (Caut et al., 2009; Post, 2002; Vander Zanden & Rasmussen, 1999).

Stable nitrogen isotope value (δ^{15}N) is a proxy of the trophic level of a species so it can be used to identify the position of a species within a trophic food web (Post, 2002; Vander Zanden & Rasmussen, 1999). The stable carbon isotope value (δ^{13}C) is more helpful to determine the origin of sources of food for an organism (Pinnegar & Polunin, 2000; Vander Zanden & Rasmussen, 1999) and to discriminate between a benthic or a pelagic origin of food, or continental *vs.* marine inputs (France, 1995) (Fig. 1.14). The difference in δ^{15}N and δ^{13}C between consumers tissues and their diet is termed Trophic Enrichment Factor (TEF) and it corresponds to values between 2.5 and 5‰ for δ^{15}N (Deniro & Epstein, 1981; Post, 2002; Vander Zanden & Rasmussen, 1999) and <1‰ for δ^{13}C (DeNiro & Epstein, 1978; Post, 2002; Vander Zanden & Rasmussen, 1999). From this, it is easy to understand why nitrogen can better clarify the trophic position instead of carbon (Fig 1.14).

Stomach contents analyses reflects the food ingested in a unique or few points in the space and in a more or less restricted time, due to fast turnover times of gut contents. So, it provides only a snapshot of the species dietary habits, showing the last food intakes. Moreover, specimens of a determined species can present an empty stomach. This is why SCA may offer a snapshot of the diet of an individual in a precise time and space, while SIA provide time-integrated information, especially if SIA is run on low turn-over rate tissues,

such as muscle or hard structures like vertebrae, otoliths etc. (Caut et al., 2009; Fanelli, Badalamenti, et al., 2011; Post, 2002; Paola Rumolo et al., 2018). With this integrated dual approach, schemes and knowledges about resource partitioning, prey-predator relationships and trophic dynamics of energy flows inside food webs can be better understood or even totally overturned.
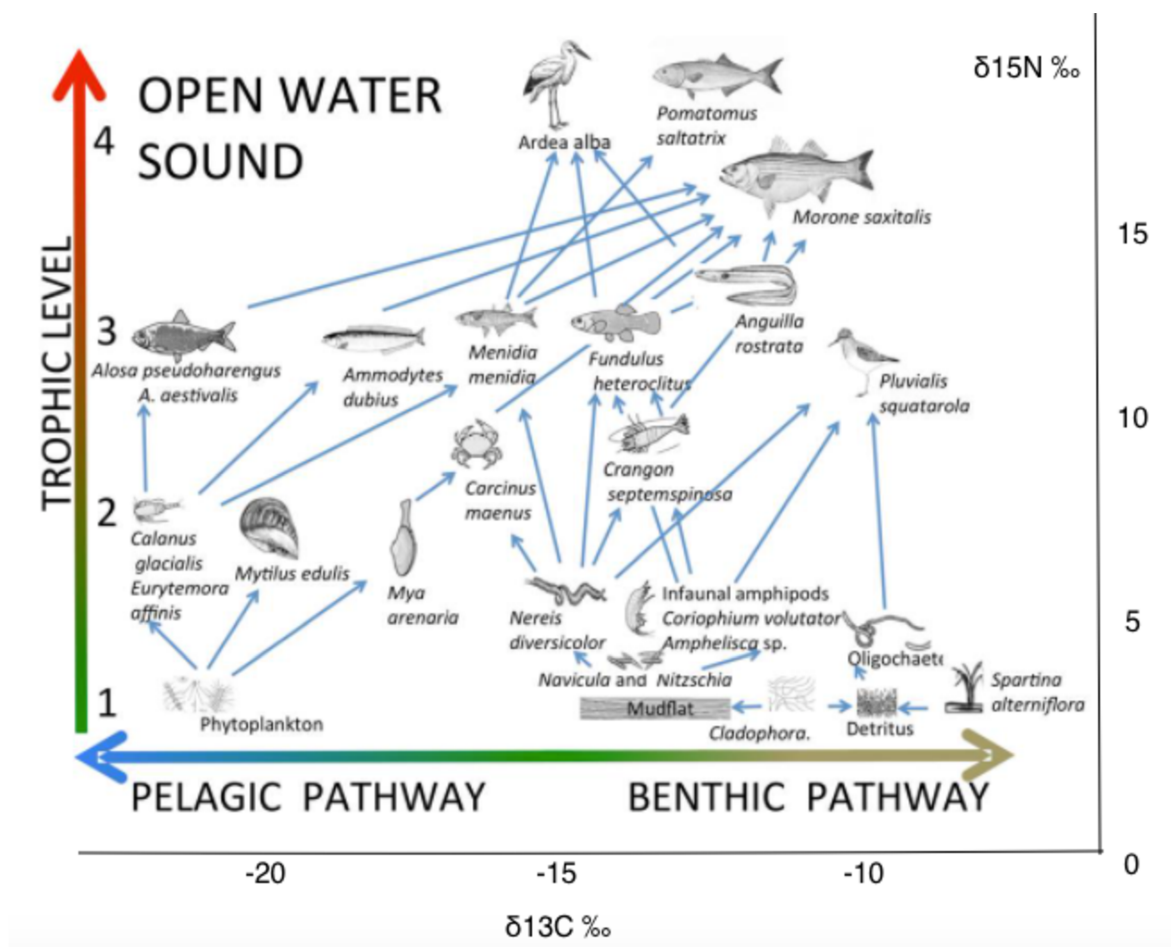
Fig. 1.14 Trophic web of open waters of Plume Island (source: Marine Biological Laboratory, University of Chicago)

1.3 Aim of the thesis

Taking into consideration all the aspects covered in the introduction, some gaps regarding these species and especially their trophic ecology emerge in the scientific literature. At the Mediterranean and, even more at the Adriatic level, there are very few studies concerning feeding habits and resource partitioning among middle-sized pelagic fishes, such as mackerels and horse mackerels. The goal of this thesis is to adopt the SCA/SIA integrated approach to the four fish species, in order to:

- o advance our knowledge on their trophic ecology, taking into account possible ontogenetic shifts,
- o analyze mechanisms of resource partitioning,
- o analyze also possible diet's environmental drivers.

Consequently, it will result easier to:

- o confirm their key-role in the pelagic food web (*i.e.* wasp-waist control),
- o put forward new hypothesis on their importance in the Adriatic trophic sea web.

Chapter Two

2. MATERIALS AND METHODS

2.1 Study area: The Adriatic Sea

2.1.1 A general overview

The Adriatic Sea represents less than 5% of the Mediterranean Sea surface (2.500.000 km^2) and 1% of the total volume (3.500.000 km^3), may be considered as a semi-enclosed basin within the Mediterranean Sea, extending from the Gulf of Trieste to the Strait of Otranto and separating the Italian Peninsula from the Balkans (Russo & Artegiani, 1996). It extends along its major axis, which is NW-SE oriented, for about 800 km long and around 150–200 km wide, with a surface of about 138,600 km^2 and a volume of roughly 35,000 km^3 (McKinney, 2007) (Fig. 2.1).

Fig. 2.1 Geo-localization of Adriatic Sea within Mediterranean Sea

About 74% of the Adriatic Sea bottom is a shallow continental shelf, therefore is the most extensive one in the central Mediterranean Sea (Danovaro & Boero, 2018). Authors (Artegiani et al., 1997a) divided the basin almost always in three sub-basins (or sectors), from north to south: northern, central and southern Adriatic Sea. The northern sub-basin has an average depth of 35 m with a maximum depth of 75-100 m, approximately between Pescara and Sibenik, where a slope gently leads to the central basin at depths of 140–150 m. In this sector tides can be particularly relevant, contrary to the rest of the Mediterranean Sea, where tides are negligible (Danovaro & Boero, 2018). The central Adriatic has an average depth of 130–150 m and is characterized by the presence of the deep Pomo Depression that reaches 273 m depth. The southern sub-basin can contain up to 80% of the entire Adriatic basin volume, due to an average depth of 450 m and a wide depression that reaches 1233 m in its deepest point (Fig. 2.2 and 2.3).
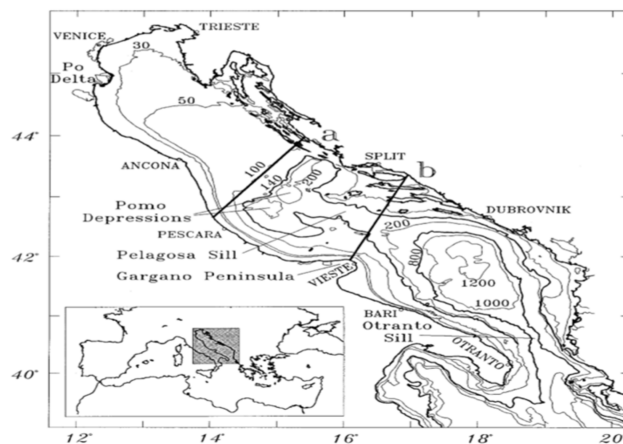
Fig. 2.2 Adriatic Sea's sectors and bathymetry (from Artegiani et al., 1997a)
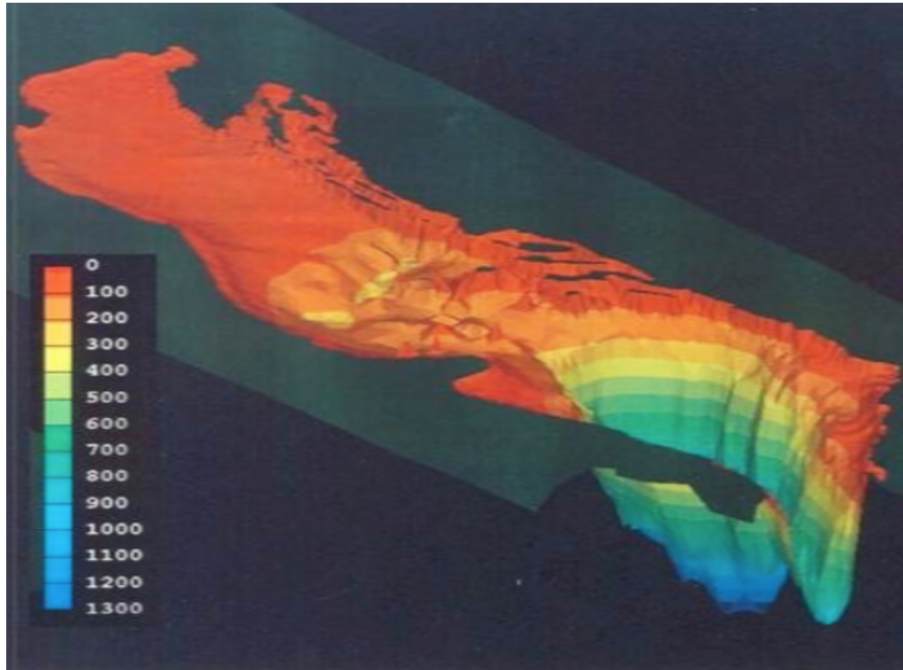
Fig. 2.3 Bathymetry of the Adriatic Sea (credit: engineering.dartmouth.edu)

2.1.2 Temperature and Salinity

The sea surface temperature and salinity exhibits a pronounced seasonal cycle and strong longitudinal gradients, particularly evident for salinity throughout the year, and only in winter and autumn in the case of temperature (Lipizer et al., 2014).

In the northern Adriatic, a significant cooling begins close to the surface in autumn, with the cooling of the whole water column in winter. The surface temperature exhibits a pronounced north–south gradient and a less pronounced, but still well recognizable, west–east gradient in surface temperature, with the lowest values (< 9 °C) in the northernmost part of the basin and close to the

north-western Italian coast (Fig. 2.4). In spring, due to increased river runoff, the freshwater effect is evident: waters with lower salinity waters (S < 37 psu) are distributed over a larger area and tend to occupy a wide part of the northern Adriatic (Po River plume shows a north-eastward propagation up to the eastern Croatian coast). In summer, is evident a seasonal layer of Northern Adriatic Surface Water (NAdSW), which corresponds to low salinities and relatively high temperatures (25 °C), even if the coldest summer waters are present along the north-eastern coast (23 °C) (Artegiani et al., 1997a; Lipizer et al., 2014) (Fig. 2.4).
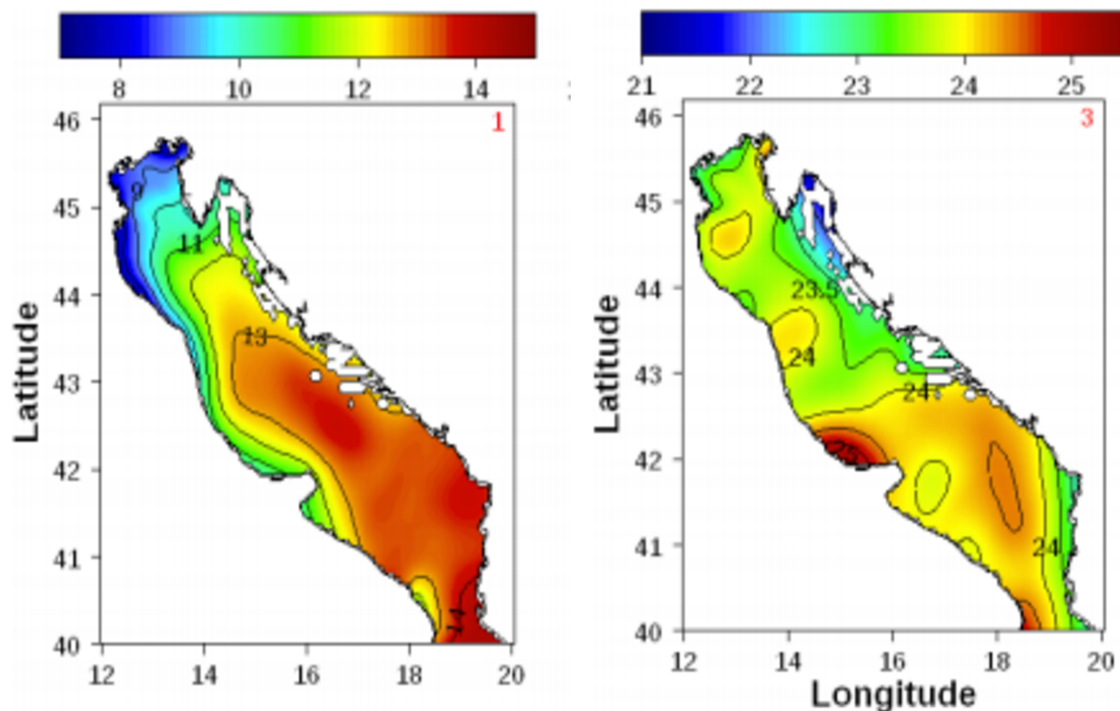
Fig. 2.4 Climatological map of Adriatic surface T° in winter (left) and summer (right) (adapted from Lipizer et al., 2014)

In the middle Adriatic, winter surface temperatures are slightly higher than in the north. The surface waters are freshened during spring and summer, due to river runoff, as in the northern Adriatic. Salinity is higher in the northern basin and a little bit lower in the southern basin, especially in open waters, during all the year. A thermocline is formed down to a depth of 50 m; under this thermocline a mass of water called Mid-Adriatic Dense Water (MAdDW) with relatively low average temperature (11.62 ± 0.75 °C) and substantially higher average salinity (38.47 ± 0.15 psu) occurs. This water originates from the winter Northern Adriatic Dense Water (NAdDW) that moves southward and experiences substantial mixing. From spring to autumn, the MAdDW is the coldest bottom water mass in all the Adriatic basin.

Surface waters in the southern basin have the highest temperature during winter (Fig. 2.4) and spring. Salinity is high all over the year with a slight decrease, consequent to freshwater discharge, close to the Albanian coast (Artegiani et al., 1997b) (Fig. 2.5) The southern Adriatic deep water (SAdDW) is more homogeneous with T ≈ 13.5 °C, and S ≈ 38.6 psu during all the year from 150 m to the bottom, typical of the Mediterranean open sea conditions (Artegiani et al., 1997a; Lipizer et al., 2014).
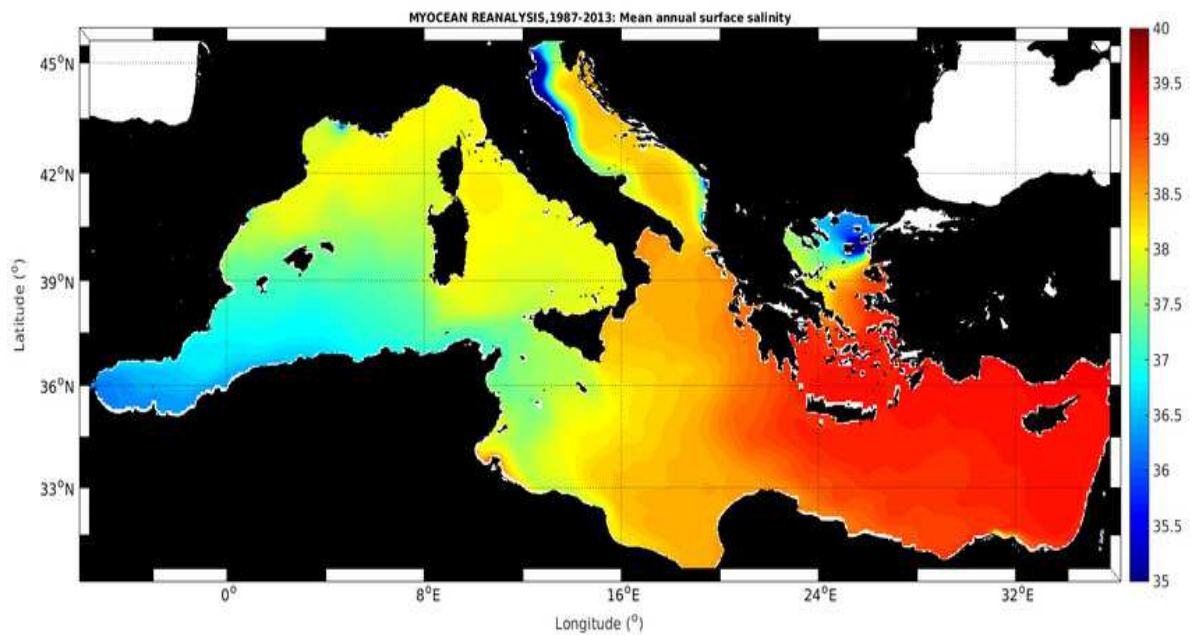
Fig. 2.5 Mean annual surface salinity in the Mediterranean Sea (adapted from Soukissian et al., 2017)

2.1.3 Coastal morphology, sediments and circulation

The western part of the Adriatic is low, with sediment-loaded beaches, which originated from Pleistocene to Holocene due to strong river discharge. The eastern Adriatic coastal morphology is rugged and rocky. The Adriatic Sea takes in up to one-third of the freshwater flow received by the entire Mediterranean, mainly from the north-western Adriatic river mouths. As a result, heavy sediments like sands deposit in coastal areas, while finer sediments (e.g. silt, clay) deposit off-shore and, thanks to main marine currents, in south-western sectors (Artegiani et al., 1997a; Marini et al., 2008). This led to predominantly sandy-muddy bottoms (70% of marine seafloor) especially

35

along the western coast, toward south, where finer sediments increase, reaching the dimensions of clay (Vdovic & Juracic, 1993).

The general Adriatic Surface Water (ASW) circulation is counterclockwise or cyclonic, with fast northward currents along the eastern side, the East Adriatic Current (EAC), and a swift return flow along the Italian Peninsula on the western side, the West Adriatic Current (WAC) (Poulain, 2001) (Fig. 2.6). The ASW circulation is affected by the inflow of Mediterranean water through the Otranto Strait, winds and the Po river seasonal discharge. The latter regulate the content of much of the sea and is a major factor in the circulation, driving the EAC (Orlic et al., 1992).

Headlands such as those present at Istria, Conero and Gargano leads to the formation of a northern, a central, and a southern gyre, with horizontal currents between the western and the eastern coasts. As a result, the circulation in the three sub-basins is often dominated by their own cyclonic gyre, that vary in intensity according to the season (Russo & Artegiani, 1996).

The Adriatic Sea is also known to be the major source of the Eastern Mediterranean Deep Water (EMDW). The strongest supply to this water-mass derives from the Adriatic deep water (ADW), a high density, low temperature and low salinity current on the western slope, heading south (McKinney, 2007).

The ADW forms mainly in the northern Adriatic basin associated with cold and dry winter wind (*e.g.* Bora) (Orlic et al., 1992).

Given all these aspects, *i.e.* bathymetry, temperatures, currents and, in particular, freshwater inputs, the Adriatic Sea is one of the most productive regions of the entire Mediterranean Sea. Western runoffs, especially from the Po River, notably influences the productivity of the Adriatic marine ecosystem, affecting primary and secondary production. As a consequence, the trophic web structure and biomass of this sea depends also on the annual productivity.
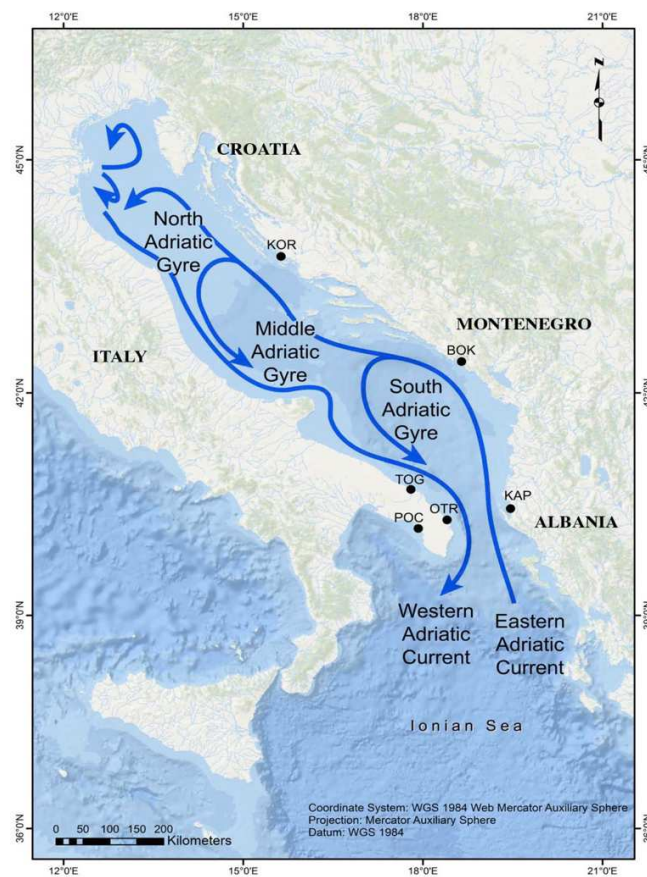
Fig. 2.6 Main Adriatic surface currents (adapted from Melià et al., 2016)

2.2 Samples collection

2.2.1 Mediterranean geographical subareas (GSAs)

Since 1949, the Food and Agriculture Organization of the United Nations (FAO) established the General Fisheries Commission for the Mediterranean (GFCM), one of regional fisheries management organizations (RFMOs) regulating the fishing activity in high seas. The GFCM plays a critical role in Mediterranean and Black Sea fisheries governance and conservation and aquaculture development. The GFCM divided this area into five subregions: Western, Central and Eastern Mediterranean, the Adriatic Sea and the Black Sea. These subregions are divided into 30 Geographical Sub-Areas (GSAs) (Fig. 2.7), commonly used in the GFCM as the minimal management unit (http://www.fao.org/gfcm/about/area-of-application/en/).

2.2.2 The MEDIAS project

Samples have been collected between 1 June and 15 July 2019, during the GSA 17 and GSA 18 acoustic survey of small pelagic fish, conducted in the framework of the MEDiterranean International Acoustic Survey (MEDIAS) project (http://www.medias-project.eu/medias/website/ MEDIAS, 2019; Leonori et al., 2020a) in the framework of the Italian National Program for Fisheries Data Collection (PLNRDA).
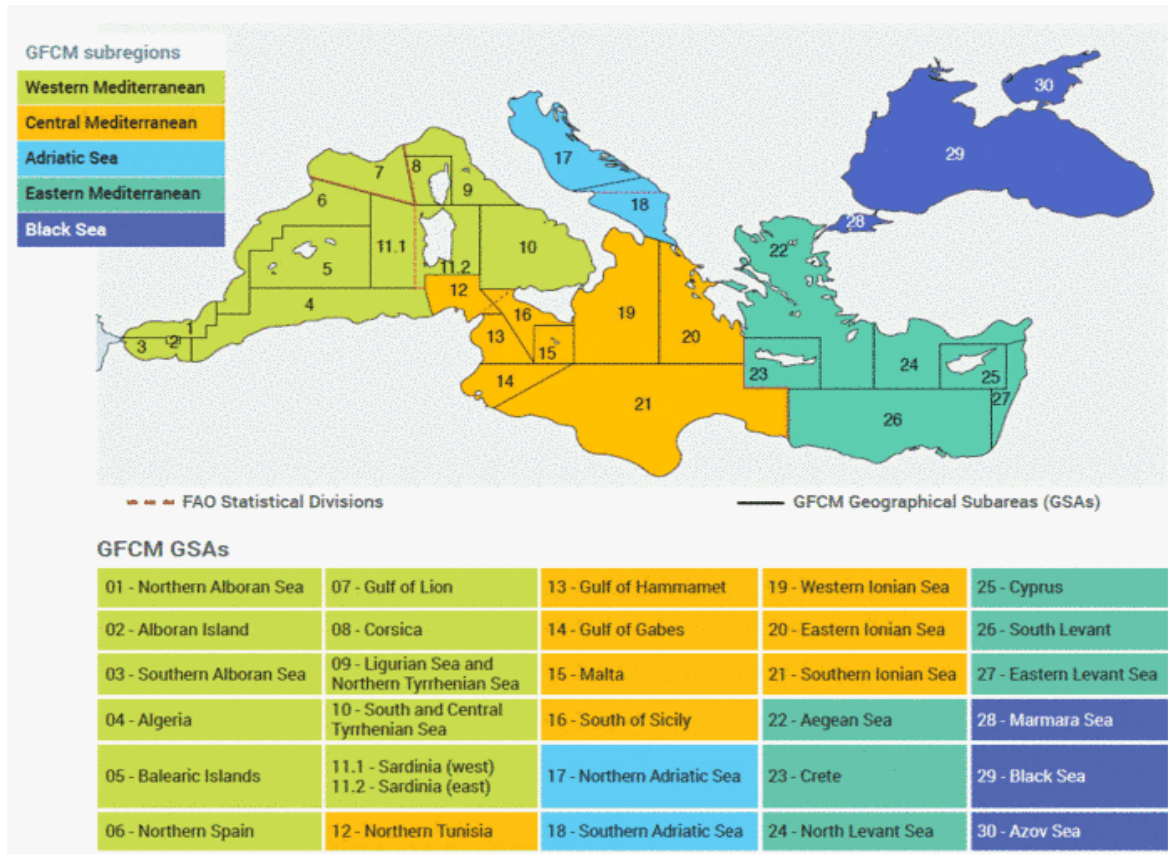
Fig. 2.7 GFCM 5 subregions and 30 GSAs

(source: http://www.fao.org/gfcm/about/area-of-application/en/)

This ensemble of marine acoustic surveys covers several areas of the Mediterranean and Black Sea, involving different nations. MEDIAS is listed by the EU Fisheries Data Collection Framework (DCF) as an annual and mandatory survey. Its aim is to evaluate biomass levels and spatial distribution of small pelagic fishes, in order to supply information to fisheries decision makers for stocks management. Moreover, it provides input for stocks assessment. Nations involved in this acoustic surveys project follow a common and standardized protocol, the MEDIAS Handbook, frequently reviewed and

available on the project website (http://www.medias-project.eu/medias/website/handbooks-menu.html). The marine acoustic group of CNR-IRBIM of Ancona performed the MEDIAS 2019 surveys in the Italian western sectors of GSAs 17 and 18, including also Slovenian waters (Leonori et al., 2020b) and an acoustic survey in Albania as extension of MEDIAS to not EU waters (De Felice et al., 2020).

2.2.2 The oceanographic research vessel and its equipment

CNR-IRBIM of Ancona performs MEDIAS acoustic surveys on board of the R/V (Research Vessel) "G. Dallaporta" (Fig. 2.8) in Adriatic. Its general characteristics are listed in Tab. 2.1.

Fig. 2.8 R/V "G. Dallaporta" (source: https://www.cnr.it/en/node/154)

Tab. 2.1 R/V "G. Dallaporta" general characteristics (source: Leonori et al., 2012)

Characteristics	R/V "Dallaporta"
Length (m)	35.30
Width (m)	7.65
Draught (m)	3.40
Gross tonnage (GRT)	285
Max speed (km/h)	25.93
(knots)	14

This oceanographic vessel presents on board all the most modern scientific equipment, crucial to apply the multidisciplinary approach performed by CNR-IRBIM of Ancona for the evaluation of small pelagics stocks. This approach foresees the acquisition of:

- acoustic data, to estimate the abundance, biomass and spatial distribution of small pelagic fishes;

- biological samples of small pelagic fishes for demographic information (species composition, mean size, sex and age structure of the populations).

- zooplankton and anchovies' eggs and larvae, for auxiliary biological data and indexes;

- environmental data, in order to get information about oceanographic parameters of the surveyed area.

The equipment necessary to perform this approach is constituted by a SIMRAD split-beam multi-frequency scientific echosounder, a pelagic trawl net with a

SIMRAD "trawl eye" net control system, a WP2 plankton net and a CTD multiparametric probe (Leonori et al., 2018; Leonori et al., 2017).

2.2.3 Sampling strategy

Acoustic data were collected following a survey design of systematic sampling along a trajectory of parallel transects, perpendicular to the coastline starting from 10-15 meters depth, and ending at 200 meters depth or at the Adriatic Midline for shallower areas. The inter-transect distance was between 8 and 10 nautical miles (NM) with a sampling speed of 9.5 knots (MEDIAS, 2019) (Fig. 2.9). The vessel is equipped with two split-beam multi-frequency echo-sounder, the SIMRAD EK60 echosounder equipped with 38 and 200 kHz and the EK80 with 70 and 120 kHz. The 38 kHz frequency is used to estimate small pelagics biomass while the others are complementary frequencies, useful to discriminate small pelagics from fishes without swim bladder, plankton and noise.

Biological samples collection of small pelagic fishes was performed with a midwater trawl net, designed by CNR-IRBIM, with 10 m vertical opening, 12 m horizontal opening and codend mesh size of 18 mm. A SIMRAD "trawl eye" system mounted on the net, helps operators to understand if the net was correctly opened and if fishes were entering. Sampling hauls were carried out

along acoustic transects (Fig. 2.9) taking into account the location of the main aggregations of fish visible with the echosounder. Hauls were performed at different times of the day, in different light conditions, trying to uniformly cover the area from a spatial point of view (inshore and offshore and at different depths). Hauls maximum duration was around 30 minutes.
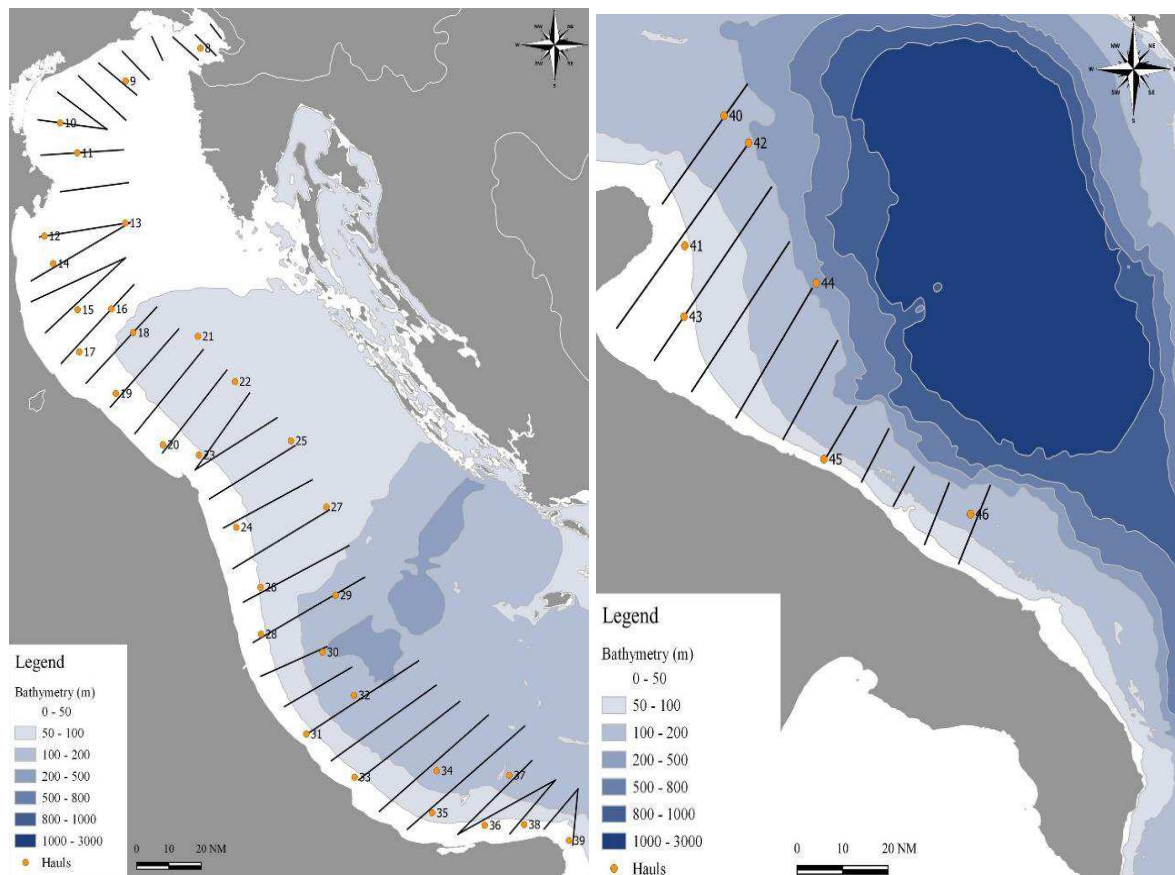
Fig. 2.9 Numbered hauls performed for GSA 17 (left) and GSA 18 (right). Black lines represent transects performed for acoustic sampling

The total catch of each haul was weighted with precision instruments calibrated for machine drift due to navigation, divided by species and measured on board

for length frequency distributions. Sardines (*Sardina pilchardus*), anchovies (*Engraulis encrasicolus*) and sprats (*Sprattus sprattus*), which are MEDIAS target species, were collected. 10 individuals per length class (0.5 cm) were frozen for subsequent laboratory analyses aimed to collecting information on sex determination and gonadal maturity stage as well as information on age classes by otolith reading to determine the age-length keys.

Some hauls were characterized by the presence of other species of pelagic fishes that have been identified, collected and grouped for each haul under the name of OPS (Other Pelagic Species). Mackerels (*Scomber colias, Scomber scombrus*) individuals and horse mackerels (*Trachurus mediterraneus, Trachurus trachurus*) individuals were used as biological samples for this thesis. All collected OPS samples were immediately frozen on board at -20 °C.

Fig. 2.10 Left: fishing operation for biological sampling. Right: small pelagic fishes

2.2.4 Samples selection

The whole surveyed area (Western Adriatic GSAs 17 and 18) has been divided for this study in four different geographic sectors or subareas, on the basis of bathymetric and rivers runoff characteristics:

o   GSA 17 north: characterized by shallower depths, up to ~ 60 m and by the occurrence of large river with strong freshwater input (*i.e.* the Po river);

o   GSA 17 central: with deeper sea-bottoms, up to ~ 100 m and lower but locally important outflows;

o   GSA 17 south: with depths that can reach up to 300 m in the Pomo Pit and very scarce river inputs;

o   GSA 18: with a narrow continental shelf and depths of 1300 m in the South Adriatic Pit and low rivers runoff.

In order to obtain the best representative sample of each sub-area and of the inshore-offshore condition, a sub-set of hauls were selected, covering the whole studied area (Fig. 2.11). From these selected hauls, all the individuals were analyzed for length and weight measurements, determination of biological indexes, stomach contents analyses and muscle tissue extractions.

After the survey, samples were analyzed at the Laboratory of Marine Biology and Ecology of the Department of Life and Environmental Sciences of the Polytechnic University of Marche, and at CNR-IRBIM of Ancona laboratories.
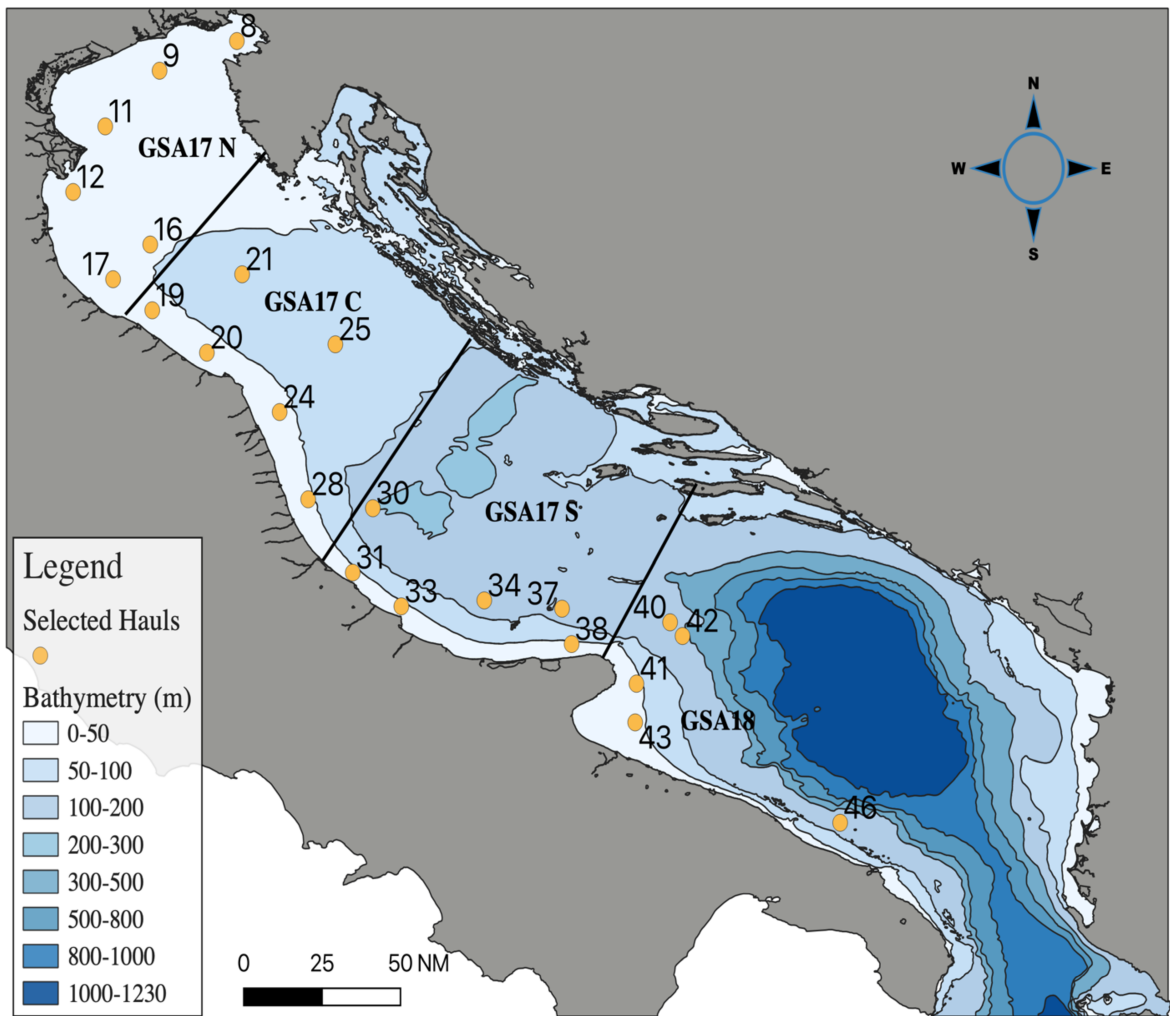
Fig. 2.11 Surveyed subareas and their selected hauls

2.3 Data collection

2.3.1 Morphometric measurements and biological indexes

Every individual was processed as follows:

1. After being carefully thawed and confirmed that the individual belonged
   to the species identified on board, each individual was labelled with an
   identification code;

2. each individual was weighed (Wet Weight, WW, in g) with a weight scale (± 0.01 g) and measured (Total Length, TL, in cm) with an ichthyometer (± 0.1 cm) (Fig. 2.12);

3. starting from the cloaca the abdominal cavity of the fish was opened to extract the bowels with scalpel, scissors and tweezers. With great care, liver, stomach and gonads (when possible) were extracted and placed in a Petri dish;

4. in adults, gonads were observed to determine sex and maturity stage of the individual, according to Walsh et al. (1990) maturity scale (Fig. 2.13), commonly used for mackerels and recommended also for horse mackerels (ICES, 2015). Otherwise, individual was classified as undetermined (IND);

5. liver, stomach and gonads were weighed with an analytical weight scale with sensitivity 10^{-5} g (Fig. 2.14);

6. extracted stomach was preserved at -20 °C in 70% ethanol inside a plastic test tube with the identification code previously assigned;

7. a portion of muscular tissue under the dorsal fin was extracted with scalpel and scissors and preserved at -20 °C inside a plastic test tube with the same identification as in step 6.
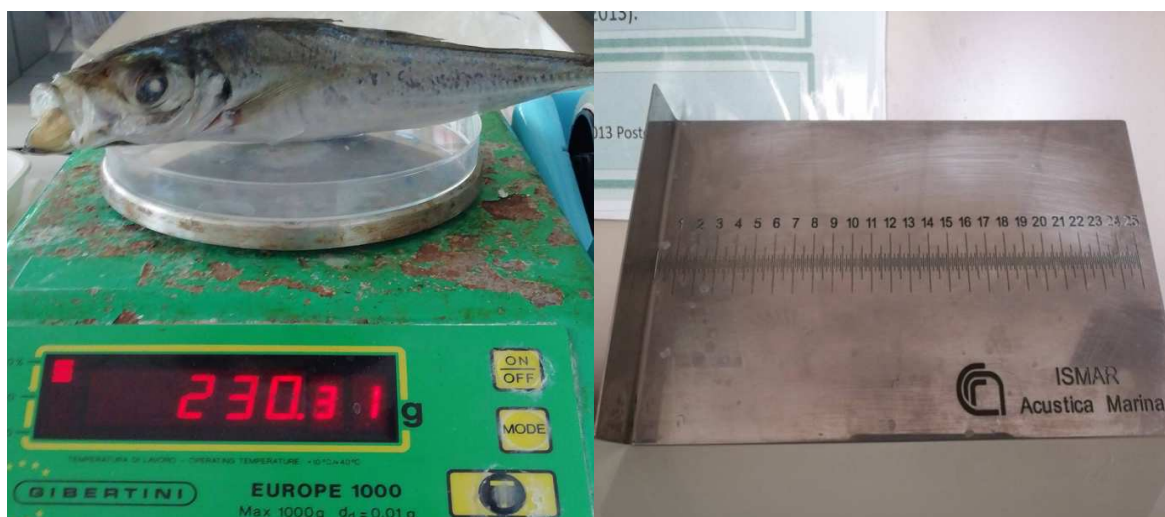
Fig. 2.12 Left: weight scale. Right: ichthyometer for length measurements

Stage	State	External appearance
1	Immature	Gonads small. Ovaries wine red and clear, torpedo shaped. Males pale, flattened and transparent.
2	Early ripening	Gonads occupying 1/4 to 3/4 body cavity. Opaque eggs visible in ovaries giving pale pink to yellowish colouration, largest eggs without oil globule. Testes off-white, milt not running.
3	Late ripening / Partly spent (early)	Gonads occupying 3/4 to almost filing body cavity. Ovaries yellow to orange. Largest eggs may have oil globules. Testes creamy white.
4	Ripe	Testes filling body cavity, milt freely running. Ovaries characterized by externally visible hyaline eggs no matter how few or how early the stage of hydration. Ovaries with hyaline eggs only in the lumen are not included. Ovary size variable from full to 1/4.
5	Partly spent (late)	Gonads occupying 3/4 to < 1/4 body cavity. Ovaries slacker than in stage 3 and often bloodshot. Testes with free running milt and shrivelled at anus end.
6	Spent / Recovering spent	Gonads occupying 1/4 or less of body cavity. Ovaries reddish and often murky in appearance, sometimes with a scattering or patch of opaque eggs. Testes opaque with brownish tint and no trace of milt.

Fig. 2.13 Maturity scale of Walsh et al. (1990)
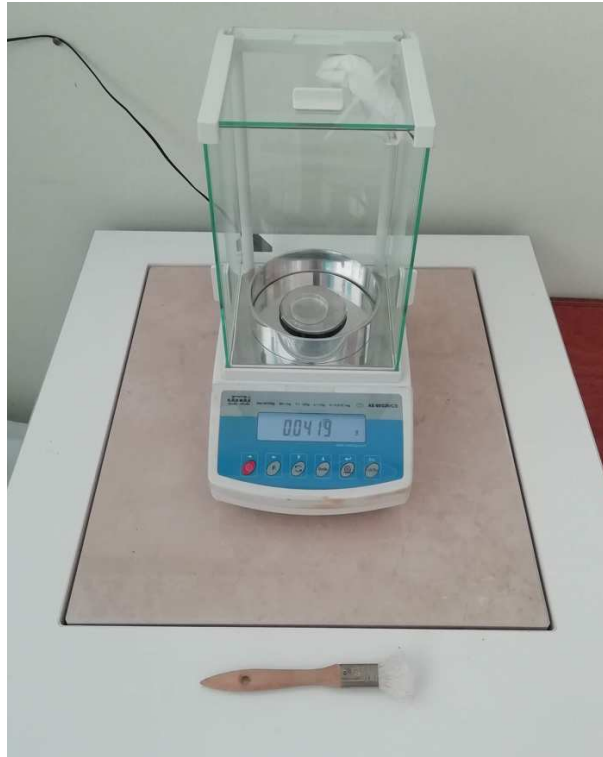
Fig. 2.14 High sensitivity weight scale for accurate and precise weighting

2.3.2 Stomach contents analyses

For stomach content analyses, each sample was analyzed as follows:

1. using blotting paper, stomach was dried up by ethanol, putted in a Petri dish and weighted again, using the analytical weight scale with 10^{-5} g sensitivity (Fig. 2.14);

2. each stomach was gently opened with scissors and tweezers (Fig. 2.15) and then emptied from its content in a Petri dish. Then, the stomach pouch was carefully washed with deionized water, dried up and weighted using the weight scale of step 1;

3. using a ZEISS Stemi 2000 stereomicroscope with 6.5-50x magnification (Fig. 2.15), in Petri dishes with deionized water, stomach contents were firstly observed, then sorted by commons high taxonomic levels (Fig. 2.16) and lastly identified to the lowest taxonomic level as possible (Fanelli, Badalamenti, et al., 2011);

4. to give an idea of the level of digestion of the contents, on the basis of a visual estimate, a value from 0 (undigested) to 3 (highly digested) was assigned;

5. identified and non-identified stomach contents were counted and weighed (using the weight scale of step 1) one by one;

6. finally, stomach contents were preserved in 70% ethanol at -20 °C, inserted in the same plastic tube where the stomach was preserved.
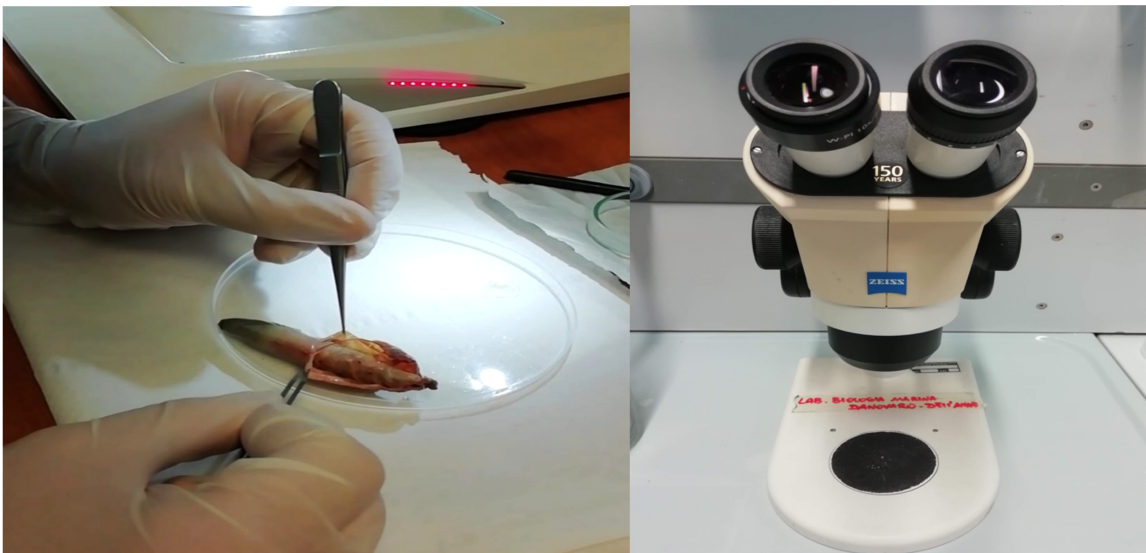
Fig. 2.15 Left: *Scomber colias* stomach opening. Right: the stereomicroscope used for stomach contents sorting and identification
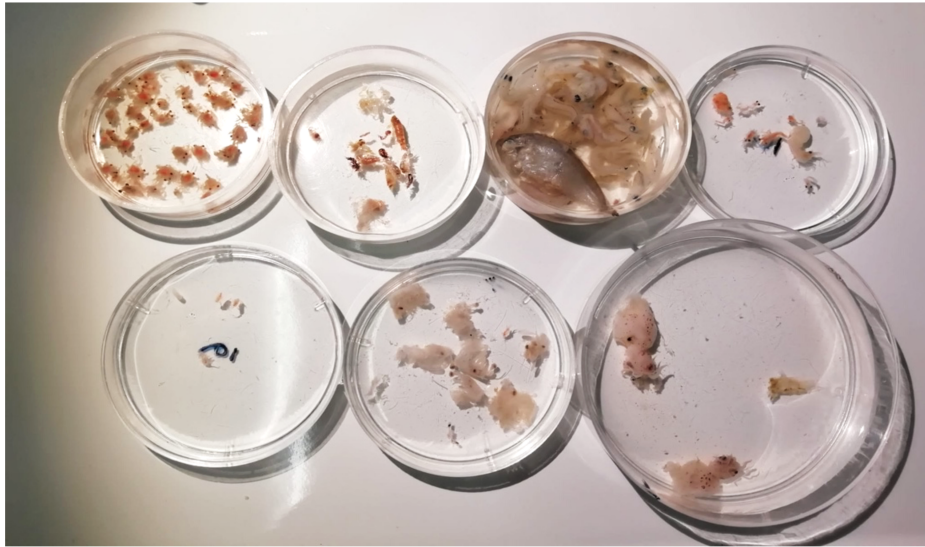
Fig. 2.16 Example of stomach contents sorted by commons high taxonomic levels

In order to identify organisms found in stomach contents, the following identification keys were used:

- Chevreux, E. & Fage, L. 1925. Faune de France. Amphipodes. 9. Lechevalier, Paris, pp. 488

- Costa F., Krapp T., Ruffo S., 2009. Atlante degli anfipodi mediterranei. Guida illustrata a colori. Milano, pp. 221

- Fage L., 1951. Cumacès, Faune de France. Fèdèration Française des Sociètès de Sciences Naturelles. Office Central de Faunistique. pp. 136.

- Falciai L., Minervini R., Fortes M.J., 1995. Guía de los crustáceos decápodos de Europa. Ediciones Omega, S.A. 1. ed. 1995

- ICRAM, 2006. Guida al riconoscimento del plancton neritico dei mari italiani, Volume II - Zooplancton Neritico - Tavole pp. 196

- Mauchline J., 1984. Euphausiid, Stomatopod and Leptostracan Crustaceans. Key and notes for the identification of species. London, Leiden, Koln

- Naylor E., 1972. British Marine Isopods. Key and Notes for the Identification. Synopes of the British Fauna n°3. Accademic Press, London and New York. pp. 80.

- Rodriguez, J.M., Alemany, F. and Garcia A. 2017. A guide to the eggs and larvae of 100 common Western Mediterranean Sea bony fish species. FAO, Rome, Italy, 256 pp.

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2.3.3 Stable isotopes analyses of Carbon and Nitrogen

For each subarea and for each of the four species present from that subarea, 5 specimens (when available) for each of the three length categories, established on the basis of frequency length distribution (small, medium and large) were selected. Due to the low number of *Scomber scombrus* individuals, all muscle samples of this species were utilized. Samples were oven-dried for 24 hours at 60 °C, then converted to a fine powder with mortar and pestle (Fig. 2.17b) and ca. 0.8-1.3 mg were weighed with an analytical balance (10^{-5} g) and put in tin capsules (Fig. 2.17c) for subsequent analyses. Tin capsules were inserted in a numbered rack that allowed to recognize the corresponding position of a given muscle sample (Fig. 2.17d). All instruments used to powder samples (*i.e.* mortar, pestle and tweezers) were cleaned with deionized water and wiped with paper after each sample, in order to avoid contaminations.

Racks were then sent to the University of Palermo, where samples were analyzed through an Isotope Ratio Mass Spectrometer, coupled with an Elemental Analyzer (EA-IRMS). Tin capsules were automatically loaded in the elemental analyzer (Thermo Flash EA 1112) for the determination of total carbon (%TOC) and nitrogen (%TN) and then analyzed for the values of $\delta^{13}C$ and $\delta^{15}N$ in a continuous-flow isotope-ratio mass spectrometer (Thermo Delta Plus XP).
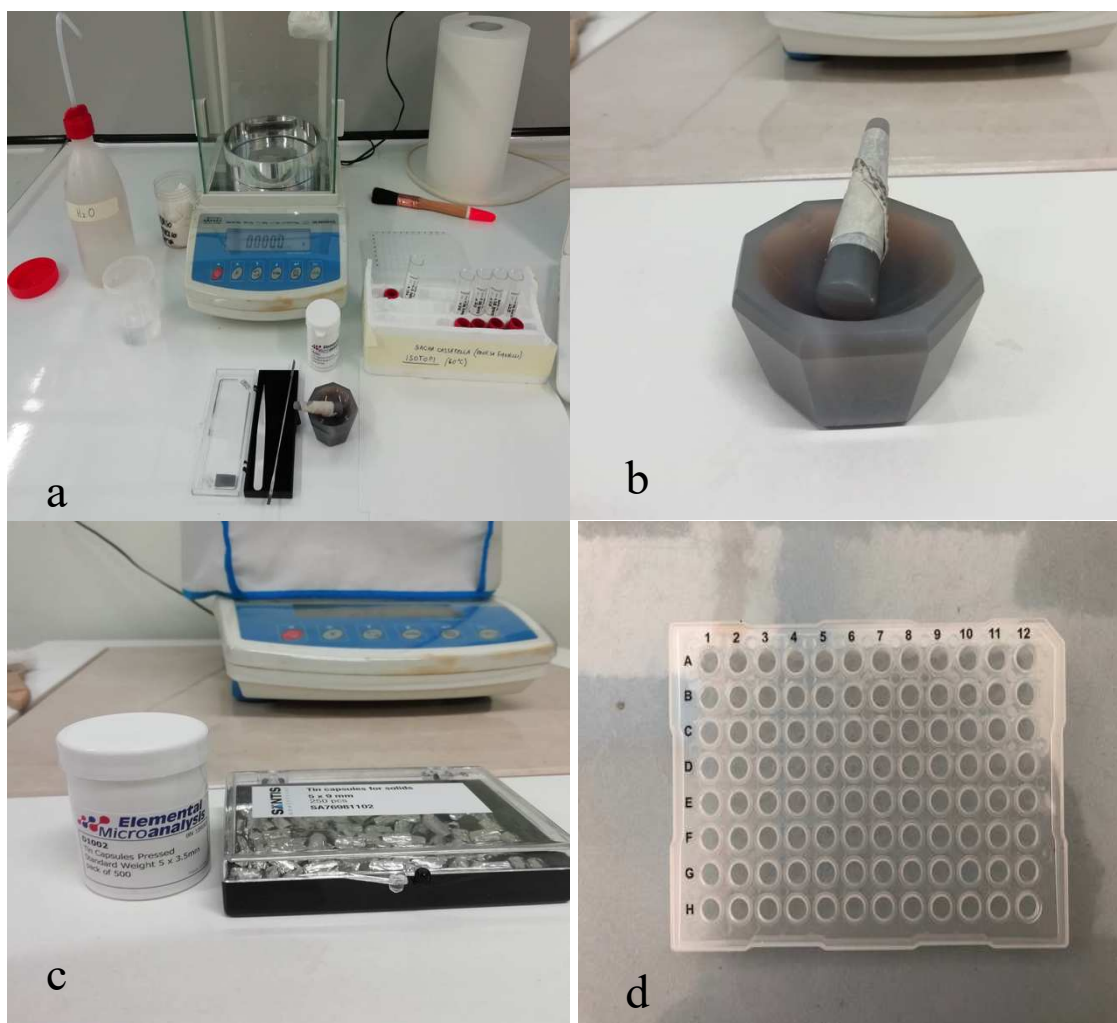
Fig. 2.17: a) work surface for samples preparation; b) mortar and pestle;

c) tin capsules for isotopic analyses; d) numbered rack for sample identification

Samples burned at 850°C, producing O_2, CO_2 and NO_x, the latter was then converted into N_2. After that, a ionizer completed separations for different masses. To conclude the analyses, a specific software linked to the instrument provided the related values of $\delta^{13}C$, $\delta^{15}N$, %TOC, %TN and a graph, to understand if there were problems during samples analyses. Stable isotope

ratios were expressed, in relation to reference international standards (atmospheric N_2 and PeeDee Belemnite for $\delta^{15}N$ and $\delta^{13}C$ respectively) as:

$$\delta^{13}C \text{ or } \delta^{15}N : [(R_{sample}/R_{standard})-1)]*10^3$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$.

2.4 Statistical treatment of biological indices and stomach content data

For each species, females, males and undetermined individuals were grouped per size class (1.0 cm) and their %abundance was calculated for the entire surveyed area. Then, the abundance of females, males and undetermined individuals was calculated for every subarea.

The gonadosomatic index (%GSI), a proxy of gonadal development, was calculated considering only female individuals, as follows:

%GSI = gonads weight / individual weight *100

The hepatosomatic index (%HSI), a proxy of lipid content, was calculated for all the individuals, when livers were not damaged, as follows:

%HSI = liver weight / individual weight *100

Calculating the mathematical difference between weights of the full stomach and the stomach pouch respectively, stomach content weight was obtained. Through this data the fullness index, a proxy of feeding intensity, was calculated as follows:

$$\%FULLNESS = \text{stomach content weight} / \text{individual weight} *100$$

These three indexes (*i.e.* %GSI, %HSI, %FULLNESS) were then analyzed with two-way univariate Permutational Multivariate Analysis of Variance (PERMANOVA). Univariate PERMANOVA tests were run on the Euclidean distance resemblance matrix of untransformed data for each index, separately, according to the following sampling design:

- subarea as a fixed factor with four levels (GSA 17 N, GSA 17 C, GSA 17 S and GSA 18)

- inshore *vs.* offshore location as a fixed factor with two levels (inshore and offshore).

The permutation method was "unrestricted permutation of raw data", with 9999 permutations. The design used was crossed, a part when one or more inshore offshore location levels were absent, thus a nested design was used with "inshore *vs.* offshore" as a random factor. Both main-test and pair-wise comparison were run, the latter only when design factors were both fixed.

Based on stomach contents data, the following indices were calculated, in order to estimate the contribution of each prey to the species diet (Pinkas, 1971), in all the sampled area and for each of the four subareas:

1. Weight percentage of prey (%W)

    %W = weight of prey x / weight of all preys *100

2. Numeric percentage of prey (%N)

    %N = number of prey x / total number of preys *100

3. Frequency of occurrence of prey (%F)

    %F = number of stomachs with prey x / total number of stomachs *100

Considered separately, these indices may produce some bias, especially if a prey is very large but rare, or conversely, if a species is too small but occur frequently. For this reason, they were related in a single trophic statistic index, the Index of Relative Importance (% IRI) (Pinkas, 1971), which was calculated as follows:

$$IRI = (\% \ W + \% \ N) * \% \ F$$

$$\%IRI = (IRI \ / \ \sum IRI) * 100$$

Multivariate analyses were run for each species considering preys biomasses, as variables. First, biomass data were logarithmically transformed to reduce the contribution of highly abundant preys or with a remarkable biomass. A resemblance matrix on these data was obtained, using the Bray-Curtis distance, and a nMDS (non-metric Multi-Dimensional Scaling) was performed, to visualize the level of diet similarity/dissimilarity between individual samples.

A PERMANOVA was then run to test for differences using the same design previously described for univariate analyses, with 9999 permutations but with "permutation of residuals under a reduced model" as permutation method. Main-test and pairwise comparison were performed, the latter when possible. Differences were considered significant when P values resulted < 0.05, both for univariate and multivariate analyses.

On the factors found to be significant by PERMANOVA, CAP (Canonical Analysis of Principal Coordinates, Anderson and Willis, 2003) analyses were done, in order to visualize samples separation on the basis of putative factors.

After that, SIMPER (Similarity Percentage) analysis was performed, to obtain the percentage contribution of different preys to the average similarity of diet among samples in each subarea. The analysis was conducted using Bray-Curtis similarity with a cut-off of percentage contribution at 60%.

Further, in order to assess the trophic diversity of each species in each subarea, the Shannon-Wiener index (H') was calculated for each sample and univariate PERMANOVA analyses were performed with the same design and method used for %GSI, %HSI and %FULLNESS.

Finally, the resemblance matrix of transformed biomass data was correlated to environmental data such as temperature (°C), dissolved oxygen (mg/L), salinity (psu) and chlorophyll-a (mg/m^3), in order to identify the main environmental

drivers of the diet across the subareas. Data of temperature, dissolved oxygen and salinity were collected through a CTD probe during 2019 MEDIAS survey, while a-chlorophyll values (from five to one month before sampling) were obtained on a NASA website (https://giovanni.gsfc.nasa.gov/giovanni/). Environmental data were first tested for collinearity by using a Draftsman plot to assess co-varying variables. Then, the matrix was used for a DistLM (Distance based linear models) analysis with best fitted environmental data from Draftsman plot used as environmental variables. DistLM was run using "stepwise" as selection procedure and "AIC" (Akaike Information Criterion) as selection criterion.

All these analyses were also applied combining together data of the four species. For univariate analyses, the univariate PERMANOVA was run in the same way but with the addition of "species" as a fixed factor in the PERMANOVA design, while sub-area and inshore-offshore location factors were random factors, nested within species and subarea factors, respectively. Thus, also multivariate analyses were conducted with this new design, performing all the tests and adding the PERMDISP multivariate dispersion test, to evaluate the homogeneity of multivariate dispersions between species on the

basis of resemblance measures. PERMDISP mean values can be considered as indicators of diet generalism *vs.* specialism.

2.5 Statistical treatment of stable isotopes data

Isotopic analyses provided for each sample $\delta^{13}C$ and $\delta^{15}N$ values, percentage of carbon (%C) and nitrogen (%N) and C/N ratio. Taking into account that high lipid concentrations can alter $\delta^{13}C$ values (Post et al., 2007), samples with high lipid concentration can be defatted to avoid ^{13}C depletion. However, lipid extraction can alter $\delta^{15}N$ values and can complicate sample preparation, for these reasons $\delta^{13}C$ of samples rich in lipids was normalized according to the Post *et al.,* (2007) equation:

$$\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + 0.99 \, C/N_{sample}$$

C/N ratio values were used as a proxy of fat content to evaluate which samples were rich in lipids. The normalization was applied to samples with a C/N ratio > 3, according to Post *et al.*, (2007).

For each fish species, scatterplot of $\delta^{13}C$ and $\delta^{15}N$ values related to fish length was created, for the whole sampled area (without subareas division). R and P values were calculated, considered their importance as statistical correlation factors.

δ^{13}C and δ^{15}N values of the species were then all combined and analyzed with univariate and multivariate analyses. Univariate analyses were performed with a univariate Euclidean distance resemblance matrix on untransformed data, one on δ^{13}C and one on δ^{15}N values. On each matrix, a univariate PERMANOVA was run with a three-way design: species as a fixed factor with four levels, subarea as a random factor with four levels (GSA 17 N, GSA 17 C, GSA 17 S and GSA 18) and inshore-offshore location as a random factor with two levels (inshore *vs* offshore), the last two nested within species and subarea factors respectively. The permutation method was "unrestricted permutation of raw data", runed with 9999 permutations. Main test and pairwise comparison were performed.

Multivariate analyses were performed creating a bivariate Euclidean distance resemblance matrix of δ^{13}C and δ^{15}N, without transforming data. This matrix underwent to nMDS and to multivariate PERMANOVA with the same design used for univariate analyses but with "permutation of residuals under a reduced model" as permutation method. CAP analyses were then conducted on factor resulted significant from PERMANOVA main test.

Variations of δ^{15}N and δ^{13}C were then plotted together, to examinate trophic niche partitioning between fish species of this study.

A final scatterplot was create with $\delta^{13}C$ and $\delta^{15}N$ mean values of 4 trophic groups of zooplankton, isotopic $\delta^{13}C$ and $\delta^{15}N$ mean values of sardines and anchovies, isotopic values of this study and $\delta^{13}C$ and $\delta^{15}N$ mean values of mackerels predators. Zooplankton, sardines and anchovies isotopic values were taken from Menicucci S. MSc degree thesis (2021). $\delta^{13}C$ and $\delta^{15}N$ values of *Sarda sarda* and *Euthynnus alletteratus* were taken from Fanelli, E. (unpubl.data). Values of *Thunnus thynnus* were taken from Vizzini *et al*. (2010) while, *Tursiops truncatus* values were taken from Fortibuoni *et al.* (2013). All the analyses were done using the software PRIMER6&PERMANOVA+ and PAST4.04.

Chapter Three

3. RESULTS

3.1 Biological indexes and diet composition

3.1.1. Scomber colias

A total of 62 individuals were sampled: 8 in the GSA 17 C, 19 in the GSA 17 S and 35 in the GSA 18. Out of these 62 individuals, 51 were undetermined (IND), mainly present in the GSA 17 S and in the GSA 18, thus adults were poorly represented, with 3 females (F) and 8 males (M). (Fig. 3.1).
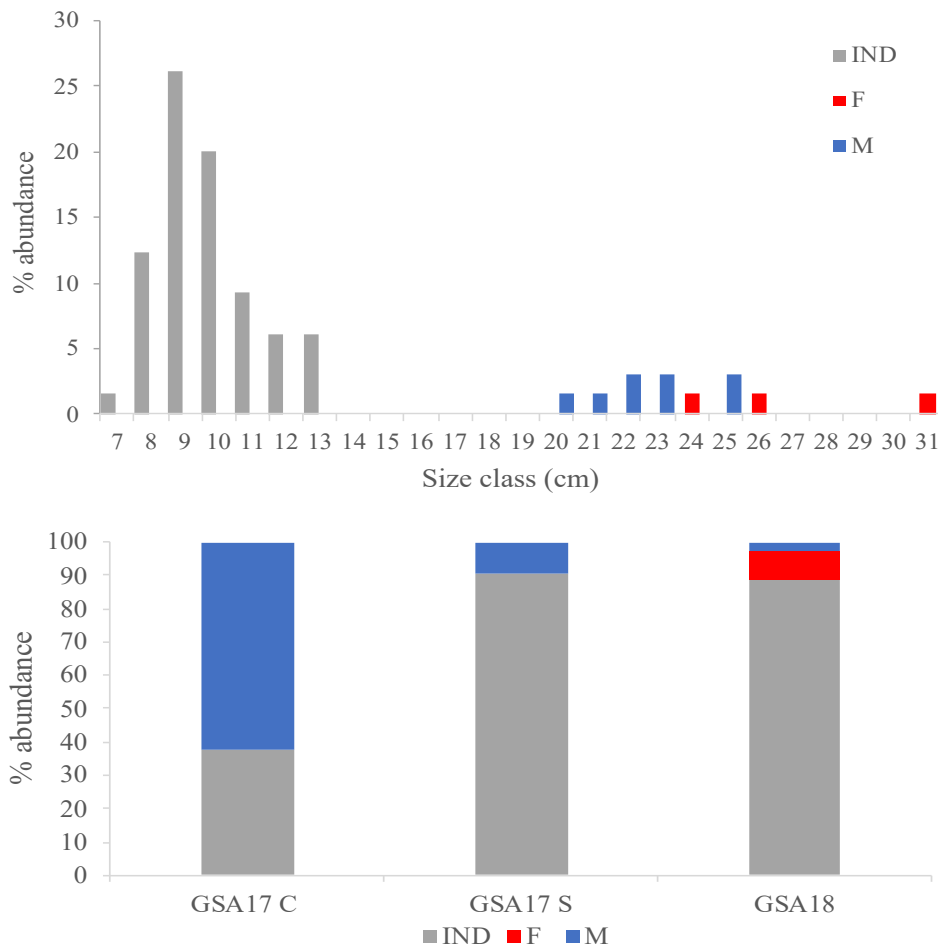
Fig. 3.1 *S. colias* % of abundance by size classes (up) and sex in the subareas (bottom)

Females were available only in the GSA 18, as a consequence %GSI was obtained only for this subarea. On the contrary, %HSI and %FULLNESS were obtained for each subarea. For %HSI, similar mean values were obtained among subareas while, %FULLNESS mean values showed a peak in the GSA 18 offshore and the lowest value in the GSA 17 S offshore (Fig. 3.2).
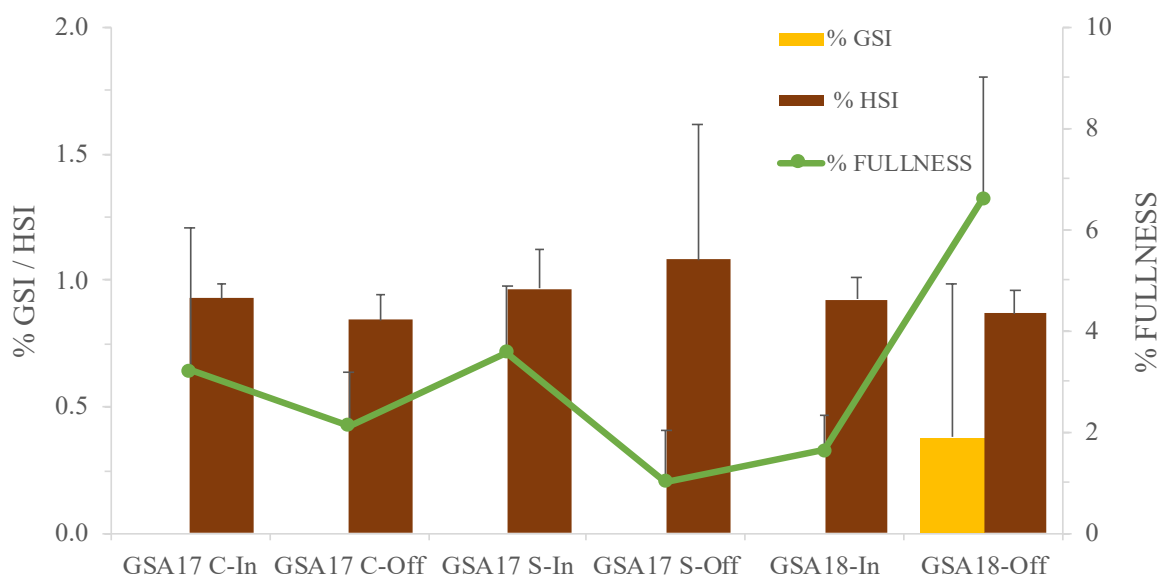
Fig. 3.2 *S. colias* %GSI, %HSI and %FULLNESS mean values (±CI) in the subareas

Univariate PERMANOVA analyses showed that %HSI did not vary significantly with respect of the factors considered (Tab. 3.1). Taking into account only comparisons between contiguous subareas, the pairwise test of %FULLNESS (Tab. 3.2b) showed how variations were significant between inshore levels of GSA17 S and GSA 18 and between inshore and offshore levels within GSA18.

Tab 3.1 %HSI PERMANOVA main test result

Source	df	MS	Pseudo-F	P(MC)
Subarea	2	0.07	1.17	0.31
In vs Off	1	0.00	0.01	0.91
Subarea x InvsOff	2	0.03	0.54	0.59
Residuals	56	0.06		
Total	61			

Tab. 3.2 %FULLNESS PERMANOVA results: a) main test b) pairwise comparisons

a)

Source	df	MS	Pseudo-F	P(MC)
Subarea	2	16.36	1.12	0.33
In vs Off	1	1.70	0.12	0.73
Subarea x InvsOff	2	69.41	4.73	0.0134
Residuals	56	14.67		
Total	61			

b)

Groups	t	P(MC)
GSA17 C In, GSA17 S In	0.23	0.82
GSA17 S In, GSA18 In	2.43	0.0232
GSA17 C Off, GSA17 S Off	1.38	0.22
GSA17 S Off, GSA18 Off	1.67	0.10
GSA17 C In, GSA18 In	1.62	0.13
GSA17 C Off, GSA18 Off	1.73	0.10

Groups	t	P(MC)
GSA17 C In, GSA17 C Off	0.85	0.37
GSA17 S In, GSA17 S Off	1.64	0.10
GSA18 In, GSA18 Off	3.09	0.0038

All stomachs of *Scomber colias*' were full. A total number of 39 Taxa were identified (listed in the Annex 1), most of them found in the GSA 18. In terms of %IRI (Fig. 3.3), more representative preys were represented by thaliaceans, in particular Salpidae, in all the subareas. Several Osteichthyes were also found, especially in the GSA 17 S and GSA 18, with *Engraulis encrasicolus* larvae as the main prey. Crustaceans were mainly represented by several

species of amphipods, in all the subareas, and euphausiids, the latter only in the GSA 18. Preys of secondary importance were sepiolid cephalopods, Calicophorae hydrozoans and Decapoda larvae. "Others" prey category, of noticeable importance in the GSA 17 C, was represented by scales and Digenea Trematoda parasites.
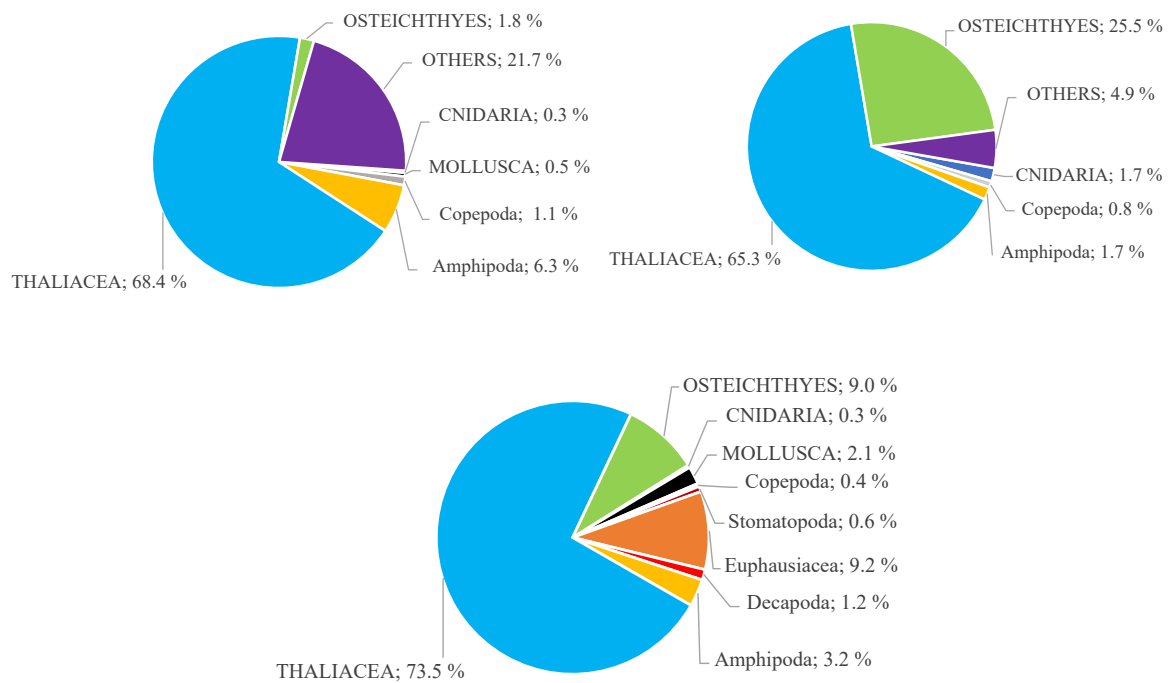
Fig. 3.3 *Scomber colias* diet composition (based on %IRI) in GSA 17 C (up-left), GSA17 S (up-right) and GSA 18 (bottom)
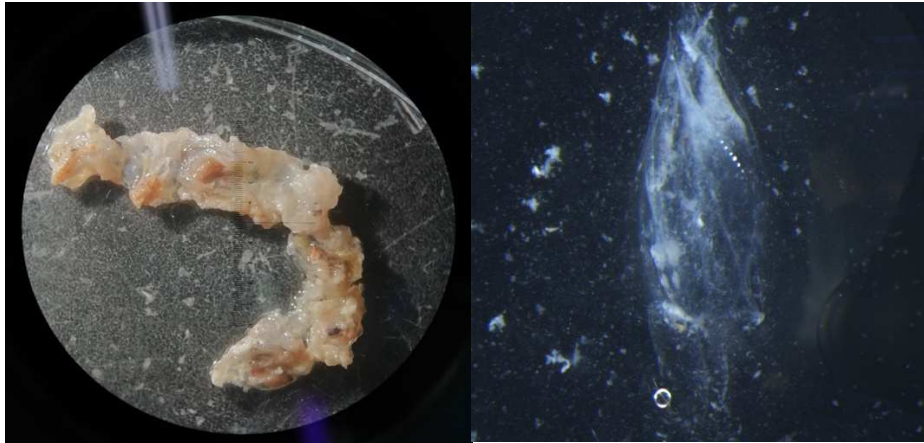
Fig. 3.4 Left: Salpidae colony (Thaliacea). Right: Calicophorae (Hydrozoa) specimen
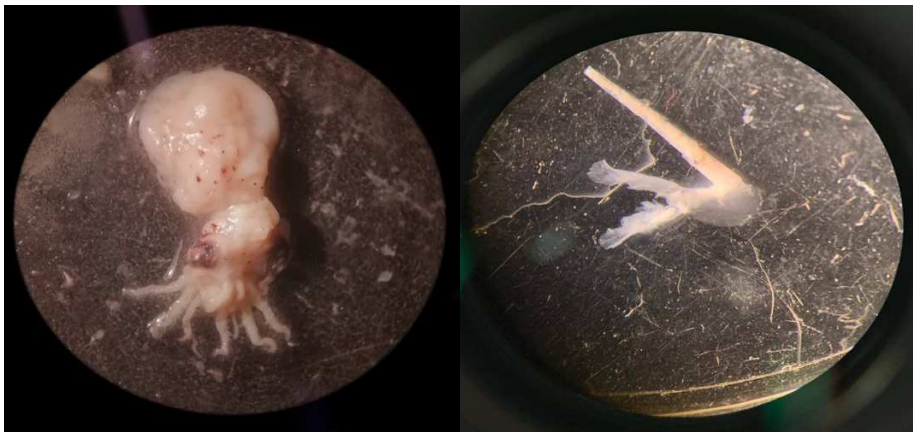
Fig. 3.5. Mollusca: A Sepiolidae cephalopod on the left and the pteropod *Creseis acicula* on the right

Fig. 3.6 Left: different species of Hyperiidea amphipods. Right: *Phronima atlantica*

Fig. 3.7 Decapoda larvae: Penaeidae sub-adult (left) and Brachiura different stages (right)
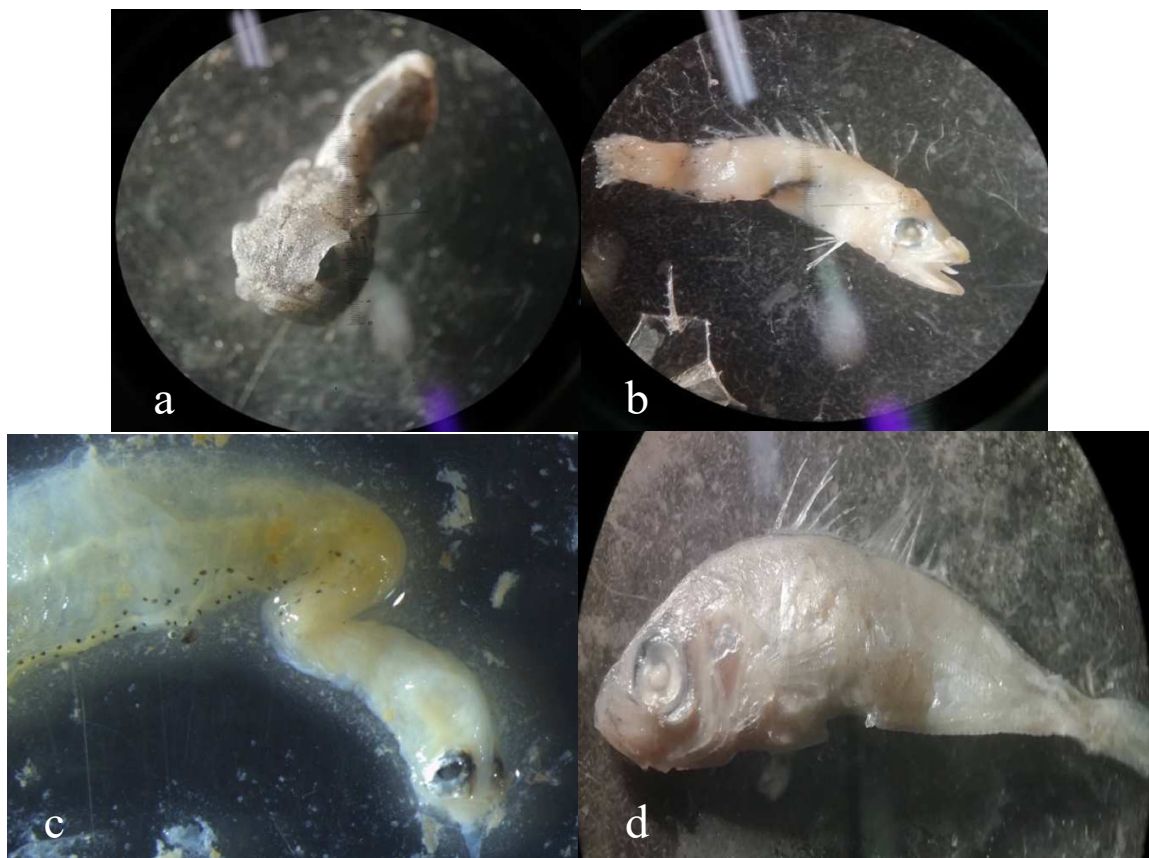
Fig. 3.8 Osteichthyes larvae: a) *Uranoscopus scaber* b) *Serranus* sp. c) *Conger conger*
d) *Trachurus mediterraneus*

The nMDS plot run on the resemblance matrix of log-transformed biomass prey data showed a separation among the subareas, although the stress is quite high (Figure 3.9).
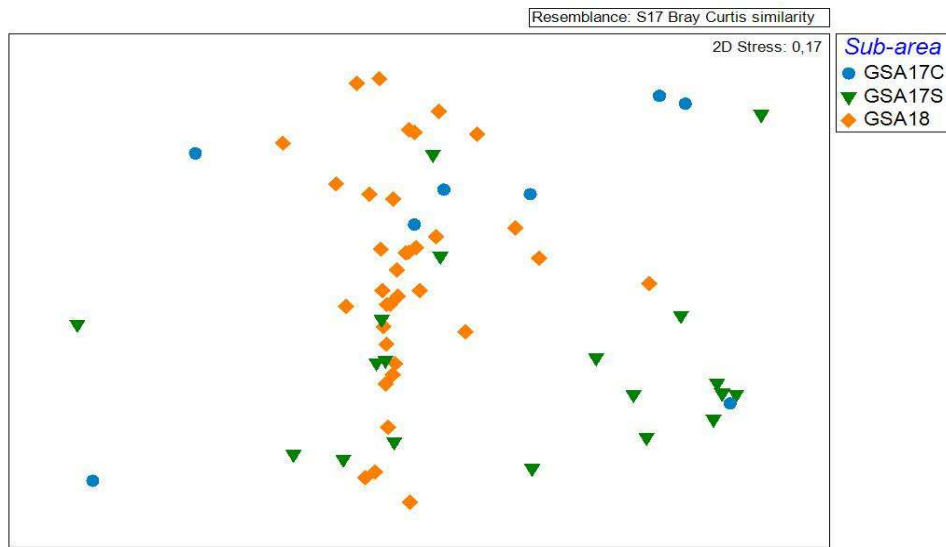
Fig. 3.9 nMDS analysis graphic representation

Two-way PERMANOVA main test revealed significant differences between all the factors (Tab. 3.3a). The pairwise test, taking into account only comparisons between contiguous subareas, showed significant differences among GSA 17 S and GSA 18, between inshore level only. Significant differences were also found between inshore and offshore levels within both GSA 17 S and GSA 18 (Tab. 3.3b).

Tab. 3.3 PERMANOVA main test (a) and pairwise comparison (b) of %W matrix analysis

a)

Source	df	MS	Pseudo-F	P(MC)
Subarea	2	9010.8	3.14	0,0003
In vs Off	1	14977	5.22	0,0001
Subarea x InvsOff	2	6956.3	2.42	0,0026
Residuals	56	2869.8		
Total	61			

b)

Groups	t	P(MC)
GSA17 C In GSA17 S In	1.06	0.32
GSA17 S In, GSA18 In	2.50	0.001
GSA17 C Off, GSA17 S Off	0.90	0.50
GSA17 S Off, GSA18 Off	1.25	0.14
GSA17 C Off, GSA18 Off	1.63	0.0161
GSA17 C In, GSA18 In	2.27	0.0017

Groups	t	P(MC)
GSA17 C Off, GSA17 C In	1.39	0.13
GSA17 S Off, GSA17 S In	1.57	0.0445
GSA18 Off, GSA 18 In	2.71	0.0001

CAP analysis showed (Fig. 3.10) a clear separation of diet composition among subareas.
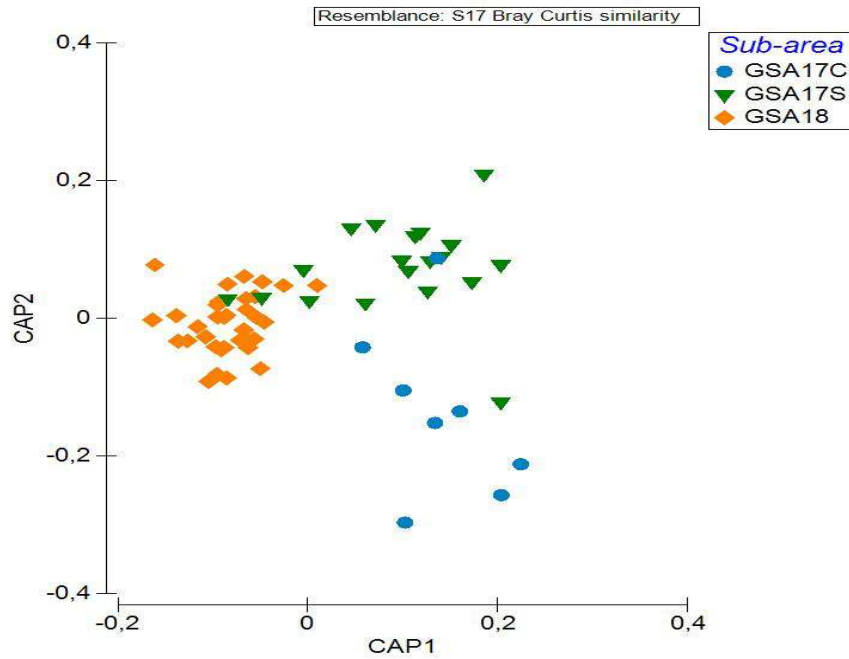
Fig. 3.10 CAP analysis graphic representation

SIMPER analysis (Tab. 3.4) revealed that Calicophorae contributed for the totality of samples similarity in GSA 17 C. In the GSA 17 S In, fish skeletons contributed for 63.83% of samples similarity while, for the remaining subareas, Salpidae explained the similarity between samples, contributing always for more than 60.0%.

Tab. 3.4 Results of SIMPER analysis per geographic subarea, with inshore-offshore levels, with a 60% cut-off

Group GSA17 C In	Average similarity: 0.49			
Species	Av.Abund	Av.Sim	Contrib%	Cum.%
Calicophorae	0.01	0.49	100.00	100.00
Group GSA17 C Off	Average similarity: 24.24			
Salpidae	0.16	15.43	63.68	63.68
Group GSA17 S In	Average similarity: 23.09			
Fish Skeleton	0.03	14.74	63.83	63.83
Group GSA17 S Off	Average similarity: 14.46			
Salpidae	0.12	11.92	82.46	82.46
Group GSA18 In	Average similarity: 44.02			
Salpidae	0.03	43.65	99.17	99.17
Group GSA18 Off	Average similarity: 28.54			
Salpidae	0.09	20.38	71.41	71.41

To run DistLM analysis, environmental variables were previously paired with the Draftsman plot, in order to assess correlations. If correlation value was higher than 0.7, variables were considered correlated, so excluded. Thus, for *Scomber colias*, temperature, O_2 and chlorophyll-a one month, two month and four months before sampling were used for DistLM analysis. The sequential

test showed that these five environmental variables had a cumulative contribution to variance of 28.4% (Tab. 3.5).

Tab. 3.5 Results of sequential test in DistLM analysis

Variable	AIC	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
T	505.35	20788	61.91	0.0001	0.09	0.09	60
O2	503.46	12249	38.19	0.0005	0.06	0.15	59
chla1mo	502.92	7583	24.21	0.0118	0.03	0.18	58
chla4mo	500.8	11683	39.18	0.0006	0.05	0.24	57
chla2mo	498.72	10833	38.13	0.0006	0.05	0.28	56

Then, dbRDA (Distance-based redundancy analysis) plot generated as graphical output of the DistLM showed that dbRDA1 axis explained 46.2% of fitted variation and 13.1 % of total variation, the dbRDA2 axis explained 30.2% of fitted variation and 8.6% of total variation (Fig. 3.11).
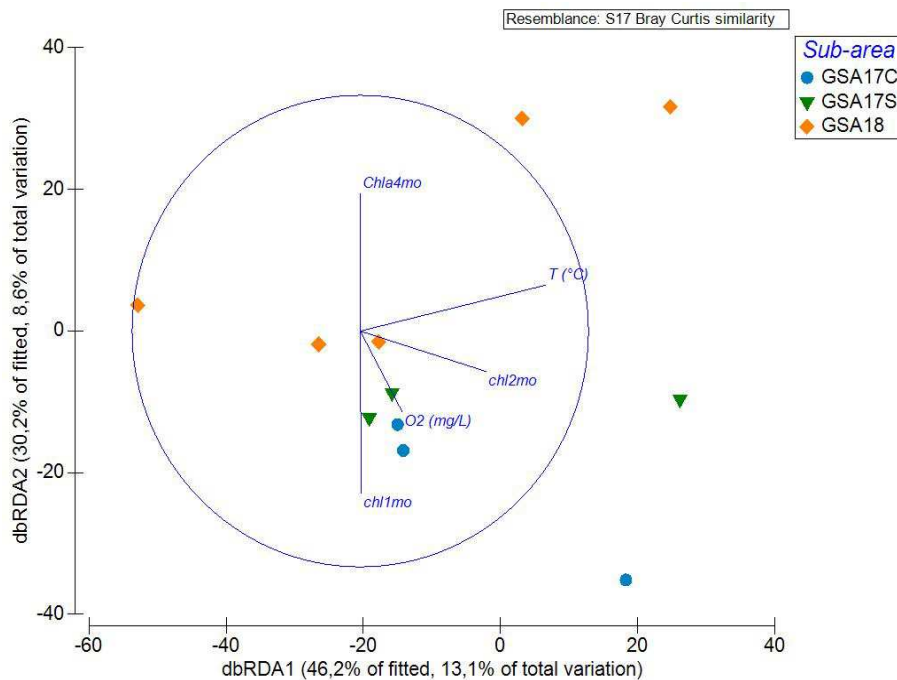
Fig. 3.11 DbRDA of DistLM analysis of environmental variables

Shannon-Wiener diversity index (H') was calculated for each stomach and then the PERMANOVA main test (Tab. 3.6a), ran on univariate H' matrix, revealed significant variations only for "In vs Off" factor. Pairwise comparison (Tab. 3.6b), run on the interaction factor, demonstrated significant differences between inshore and offshore levels inside GSA 17 S and GSA18. These differences were evident relating H' mean values with subareas (Fig. 3.12): all the offshore levels within the factor "subarea" had higher H' mean values then those in the inshore ones.
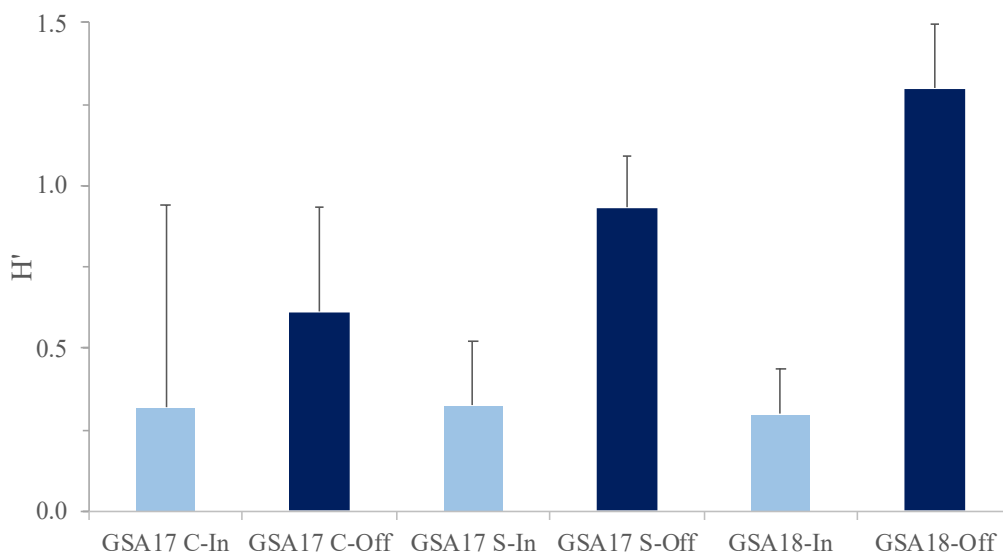
Fig. 3.12 H' mean values (±CI) in the subareas

Tab. 3.6 PERMANOVA main test (a) and pairwise comparison (b) results of H' matrix

a)

Source	df	MS	Pseudo-F	P(MC)
Subarea	2	0.38	2.38	0.10
In vs Off	1	3.43	2.13	0.0001
Subarea x InvsOff	2	0.45	2.78	0.07
Residuals	56	0.16		
Total	61			

b)

Groups	t	P(MC)
GSA17 C Off, GSA17 C In	0.92	0.36
GSA17 S Off, GSA17 S In	2.53	0.0153
GSA 18 Off, GSA 18 In	7.05	0.0001

3.1.2 Scomber scombrus

A total of 16 individuals were sampled, only inshore: 11 in the GSA 17 N and five in the GSA 17 S. Just one male and one female were present, both in the GSA 17 N while, the remaining 14 individuals were undetermined (Fig. 3.13).

%HSI and %FULLNESS were obtained for both subareas and %HSI mean values were similar among subareas while, %FULLNESS mean value was slightly higher in the GSA 17 S (Fig. 3.14).
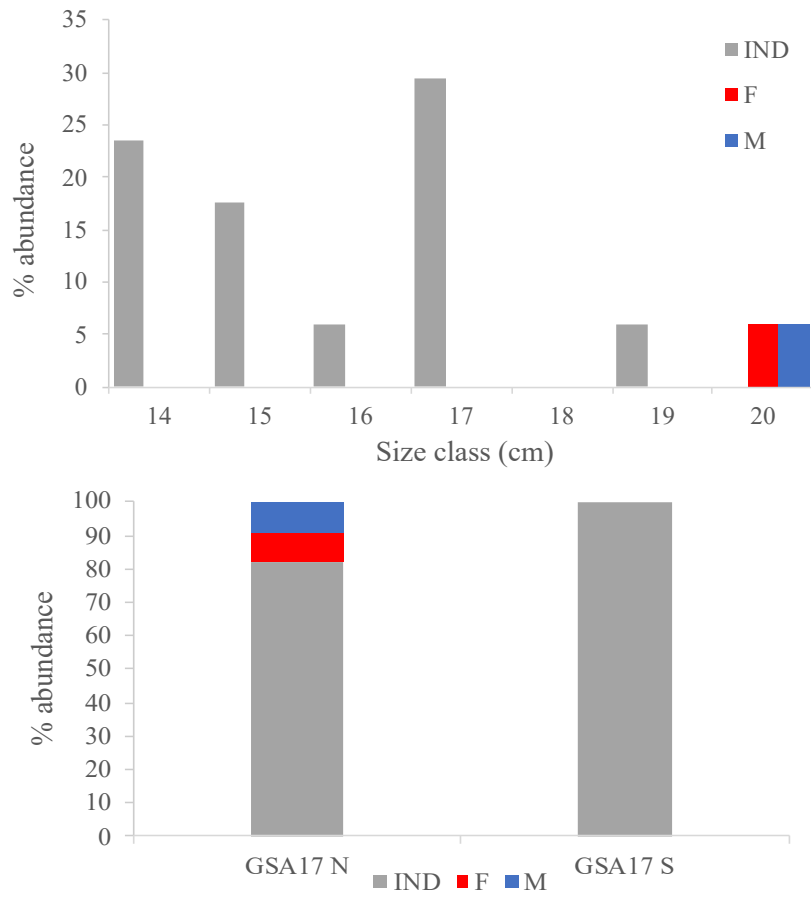
Fig. 3.13 *S. scombrus % of* abundance of by size classes (up) and sex in the two subareas

(bottom)
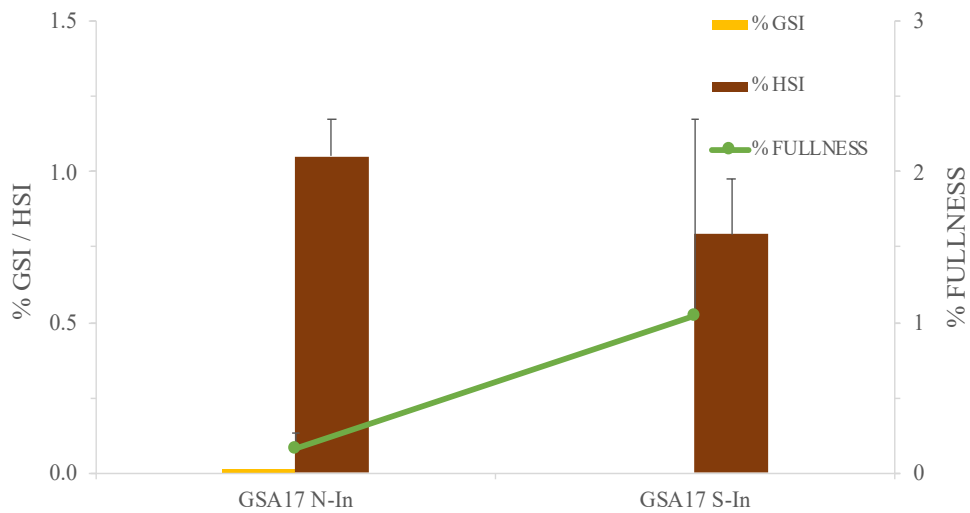
Fig. 3.14 *S. scombrus* %GSI, %HSI and %FULLNESS mean values (±CI) in the subareas

Univariate PERMANOVA analysis showed that %HSI differences between the two subareas were significant. On the contrary, %FULLNESS differences were not significant (Tab. 3.7).

Tab. 3.7 PERMANOVA main test result of %HSI (up) and %FULLNESS (bottom)

Source	df	MS	Pseudo-F	P(MC)
Subarea	1	0.23	5.38	0.036
Residuals	14	0.04		
Total	15			

Source	df	MS	Pseudo-F	P(MC)
Subarea	1	2.65	4.09	0.06
Residuals	14	0.65		
Total	15			

All 16 stomach samples had some contents, and 8 Taxa were identified (listed in the Annex 2). In terms of %IRI (Fig. 3.15), the most representative contents' category in both subareas was "Others", represented mostly by scales and Digenea Trematoda parasites. Parasites were more present in the northern subarea while scales were more abundant in the southern one. Clupeiformes and fish skeletons were also an important prey item, only in the GSA 17 S. Preys of secondary importance for both subareas were thaliaceans like *Pyrosoma* sp. in the GSA 17 N and Salpidae specimens in the GSA 17 S.
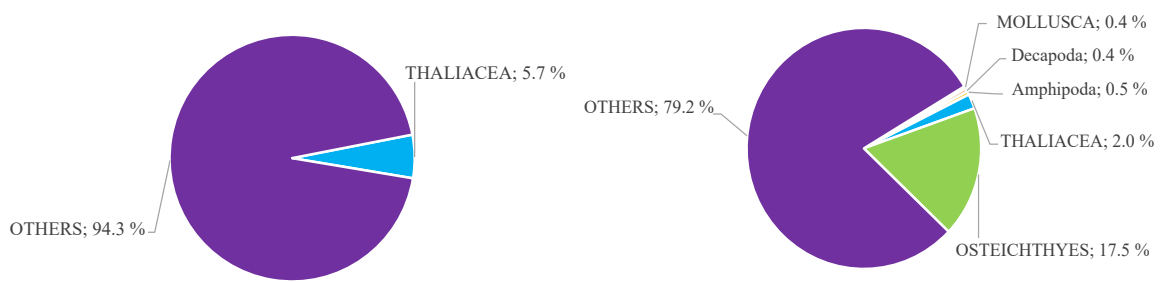
Fig. 3.15 *S. scombrus* %IRI diet composition in GSA17 N (left) and in GSA17 S (right)
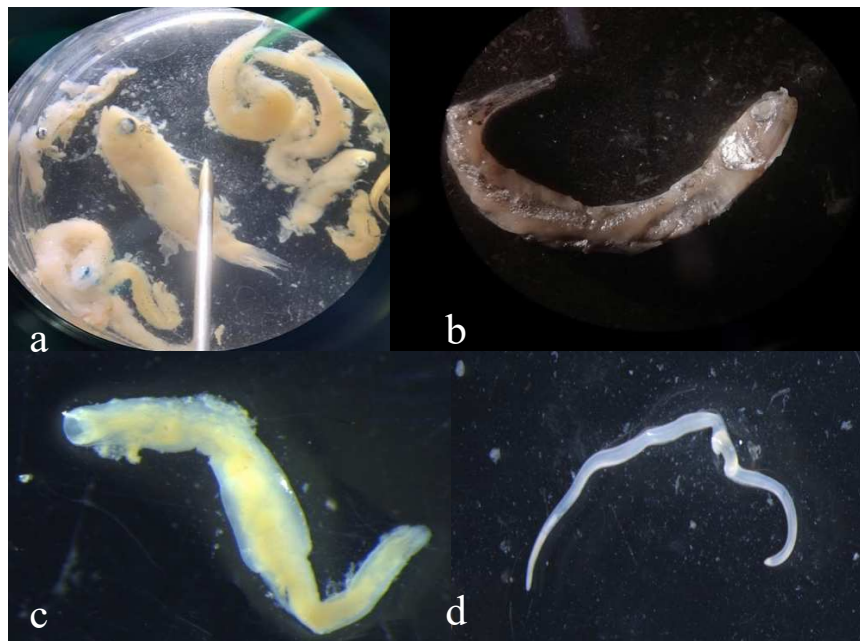
Fig. 3.16 Stomach contents: a) Clupeiformes and *Serranus* sp. b) *E. encrasicolus*

c) Digenea Trematoda parasite d) *Anisakis* sp.

Multivariate nMDS analysis on diet composition highlighted a clear separation between subareas samples (Figure 3.17).
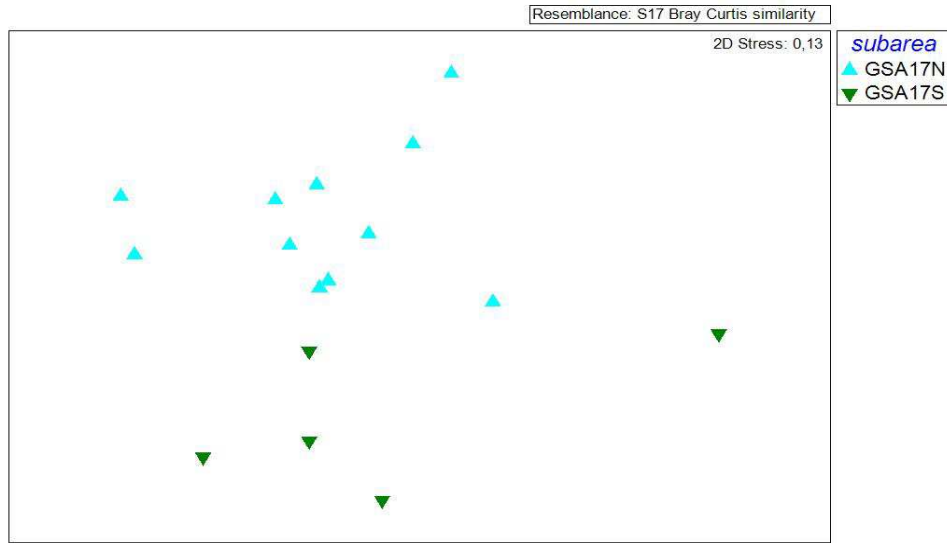
Fig. 3.17 nMDS analysis graphic representation

PERMANOVA main test revealed significant differences between the two subareas (Tab. 3.8).

Tab. 3.8 PERMANOVA main test result of %W matrix analysis

Source	df	MS	Pseudo-F	P(MC)
Subarea	1	11,657	4.19	0.0016
Residuals	14	2783.7		
Total	15			

SIMPER analysis (Tab. 3.9) showed that Trematoda parasites and scales contributed for almost the totality of samples similarity in GSA 17 N. In the GSA 17 S, scales contributed for 94.24% of samples similarity, confirming the same trend of the northern subarea.

Tab. 3.9 Results of SIMPER analysis per geographic subarea with a 60% cut-off

Group GSA17 N In	Average similarity: 31,90			
Species	Av.Abund	Av.Sim	Contrib%	Cum.%
Trematoda parasites	0	18.48	57.94	57.94
Scales	0	13.24	41.5	99.44
Group GSA17 S In	Average similarity: 21,24			
Scales	0.02	20.02	94.24	94.24

For *Scomber scombrus*, temperature, salinity and chlorophyll-a recorded three months before sampling were used for DistLM analysis. Sequential test showed that the best solution was obtained by only one variable, *i.e.* temperature, which explained 22.0% of the total variance (Tab. 3.10). According to the dbRDA, the first axis explained 100% of fitted variation and 21.8% of total variation (Fig. 3.18).

Tab. 3.10 Results of sequential test in DistLM analysis

Variable	AIC	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
T (°C)	129.03	11,018	3.89	0.0004	0.22	0.22	14
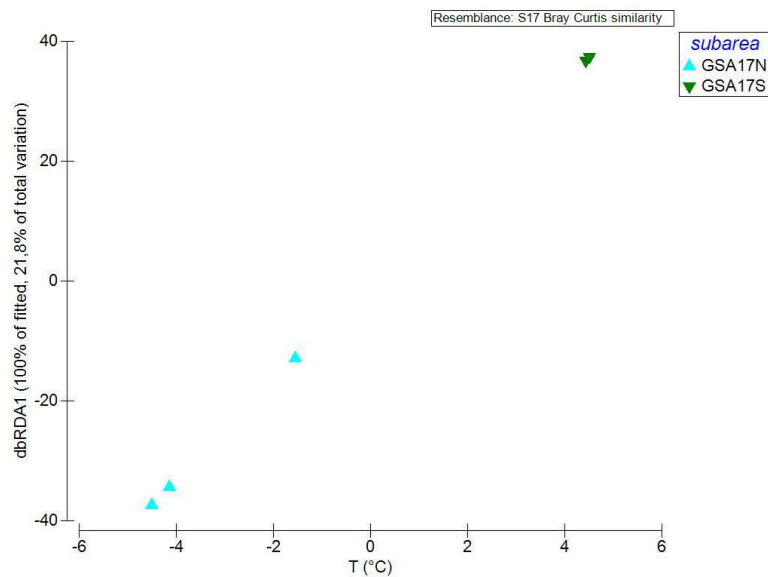
Fig. 3.18 DbRDA of DistLM analysis of environmental variables

Univariate PERMANOVA main test run on the Shannon-Wiener diversity index (H') did not revealed significant differences between GSA 17 N and GSA17 S (Tab. 3.11). H' mean value was slightly higher in the GSA 17 S ($0.67\% \pm 0.55$) than in the GSA 17 N ($0.42\% \pm 0.30$).

Tab. 3.11 PERMANOVA main test results of H' univariate matrix

Source	df	MS	Pseudo-F	P(MC)
subarea	1	0.20	1.38	0.26
Residuals	14	0.15		
Total	15			

3.1.3 Trachurus mediterraneus

A total of 93 individuals were sampled, mainly in the northern subareas. Twentyseven and 51 individuals were sampled in the GSA 17 N and in the GSA 17 C, respectively, while 11 were sampled in the GSA 17 S and four in the GSA 18. Nine individuals were undetermined, whereas, females and males, both absent in the GSA 18, were 45 and 39, respectively (Fig. 3.19). As, samples for each subarea's inshore and offshore level were not available, a PERMANOVA design with nested factors was necessary for subsequent univariate and multivariate analyses.
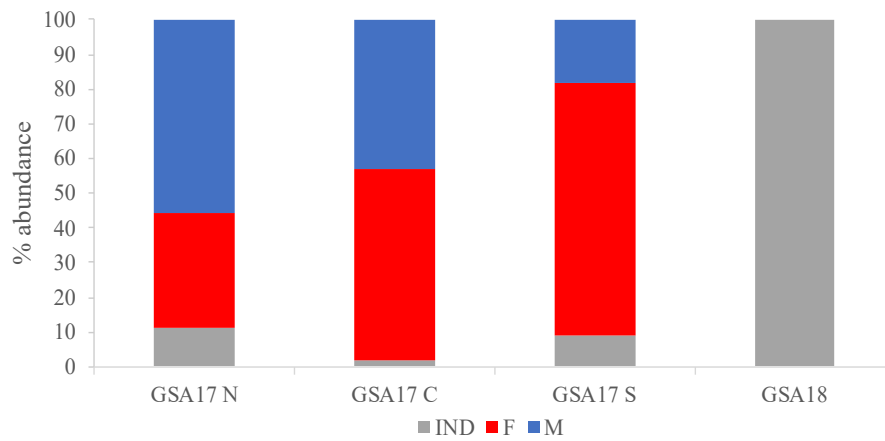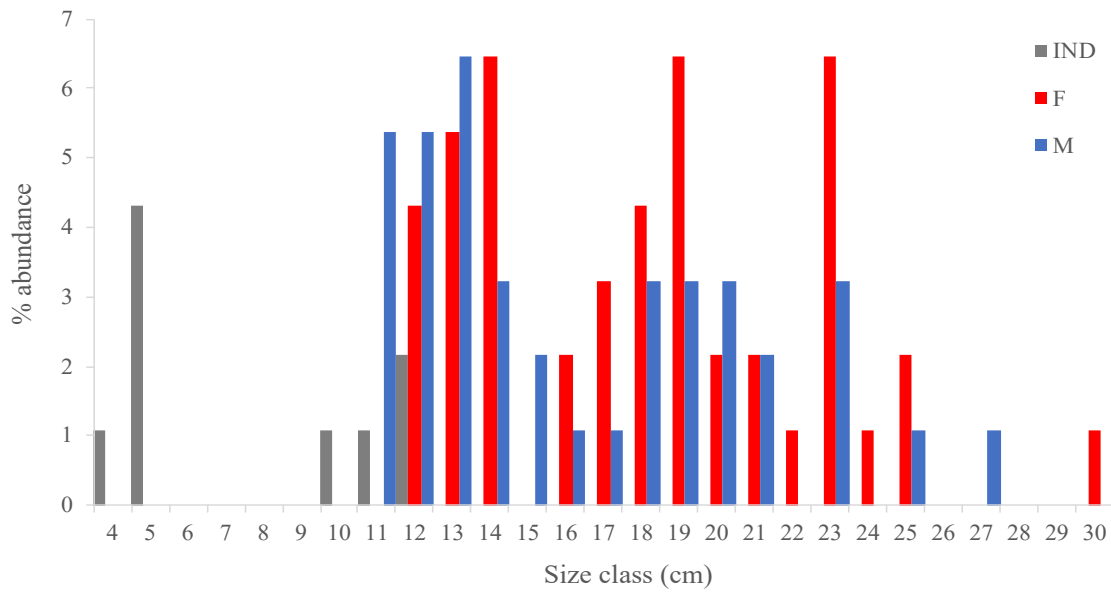
Fig. 3.19 *T. mediterraneus % of* abundance by size classes (up) and sex in subareas

(bottom)

%GSI was obtained, except for offshore levels of the GSA 17 C and GSA 18, where females were absent. %HSI and %FULLNESS were obtained for each subarea. For %HSI, similar mean values were obtained among subareas while, %FULLNESS mean values resulted very low except for the one of the GSA 18 (Fig. 3.20).
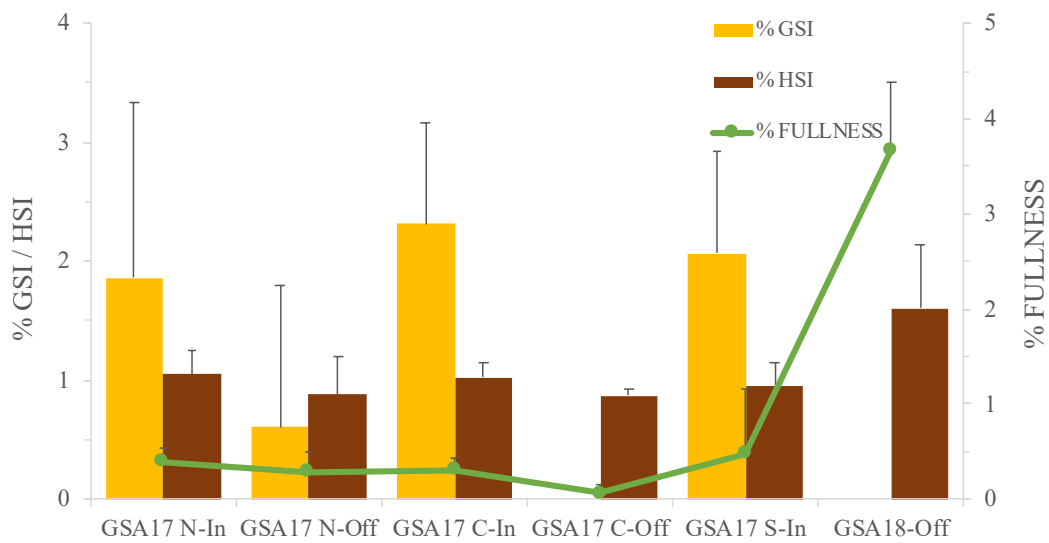
Fig. 3.20 *T. mediterraneus* %GSI, %HSI and %FULLNESS mean values (±CI) in the

subareas

Univariate PERMANOVA main tests showed that %GSI and %HSI differences were not significant for any of the factors tested (Tab. 3.12). Main test for %FULLNESS (Tab. 3.13a) showed that differences were significant among subareas and, taking into account only comparisons between contiguous subareas, between GSA 17 S and GSA 18 (Tab. 3.13b).

Tab. 3.12 %GSI (up) and %HSI (bottom) PERMANOVA main test result

Source	df	MS	Pseudo-F	P(MC)
Subarea	3	4.30	0.49	0.72
In vs Off (Subarea)	2	8.84	1.48	0.24
Residuals	70	5.96		
Total	75			

Source	df	MS	Pseudo-F	P(MC)
Subarea	3	0.51	5.26	0.15
In vs Off (Subarea)	2	0.10	0.65	0.52
Residuals	70	0.15		
Total	75			

Tab. 3.13 %FULLNESS PERMANOVA results: a) main test b) pairwise comparison

a)

Source	df	MS	Pseudo-F	P(MC)
Subarea	3	13.27	111.57	0.0038
In vs Off (Subarea)	2	0.11	0.43	0.64
Residuals	70	0.27		
Total	75			

b)

Groups	t	P(MC)
GSA17 N, GSA17 C	1.24	0.31
GSA17 C, GSA17 S	1.73	0.39
GSA17 S, GSA18	5.37	0.0003
GSA17 N, GSA17 S	0.95	0.56
GSA17 N, GSA18	16.92	0.0003
GSA17 C, GSA18	16.09	0.037

Of 93 analyzed stomach samples, 16 were empty. In the remaining 77, a total number of 44 Taxa were identified (listed in the Annex 3). In terms of % IRI (Fig. 3.21), more representative preys were pelagic copepods (mainly *Acartia* and *Euchaeta* spp.) in the GSA17 N and in the GSA18. Scales were the most representative stomach content in the GSA17 C and GSA17 S. Several *E. encrasicolus* larvae were also found, in the GSA17 S and GSA18. Amphipods were quite representative, with some benthic (Gammaridea) and pelagic (Hyperiidea) taxa. Benthic decapods like *Alpheus glaber* and *Philocheras bispinosus* were also present, as well as mysids like *Siriella* sp. (above all in the GSA17 C). Preys of secondary importance were small bivalves and gastropods, stomatopods and Foraminifera species.

Fig. 3.21 *T. mediterraneus* %IRI diet composition in GSA17 N (up-left), GSA17 C (up-right), GSA17 S (bottom-left), GSA18 (bottom-right)

Fig. 3.22 Molluscs: bivalves juv. (left), gastropod (middle), *Illex coindetii* (right)

Fig. 3.23 Stomatopoda larvae: a species of Squillidae family
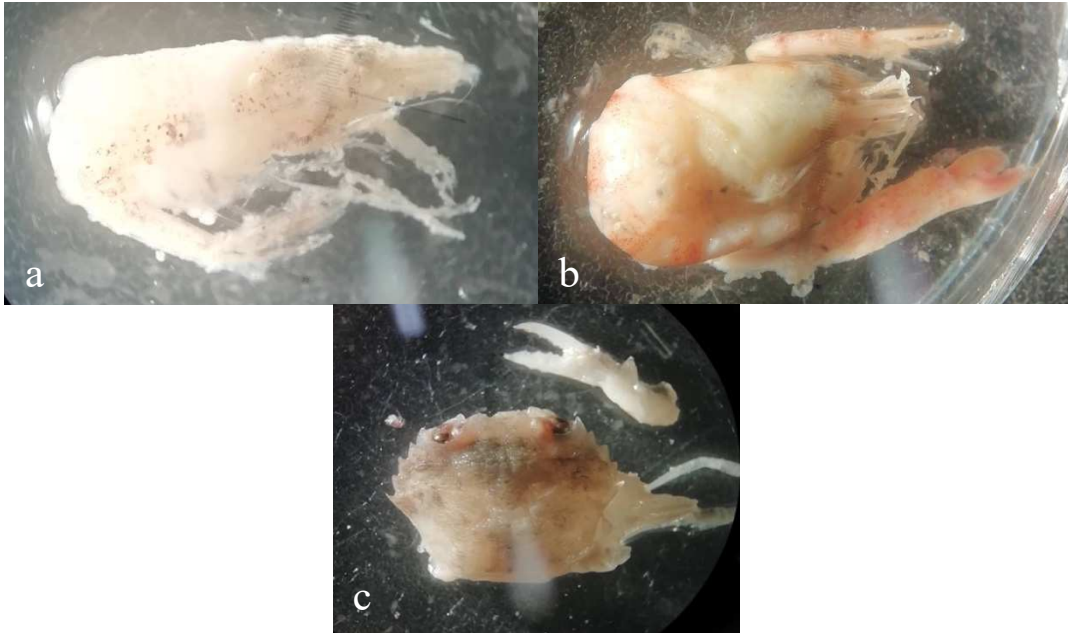
Fig. 3.24 Decapoda crustaceans: a) *Philocheras bispinosus* b) *Alpheu glaber*
c) *Leocarcinus depurator*
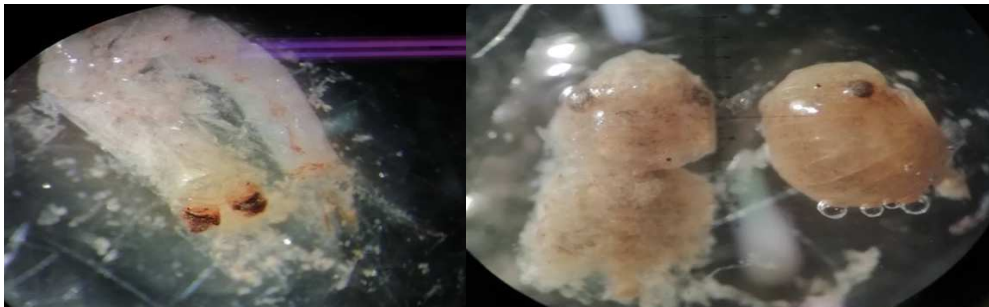
Fig. 3.25 Left: *Siriella* sp mysid. Right: *Sphaeroma* sp. isopods

Fig. 3.26 Gammaridea amphipods: *Westwoodilla rectirostris* (left), *Ampelisca* sp. (right)

Fig. 3.27 Different species of Foraminifera

The nMDS analyses did not evidenced a clear samples separation among subareas (Fig. 3.28).
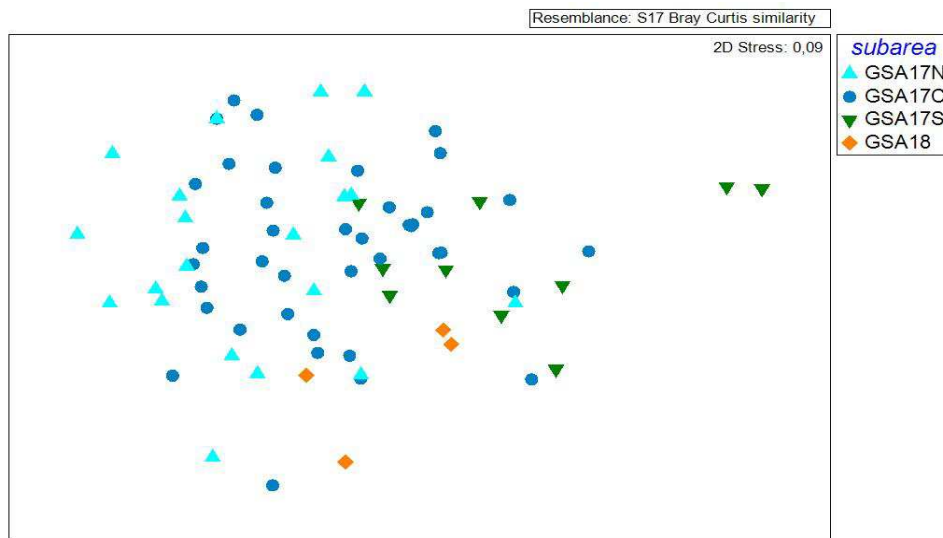
Fig. 3.28 nMDS analysis graphic representation

Accordingly, two-way PERMANOVA main test did not revealed significant differences between subareas, apart for "In vs Off" factor (nested in subarea) (Tab. 3.14a). The pairwise test showed that significant differences were present between inshore and offshore level of GSA17 C (Tab. 3.14b).

Tab. 3.14 PERMANOVA main test (a) and pairwise comparison (b) of %W matrix analysis

a)

Source	df	MS	Pseudo-F	P(MC)
Subarea	3	7549.8	1.20	0.27
In vs Off (Subarea)	2	6431.7	1.47	0.0457
Residuals	69	4363.7		
Total	74			

b)

Groups	t	P(MC)
GSA17 N Off, GSA17 N In	1.03	0.38
GSA17 C Off, GSA17 C In	1.36	0.0457

SIMPER analysis (Tab. 3.15) revealed that, in the GSA 17 N In, the copepod *Acartia* sp and the amphipod *Ampelisca* sp. contributed for 57.97% and 12.04%, respectively, to samples similarity. In the GSA 17 N Off, *Acartia* sp. and Foraminifera contributed for a cumulative value of 69.15% to samples similarity. In both inshore level of GSA 17 C and GSA 17 S, fish scales contributed to similarity between samples for 66.43% and 61.33%, respectively. For the GSA 18 Off, fish bones and *Calanus*-like copepods were the prey that mostly contributed to samples similarity, accounting for 55.09% and 15.03%, respectively.

Tab. 3.15 Results of SIMPER analysis per geographic subarea, with inshore-offshore levels, with a 60% cut-off

Group GSA17 N In		Average similarity: 5.17		
Species	Av.Abund	Av.Sim	Contrib%	Cum.%
Acartia sp.	0	3	57.97	57.97
Ampelisca sp.	0	0.62	12.04	70.01
Group GSA17 N Off		Average similarity: 5.07		
Acartia sp.	0	2.02	39.83	39.83
Foraminifera	0	1.49	29.33	69.15
Group GSA17 C In		Average similarity: 8.74		
Scales	0.01	5.81	66.43	66.43
Group GSA17 C Off		Average similarity: 31.50		
Temora stylifera	0	15.75	50	50
Creseis acicula	0	15.75	50	100
Group GSA17 S In		Average similarity: 8.30		
Scales	0	5.09	61.33	61.33
Group GSA18 Off		Average similarity: 9.09		
Fish Skeleton	0	5.01	55.09	55.09
Calanus -like	0	1.37	15.03	70.12

Environmental variables previously test with the Draftsman plot and then selected for DistLM analysis were temperature, O_2, salinity and a-chlorophyll recorded three months before the sampling. However, the sequential test showed that these five environmental variables had a cumulative contribution to variance lower than 10.0% and that only temperature significantly explained variations in diet (with a contribution of 4.9%).

Univariate PERMANOVA main test (Tab. 3.16a), revealed significant H' differences only for inshore *vs* offshore factor, nested in subareas. Then, pairwise comparison (Tab. 3.16b) showed significant differences inside GSA 17 C subarea, between inshore and offshore levels. H' mean values in offshore areas was greater than in inshore ones (Fig. 3.29).
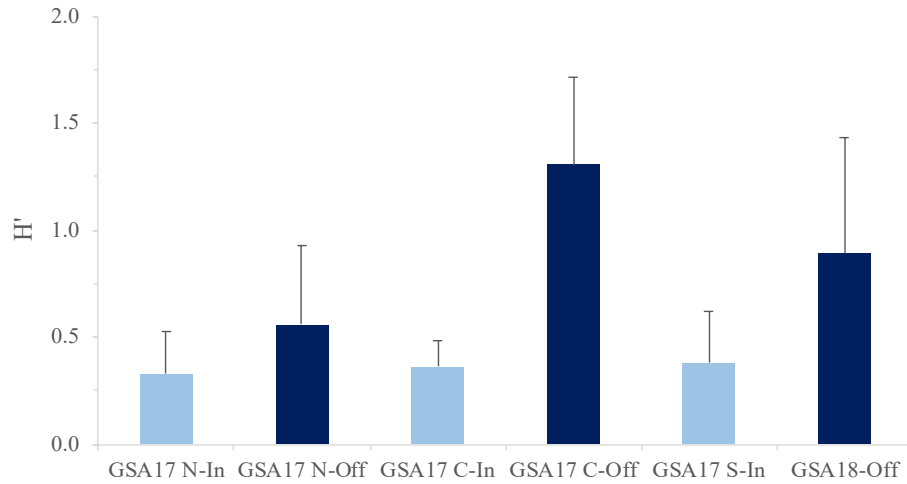
Fig. 3.29 H' mean values (±CI) in the subareas

Tab. 3.16 PERMANOVA main test (a) and pairwise comparison (b) results of H'

a)

Source	df	MS	Pseudo-F	P(MC)
Subarea	3	0.53	0.56	0.69
In vs Off (Subarea)	2	0.97	5.97	0.0039
Residuals	70	0.16		
Total	75			

b)

Groups	t	P(MC)
GSA17 N Off, GSA17 N In	1.18	0.256
GSA17 C Off, GSA17 C In	3.42	0.0019

3.1.4 Trachurus trachurus

Forty-two individuals were sampled, mainly in the southern subareas. Indeed, only two were sampled in the GSA 17 N, 7 in the GSA 17 C, 23 in the GSA 17 S and 10 in the GSA 18. The abundance of adults was very low, with just three males and no female caught. Thirty-nine undetermined individuals were caught

in all the subareas, almost half of them ranged in size between 9 and 10 cm TL (Fig. 3.30). Samples were not available for each subarea's inshore and offshore level, thus a PERMANOVA design with nested factors was necessary for subsequent univariate and multivariate analyses.

Considering that females were absent, %GSI was not calculated. %HSI mean values were quite similar among subareas, except for GSA 17 S offshore, where a slightly higher mean value was calculated. %FULLNESS mean values resulted lower in the northern subareas, compared with the offshore ones of GSA 17 S and GSA 18 (Fig. 3.31).
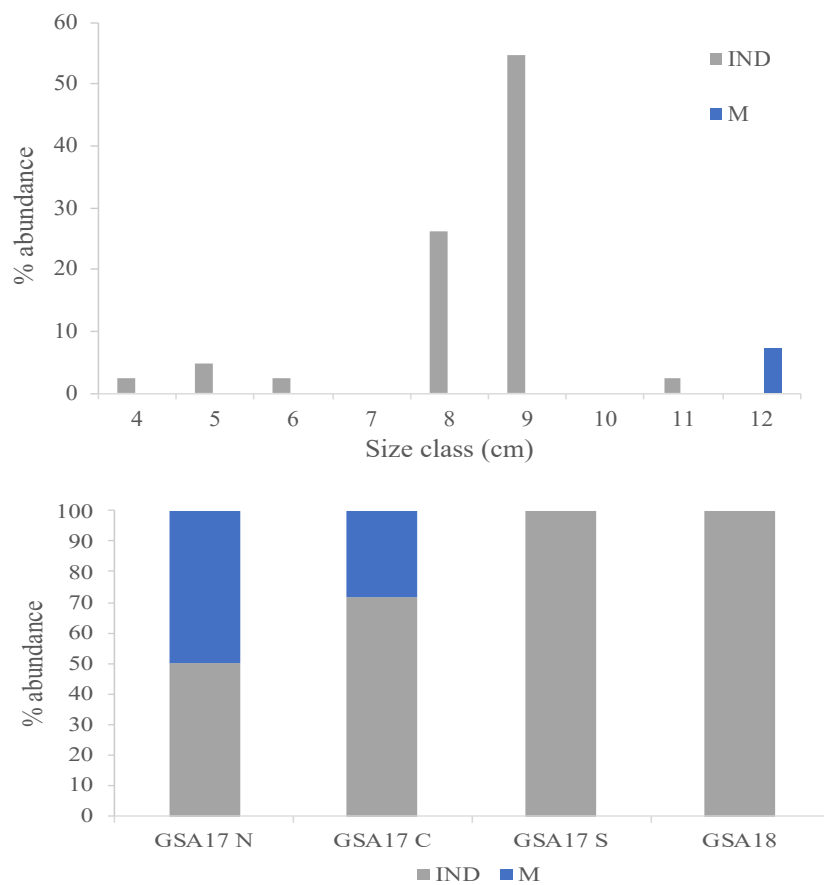
Fig. 3.30 *T. trachurus* % of abundance by size classes (up) and sex in the subareas(bottom)
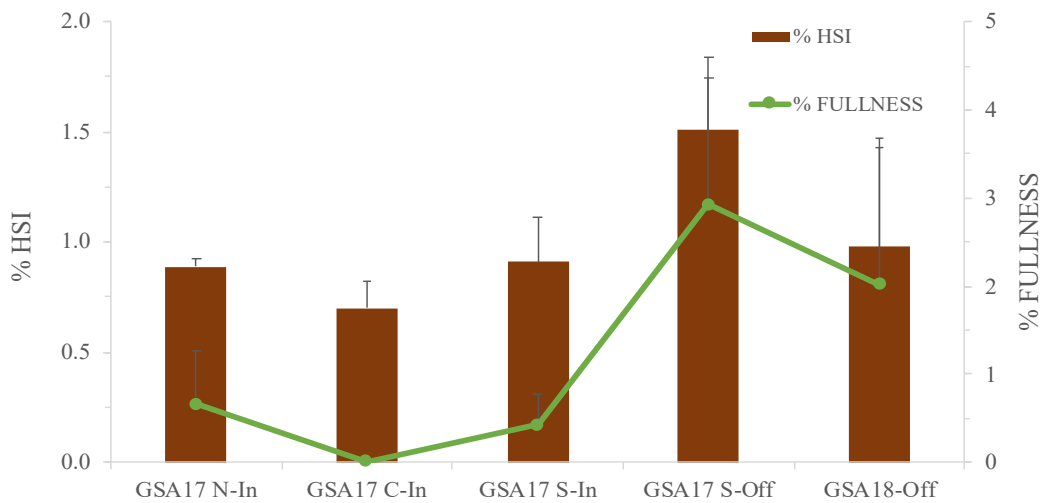
Fig. 3.31 *T. trachurus* %HSI and %FULLNESS mean values (±CI) in the subareas

Univariate PERMANOVA main tests showed that %HSI and %FULLNESS differences were significant for inshore vs offshore factor, nested in subareas (Tab. 3.17). These and following pairwise comparison tests were not run because the only subarea with both location levels represented (*i.e.* inshore and offshore) was the GSA 17 S. The results obtained from main tests suggested significant differences within the GSA 17 S, between inshore and offshore levels.

Tab. 3.17 PERMANOVA main test results for: a) % HSI b) % FULLNESS

a)

Source	df	MS	Pseudo-F	P(MC)
Subarea	3	0.17	0.27	0.85
In vs Off (Subarea)	1	0.66	6.24	0.0178
Residuals	29	0.11		
Total	33			

b)

Source	df	MS	Pseudo-F	P(MC)
Subarea	3	2.71	0.25	0.87
In vs Off (Subarea)	1	11.37	5.53	0.0266
Residuals	29	2.06		
Total	33			

Out of 42 analyzed stomachs, 34 were full. Twenty-two taxa were identified (see Annex 4). In terms of %IRI (Fig. 3.32), in the GSA 17 N, most representative preys were copepods (mostly *Acartia* sp.) whereas in the GSA 17 C, scales and Trematoda parasites were the most representative food item. The euphausiid *Nyctiphanes couchii* was the most important prey for GSA 17 S and GSA 18 samples. In the latter subarea, also crustaceans such as the copepods *Euchaeta* sp. and *Calanus* spp. and hyperiids were abundant, in terms of IRI. Osteichthyes were preys of secondary importance, occurring only in the GSA 18.
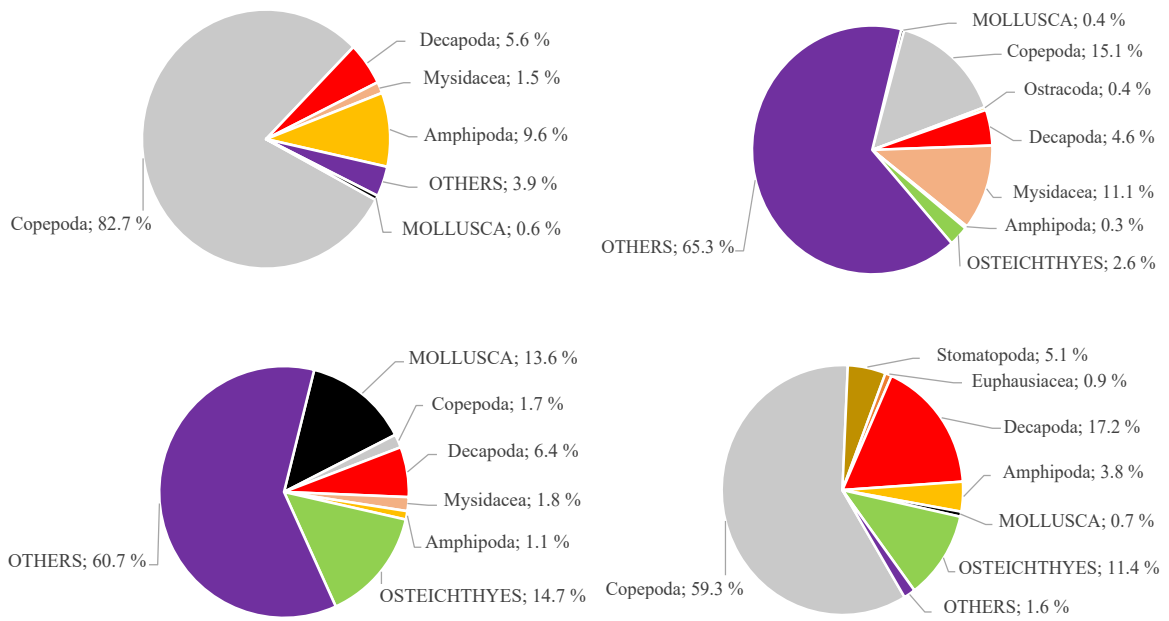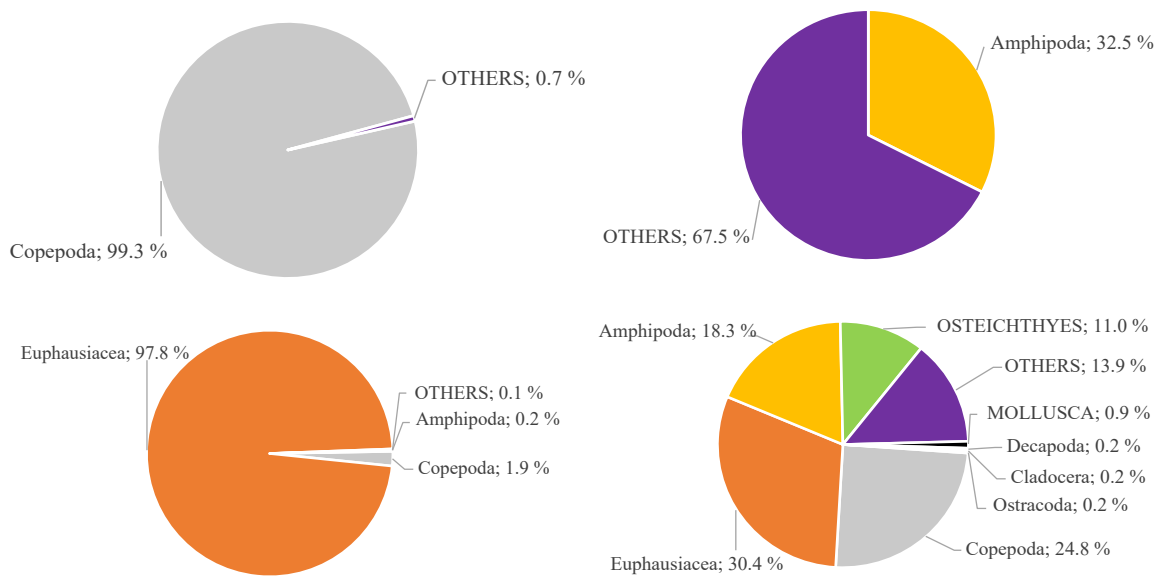
Fig. 3.32 *T. trachurus* %IRI diet composition in GSA17 N (up-left), GSA17 C (up-right),

GSA17 S (bottom-left), GSA18 (bottom-right)

Fig. 3.33 Euphausiids: *Nyctiphanes couchii* (left and middle) and *Nematoscelis* sp. (right)

Fig. 3.34 Copepods: stomach content full of *Acartia* sp. (left); *Euterpina acutifrons* (right)

The nMDS analysis on diet composition highlighted a clear separation of GSA 17 N and GSA 17 C samples with those of GSA 17 S and GSA 18 (Figure 3.35).

Fig. 3.35 nMDS analysis graphic representation

PERMANOVA main test revealed significant differences between inshore and offshore level of GSA17 S (Tab. 3.18).

Tab. 3.18 PERMANOVA main test result of %W matrix analysis

Source	df	MS	Pseudo-F	P(MC)
Subarea	3	6296.8	0.49	0.91
In vs Off (Subarea)	1	13568	4.96	0.0002
Residuals	28	2737.3		
Total	32			

Based on SIMPER analysis (Tab. 3.19), Copepoda group contributed for 77.73% to similarity of samples in the GSA 17 S In. On the other hand, in the offshore level of the same subarea, *Nyctiphanes couchii* contributed for almost the totality (98.95%) of samples similarity. In the GSA 18 Off, the same euphausiid species and unidentified digestive pulp contributed for 67.5% of cumulative samples similarity.

Tab. 3.19 Results of SIMPER analysis per geographic subarea with a 60% cut-off for low contribution

Group GSA17 N In				
Species	Av.Abund	Av.Sim	Contrib%	Cum.%
All the similarities are zero				
Group GSA17 C In				
All the similarities are zero				
Group GSA17 S In		Average similarity: 52.37		
Copepoda	0.01	40.71	77.73	77.73
Group GSA17 S Off		Average similarity: 45.07		
Nyctiphanes couchii	0.07	44.59	98.95	98.95
Group GSA18 Off		Average similarity: 5.36		
Nyctiphanes couchii	0.01	2.18	40.76	40.76
Digestive pulp	0.01	1.43	26.74	67.5

For *Trachurus trachurus*, based on the Draftsman plot results, only temperature, O_2 and a-chlorophyll recorded five months before the sampling were retained for the subsequent DistLM model. Sequential test showed that these three environmental variables accounted to 27.6% of total variance (Tab. 3.20).

Tab. 3.20 Results of sequential test in DistLM analysis

Variable	AIC	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Chla 5mo	269.4	14444	4.36	0.0004	0.12	0.12	31
O2	268.47	8695.9	2.78	0.0046	0.07	0.20	30
T	267.09	9152.8	3.13	0.0027	0.08	0.28	29

DistLM provided a dbRDA graphic (Fig. 3.36) with the first axis that explained 61.6% of fitted variation and 17.0% of total variation, and the second axis that explained 22.3% of fitted variation and 6.1% of total variation.

PERMANOVA main test on univariate H' matrix (Tab. 3.21) did not revealed significant differences among factors. H' mean values resulted higher in the GSA 17 S inshore and GSA 18 offshore (Fig. 3.37).
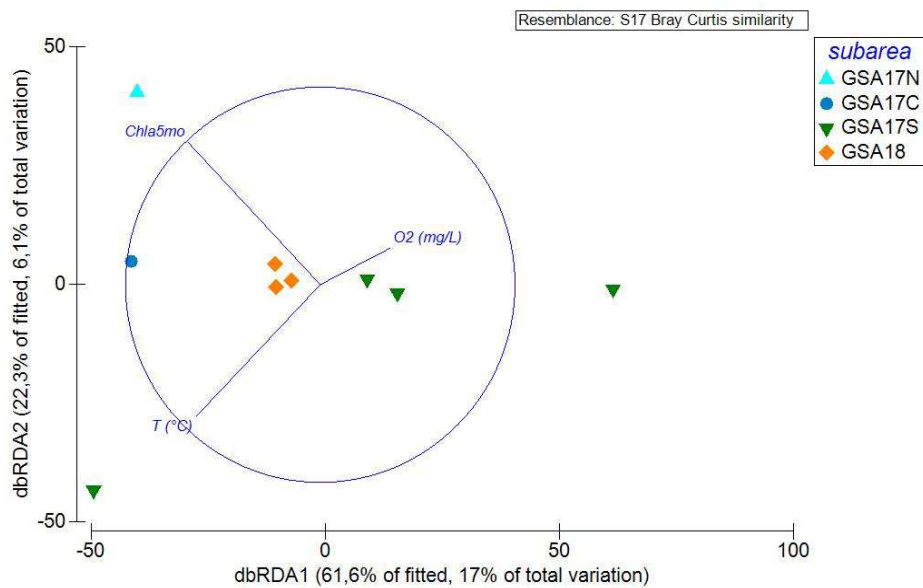
Fig. 3.36 DbRDA of DistLM analysis of environmental variables

Tab. 3.21 PERMANOVA main test results of H' matrix

Source	df	MS	Pseudo-F	P(MC)
Subarea	3	0.50	1.46	0.53
In vs Off (Subarea)	1	0.35	2.53	0.12
Residuals	29	0.14		
Total	33			
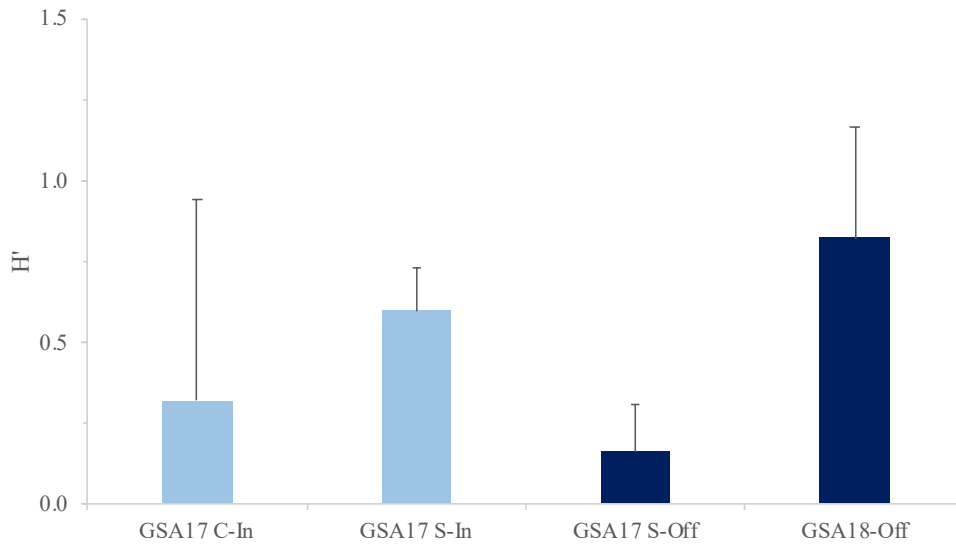
Fig. 3.37 H' mean values (±CI) in the subareas

3.1.5 Species overlap

Data of the three biological indexes were all combined in order to obtain the overall picture of the biological condition of small pelagics across the sampling areas and considering inshore *vs.* offshore conditions. The highest mean value of %GSI was obtained for the inshore level of GSA 17 C while, the lowest, for the GSA18. %HSI showed similar mean values for all the subareas. On the contrary, %FULLNESS mean values had an increasing trend toward southern

subareas, with two peaks, in the offshore levels of GSA 17 S and GSA18 (Fig. 3.38).
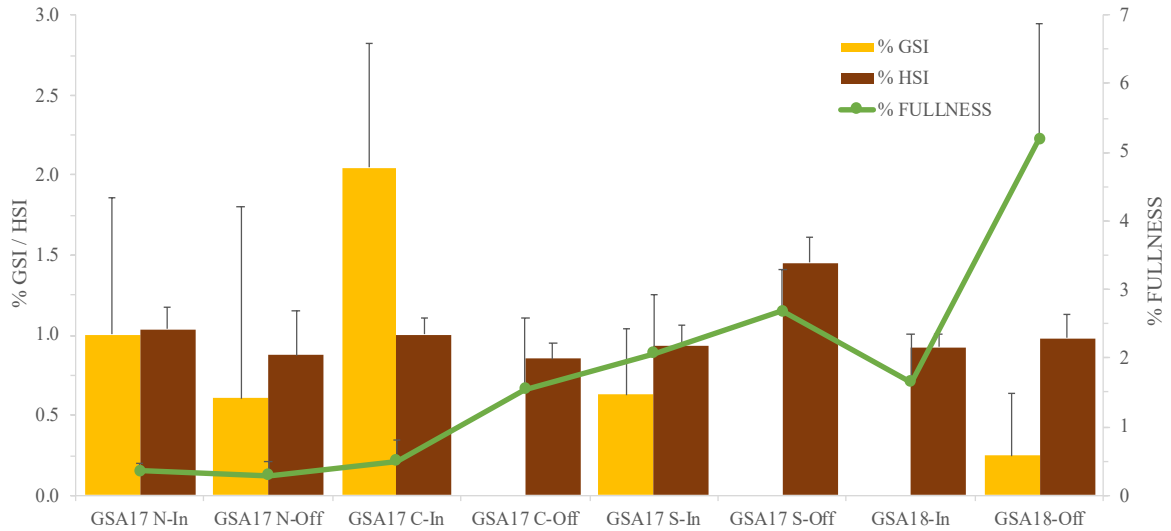
Fig. 3.38 Combination of species' %GSI, %HSI and %FULLNESS mean values (±CI) in the subareas

Univariate and multivariate analyses underwent to the previously described three-way PERMANOVA design, with the addition of "Species" as factor. PERMANOVA main test on the univariate matrix of %GSI (Tab. 3.22a) showed that significant differences were present only between species. The pairwise test comparison results (Tab. 3.22b) allowed visualizing significant differences only between *Trachurus mediterraneus* and *Scomber colias*.

Tab. 3.22 PERMANOVA tests for %GSI matrix: a) main test b) pairwise comparison

a)

Source	df	MS	Pseudo-F	P(MC)
Species	3	7.79	4.59	0.0153
Subarea (Species)	9	1.48	0.48	0.85
In vs Off (Subarea (Species))	6	3.14	1.15	0.33
Residuals	169	2.73		
Total	187			

b)

Groups	t	P(MC)
Tratra, Scocol	0.42	0.67
Tramed, Scocol	2.52	0.040
Tratra, Tramed	1.94	0.07
Scosco, Tratra	1.46	0.22
Scosco, Tramed	1.67	0.14
Scosco, Scocol	0.38	0.71

PERMANOVA main test on the univariate matrix of %HSI did not showed significant differences among factors (Tab. 3.23). Main test for %FULLNESS (Tab. 3.24) showed significant differences for "In vs Off (Subarea (Species))" factor.

Tab. 3.23 PERMANOVA main test for %HSI matrix

Source	df	MS	Pseudo-F	P(perm)
Species	3	0.22	0.90	0.48
Subarea (Species)	9	0.27	1.89	0.31
In vs Off (Subarea (Species))	6	0.15	1.50	0.19
Residuals	169	0.10		
Total	187			

Tab. 3.24 PERMANOVA main test for %FULLNESS matrix

Source	df	MS	Pseudo-F	P(perm)
Species	3	30.12	2.74	0.09
Subarea (Species)	9	9.26	0.29	0.93
In vs Off (Subarea (Species))	6	38.78	7.21	0.0016
Residuals	169	5.38		
Total	187			

nMDS analysis on diet composition highlighted well-defined separations, among the four species (Fig. 3.39)
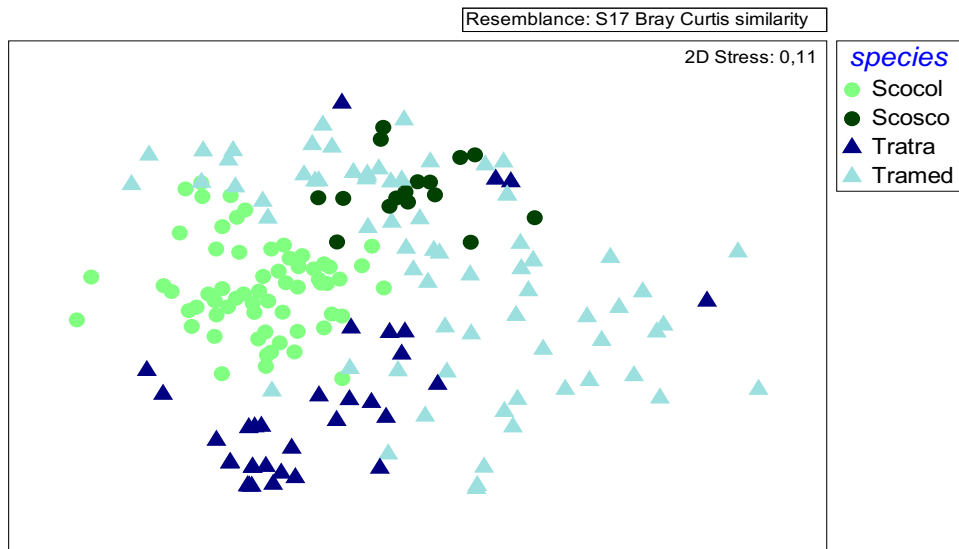
Fig. 3.39 Graphic representation of nMDS analysis separations between species

When analyzing diet overlap between co-generic species, the separation was even clearer (Fig. 3.40a-b).

a)
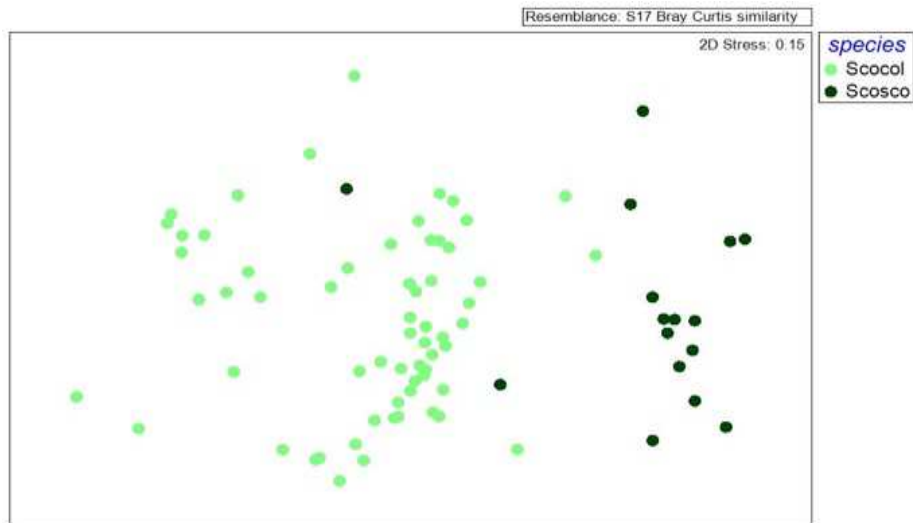
b)
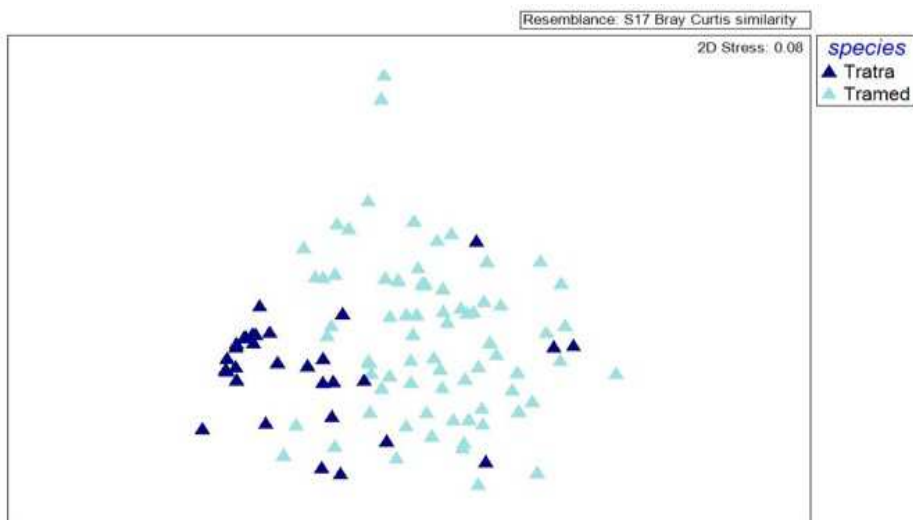
Fig. 3.40 Graphic representation of nMDS analysis separations between: *Scomber* species (a) and *Trachurus* species (b)

Three-way PERMANOVA main test, run on Bray-Curtis resemblance matrix of the log-transformed biomass data showed significant differences for factor "Species" and "In *vs.* Off (Subarea (Species))" factor (Tab. 3.25a). Pairwise comparison run on "Species" (Tab. 3.25b) demonstrated significant differences in diet composition, between *Scomber colias* and the two species of *Trachurus*.

Tab. 3.25 Main test (a) and pairwise test (b) from PERMANOVA of %W multivariate matrix

a)

Source	df	MS	Pseudo-F	P(perm)
Species	3	15991	2.11	0.0065
Subarea (Species)	9	7623.9	0.98	0.54
In vs Off (Subarea (Species))	7	9163.1	2.67	0.0001
Residuals	164	3426.4		
Total	183			

b)

Groups	t	P(perm)
Tratra, Scocol	1.58	0.043
Tramed, Scocol	1.65	0.0037
Tratra, Tramed	1.12	0.25
Scosco, Tratra	1.25	0.19
Scosco, Tramed	1.35	0.11
Scosco, Scocol	1.46	0.09

CAP analysis graphically proved a good resource partitioning among species (Fig. 3.41) accordingly to the significant results highlighted by the previous pairwise test.
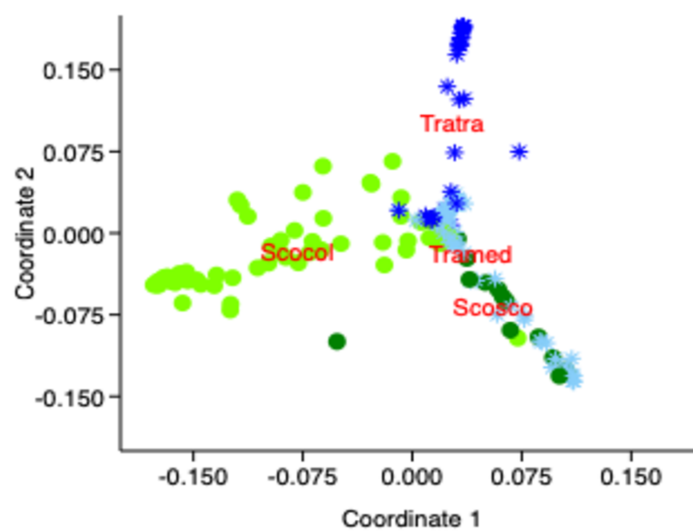
Fig. 3.41 CAP analysis graphic representation

The PERMDISP pairwise test (Tab. 3.26) showed that dispersions were significant among *Trachurus* species and among *Trachurus mediterraneus* and *Scomber* species. Considering "means and standard errors" (Tab. 3.26), a proxies of diet generalism *vs*. specialization, a more generalized diet was noticeable for *Trachurus mediterraneus*. On the contrary, *Scomber scombrus* resulted the species with the most specialized diet, although results can be biased by the poor sample size.

Tab. 3.26 PERMDISP pairwise test (left) and means and standard errors (right)

Groups	t	P(perm)
Tratra,Scocol	0.36	0.74
Tramed,Scocol	6.11	0.0001
Tratra,Tramed	4.45	0.0002
Scosco,Tratra	0.70	0.56
Scosco,Tramed	6.48	0.0001
Scosco,Scocol	1.39	0.23

Group	Size	Average	SE
Scosco	16	54.66	3.43
Scocol	62	58.98	1.32
Tramed	77	67.01	0.52
Tratra	34	58.00	2.83

SIMPER analysis (Tab. 3.27) revealed that, for *Scomber colias*, Salpidae were the prey that mostly contributed to samples similarity (80.71%). In *Scomber scombrus*, scales and Trematoda parasites accounted for a cumulative value of 99.61% to samples similarity. Scales, *Acartia* sp. and unidentified digestive pulp contributed, attained cumulatively, for 71.43% of samples similarity in *Trachurus mediterraneus*. Finally, the euphausiid *Nyctiphanes couchii* contributed for 94.9% to samples similarity in *Trachurus trachurus*.

Tab. 3.27 Results of SIMPER analysis per species, with a 60% cut-off

Group Scocol		Average similarity: 17.58		
Species	Av.Abund	Av.Sim	Contrib%	Cum.%
Salpidae	0.06	14.19	80.71	80.71
Group Scosco		Average similarity: 20.76		
Trematoda parasites	0	10.46	50.37	50.37
Scales	0.01	10.22	49.24	99.61
Group Tramed		Average similarity: 5.62		
Scales	0	2.58	46	46
Acartia sp.	0	0.79	14	60
Digestive pulp	0	0.64	11.44	71.43
Group Tratra		Average similarity: 18.48		
Nyctiphanes couchii	0.04	17.54	94.9	94.9

For species combination, salinity, O_2 and a-chlorophyll recorded one month and three months before sampling were used for DistLM model. Sequential test showed that these four environmental variables had a poor cumulative contribution to variance, explaining only 10% of the total variance (Tab. 3.28).

Tab. 3.28 Results of sequential test in DistLM analysis

Variable	AIC	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
S	1563.9	38275	8.63	0.0001	0.04	0.04	184
O2	1558.8	30402	7.08	0.0001	0.04	0.08	183
chl1mo	1558.7	8915.9	2.09	0.0014	0.01	0.09	182
chl3mo	1558.5	8795	2.07	0.0023	0.01	0.10	181

The dbRDA first axis explained 47.5% of fitted variation and 4.8% of total variation, the dbRDA second axis explained 33.0% of fitted variation and 3.3% of total variation (Fig. 3.42).
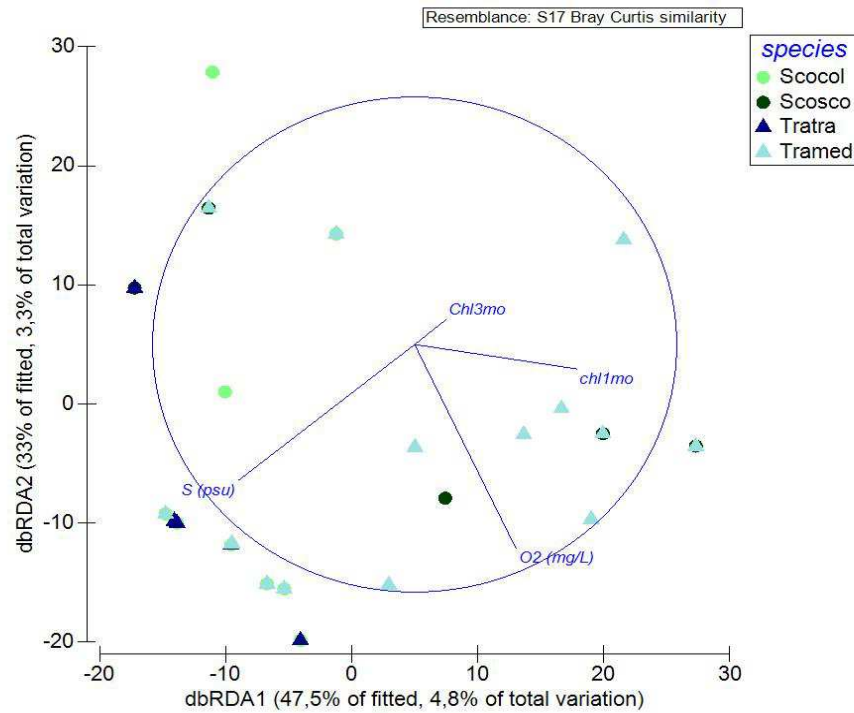
Fig. 3.42 DbRDA of DistLM analysis of environmental variables

H' diversity indexes of stomach contents were all combined and their univariate matrix was analyzed with a PERMANOVA. Its main test (Tab. 3.29) did not revealed significant differences inside species factor. Relating all species H' mean values with subareas (Fig. 3.43), a clearly trend of higher values for the offshore levels was visible, except for the GSA 17 S.

Tab. 3.29 PERMANOVA main test results of H' matrix

Source	df	MS	Pseudo-F	P(perm)
Species	3	0.31	0.61	0.62
Subarea (Species)	9	0.41	0.31	0.93
In vs Off (Subarea (Species))	7	1.69	10.76	0.0001
Residuals	169	0.16		
Total	188			
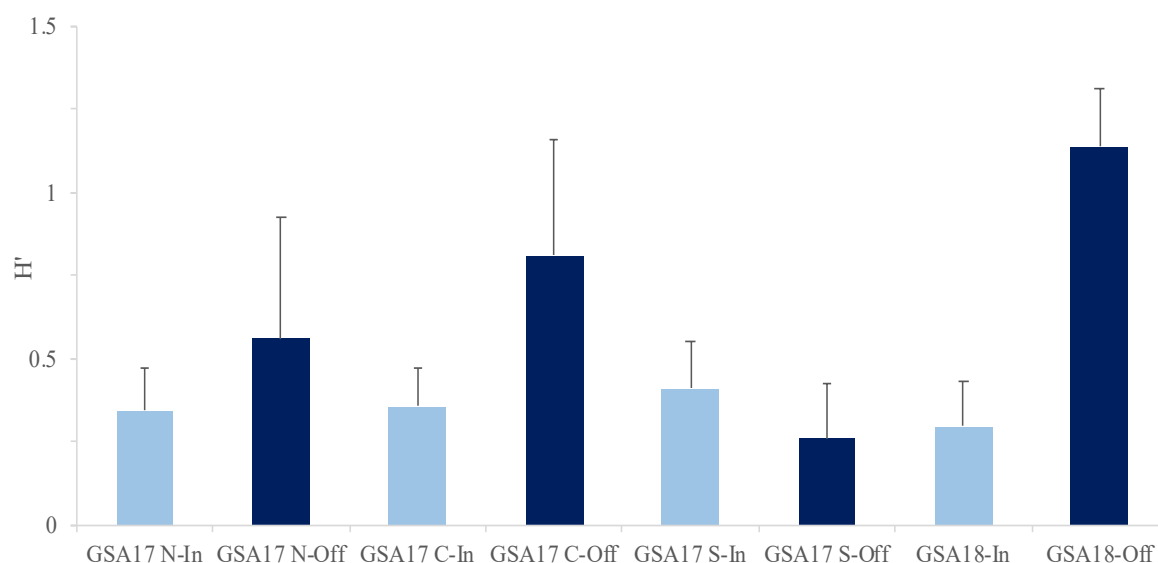
Fig. 3.43 H' mean values (±CI) in the subareas

3.2 Stable isotope analyses

3.2.1 Scomber colias

A total of 20 samples were analyzed, equally distributed among subareas. $\delta^{15}N$ values ranged from 7.04‰ to 11.56‰, with a mean value of 8.97‰ ± 1.36 (SD). On the other hand, $\delta^{13}C$ values ranged from -19.01‰ to -17.75‰, with a mean value of -18.37 ‰ ± 0.35 (SD).

$\delta^{15}N$ and $\delta^{13}C$ values were respectively plotted against fish length (Fig. 3.44) and statistical correlation factors (*i.e.* R and P values) were calculated. For $\delta^{15}N$, R = - 0.43 and P = 0.06 while, for $\delta^{13}C$, R = - 0.63 and P = 0.003.
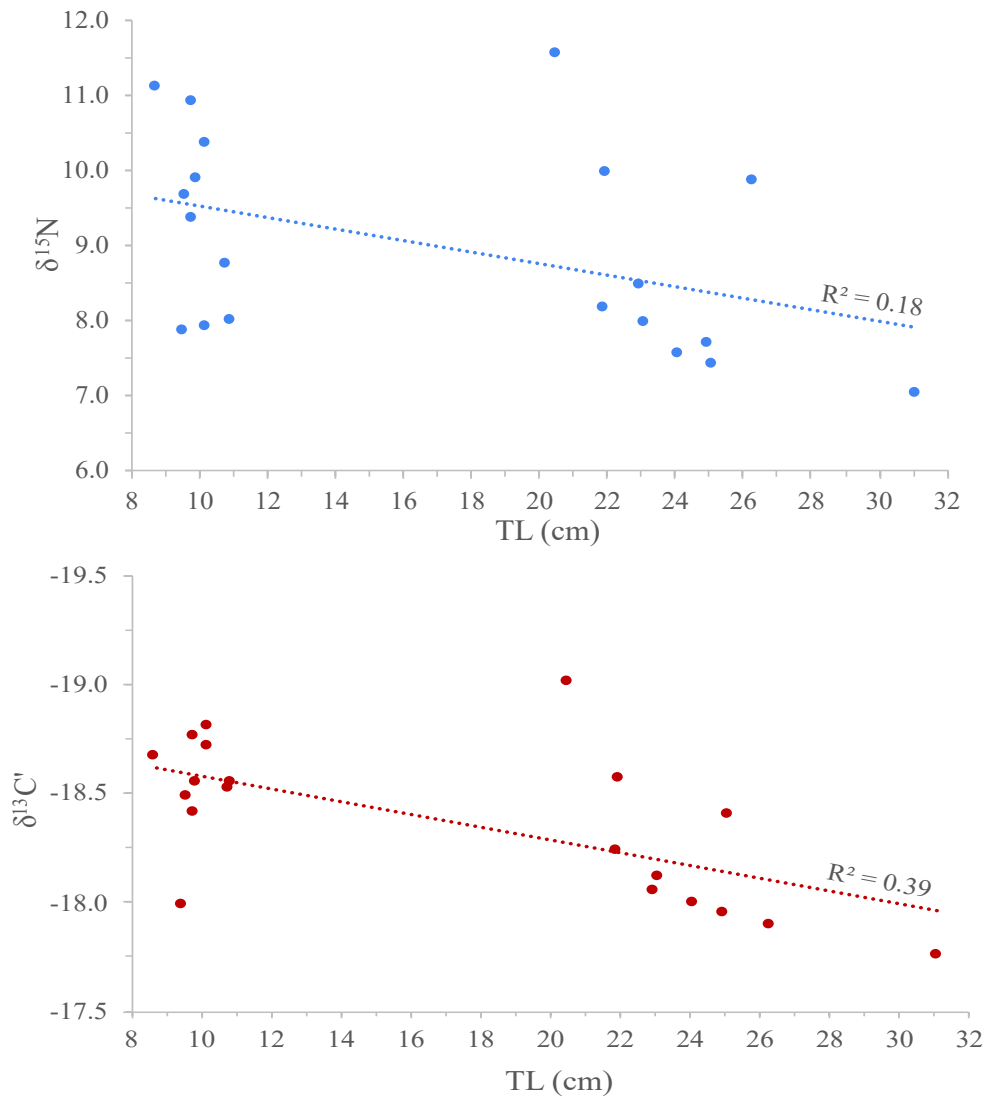
Fig. 3.44 *Scomber colias* scatterplots of $\delta^{15}N$ (up) and $\delta^{13}C$ (bottom) values related to fish length, for the entire surveyed area

$\delta^{15}N$ values showed a slight, though only almost significant, decrease with increasing fish length, while $\delta^{13}C$ values increased significantly with size.

3.2.2 Scomber scombrus

A total of 16 samples were analyzed (the totality of available *S. scombrus* samples). $\delta^{15}N$ values ranged from 9.85‰ to 14.56‰, with a mean value of 11.09‰ ± 1.03 (SD). On the other hand, $\delta^{13}C$ values ranged from -20.04‰ to - 17.24‰, with a mean value of -19.09 ‰ ± 0.74 (SD).

$\delta^{15}N$ and $\delta^{13}C$ values were respectively plotted against fish length (Fig. 3.45a-b) and statistical correlation factors were calculated. For $\delta^{15}N$, R = - 0.41 and P = 0.11 while, for $\delta^{13}C$, R = - 0.46 and P = 0.06.

The $\delta^{15}N$ trend, statistically not significant, showed a slight increase with increasing TL. Similarly, although the trend was not significant, $\delta^{13}C$ values increased with size.

a)

b)
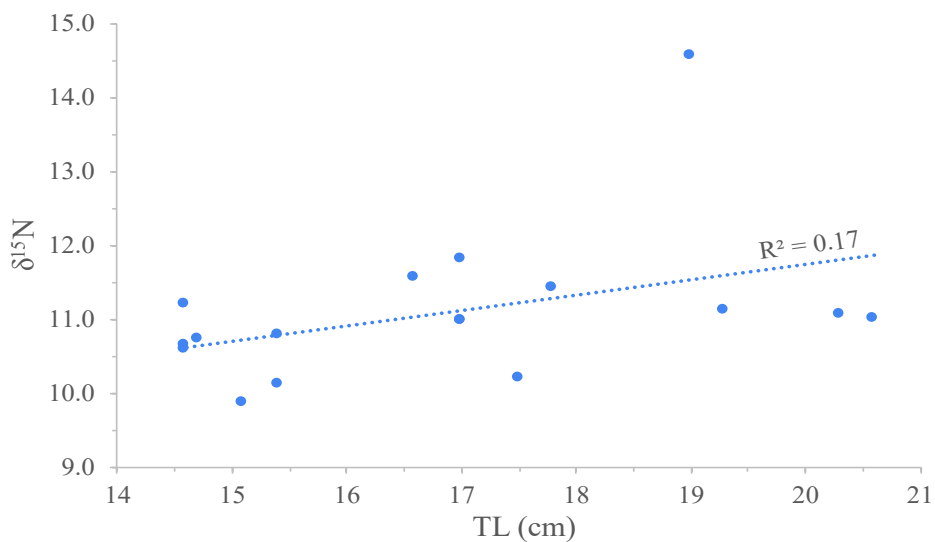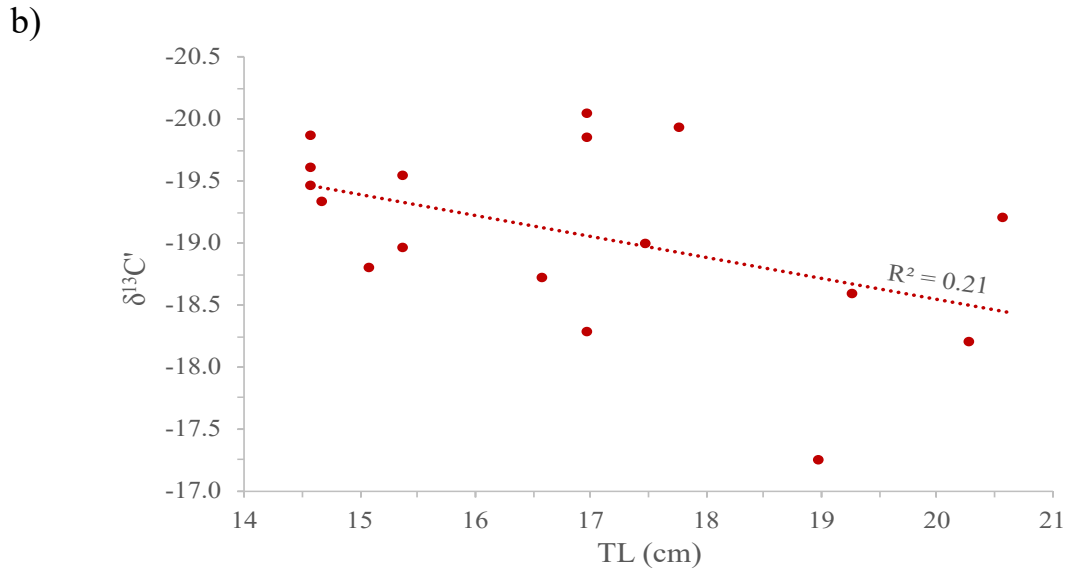
Fig. 3.45 *Scomber scombrus* scatterplots of $\delta^{15}N$ (a) and $\delta^{13}C$ (b) values related to fish length, for the entire surveyed area

3.2.3 *Trachurus mediterraneus*

A total of 33 samples were analyzed, equally distributed among subareas. $\delta^{15}N$ values ranged from 7.06‰ to 13.58‰, with a mean value of 10.37‰ ± 1.66 (SD). On the other hand, $\delta^{13}C$ values ranged from 19.58‰ to -17.22‰, with a mean value of -18.42‰ ± 0.70 (SD).

$\delta^{15}N$ and $\delta^{13}C$ values were respectively plotted against fish length (Fig. 3.46) and statistical correlation factors were obtained. For $\delta^{15}N$, R = - 0.73 and P = 1 x 10^{-6} while, for $\delta^{13}C$, R = - 0.63 and P = 9 x 10^{-5}.

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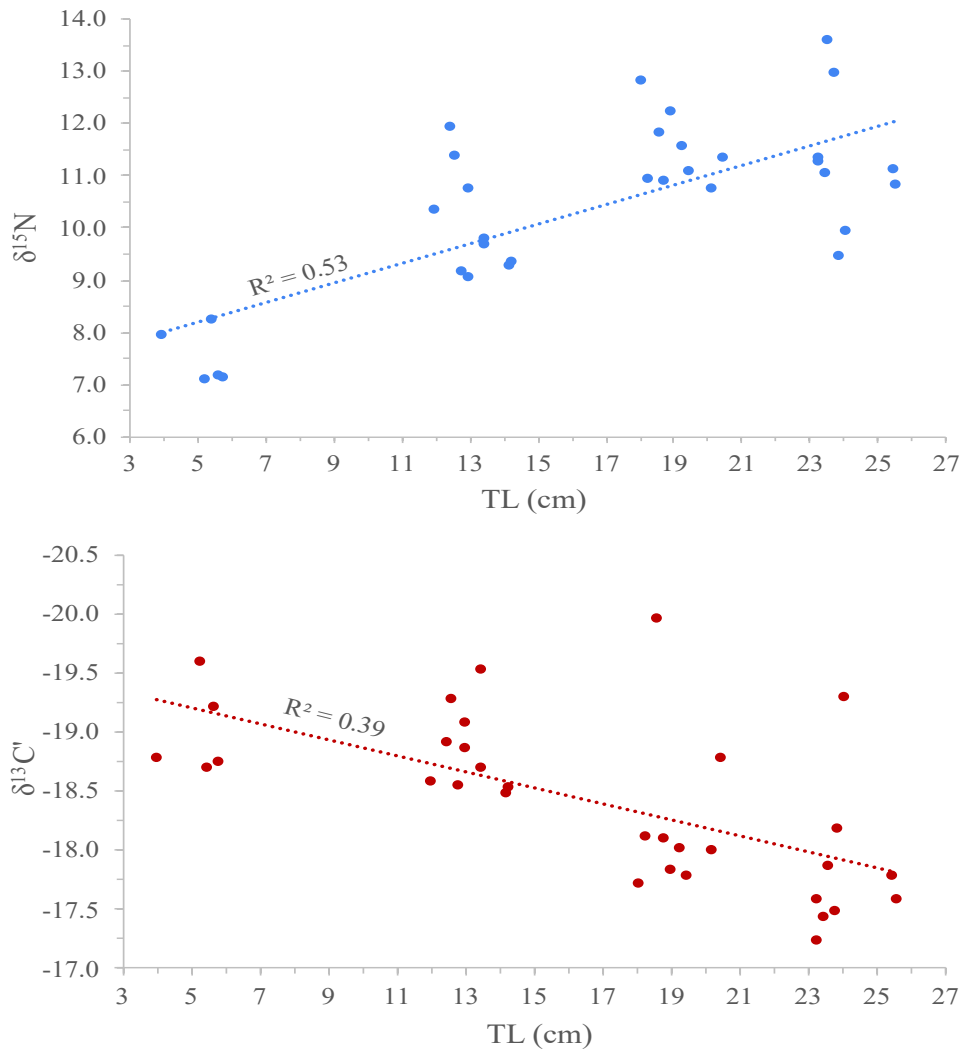
Fig. 3.46 *Trachurus mediterraneus* scatterplots of $\delta^{15}N$ (up) and $\delta^{13}C$ (bottom) values related to fish length, for the entire surveyed area

Both $\delta^{15}N$ and $\delta^{13}C$ trends with size resulted statistically significant. $\delta^{15}N$ and $\delta^{13}C$ values increased with fish size.

3.2.4 Trachurus trachurus

A total of 20 samples were analyzed, equally distributed among subareas. $\delta^{15}N$ values ranged from 6.23‰ to 11.79‰, with a mean value of 9.35‰ ± 1.55

(SD). On the other hand, $\delta^{13}C$ values ranged from -20.16‰ to -18.89‰, with a mean value of -19.29 ‰ $\pm$ 0.33 (SD).

$\delta^{15}N$ and $\delta^{13}C$ values were respectively plotted against fish length (Fig. 3.47) and statistical correlation factors were obtained. For $\delta^{15}N$, R = - 0.67 and P = 0.001 while, for $\delta^{13}C$, R = - 0.37 and P = 0.11.

$\delta^{15}N$ values increased significantly with size. The $\delta^{13}C$ trend, statistically not significant, increased with size.
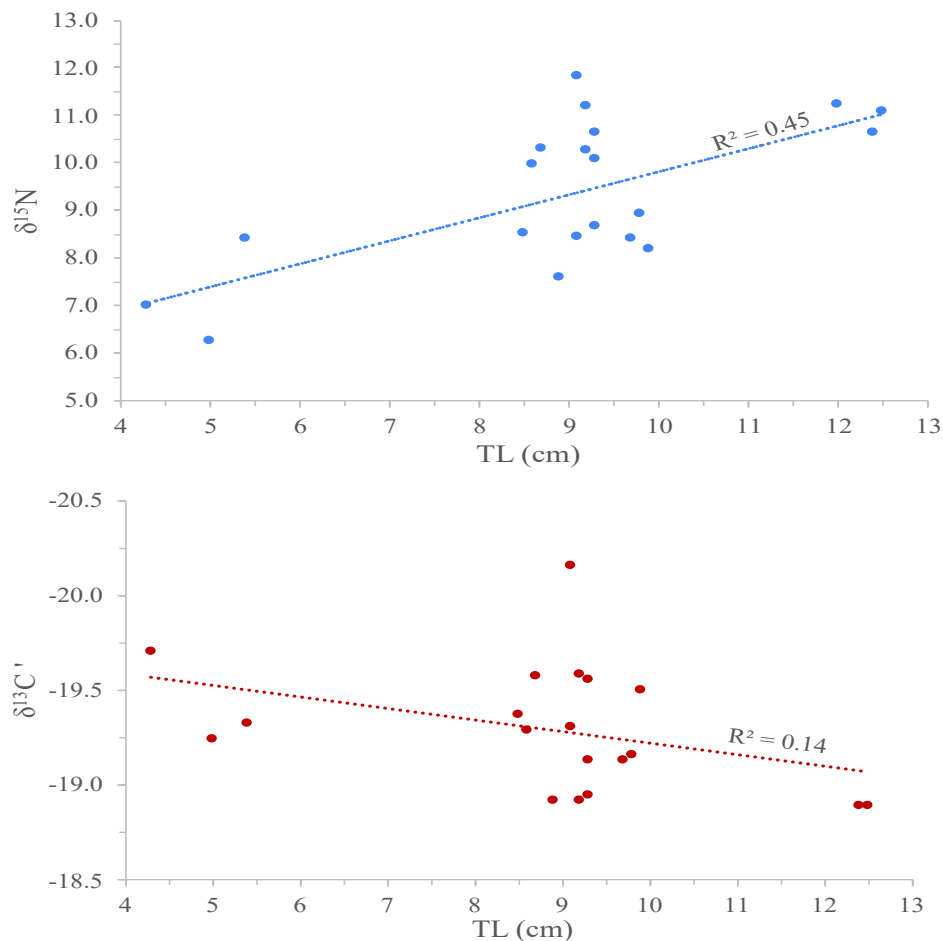
Fig. 3.47 *Trachurus trachurus* scatterplots of $\delta^{15}N$ (up) and $\delta^{13}C$ (bottom) values related to fish length, for the entire surveyed area

3.2.5 Species overlap

Both univariate and multivariate analyses underwent to the three-way PERMANOVA design. PERMANOVA main test on the univariate matrix of $\delta^{15}N$ data (Tab. 3.30a) showed significant differences only in the "In *vs* Off (Subarea (Species))" factor. However, the pairwise test comparisons (Tab. 3.30b) allowed visualizing significant results only for *Trachurus trachurus*, between inshore and offshore level of GSA 17 S.

Tab. 3.30 Main test (a) and pairwise test(b) from PERMANOVA of $\delta^{15}N$ univariate matrix

a)

Source	df	MS	Pseudo-F	P(perm)
Species	3	2.75	0.63	0.61
Subarea (Species)	11	5.16	0.83	0.61
In vs Off (Subarea (Species))	8	4.59	4.04	0.0006
Residuals	69	1.14		
Total	91			

b)

Group Scocol	t	P(perm)
GSA17 C In, GSA17 C Off	1.68	0.24
GSA18 In, GSA18 Off	0.66	0.61
Group Tramed		
GSA17 N In, GSA17 N Off	1.24	0.24
GSA17 C In, GSA17 C Off	0.21	0.93
Group Tratra		
GSA17 S In, GSA17 S Off	4.78	0.0161

PERMANOVA results on the univariate matrix of $\delta^{13}C$ data were completely different. The main test showed significant separations only for the factor "species" (Tab. 3.31a). Pairwise test comparisons listed significant differences

in $\delta^{13}C$ values between *Trachurus* species and between *Trachurus trachurus* and *Scomber colias* (Tab. 3.31 b).

The nMDS analysis did not highlighted a clear separation among species and among subareas. Separations between samples were clear when analyzing inshore *vs*. offshore factor (Fig. 3.48).

Tab. 3.31 Main test (a) and pairwise test(b) from PERMANOVA of $\delta^{13}C$ univariate matrix

a)

Source	df	MS	Pseudo-F	P(perm)
Species	3	2.94	5.90	0.014
Subarea (Species)	11	0.53	1.73	0.26
In vs Off (Subarea (Species))	8	0.27	0.78	0.63
Residuals	69	0.35		
Total	91			

b)

Groups	t	P(perm)
Tratra, Scocol	4.43	0.0061
Tramed, Scocol	1.21	0.28
Tratra, Tramed	2.87	0.0356
Tratra, Scosco	1.53	0.16
Tramed, Scosco	0.67	0.58
Scocol, Scosco	1.63	0.15

PERMANOVA main test for the bivariate matrix of $\delta^{13}C$ and $\delta^{15}N$ showed significant differences for "In *vs* Off (Subarea (Species))" factor (Tab. 3.32).

Tab. 3.32 Main PERMANOVA test on the $\delta^{15}N$-$\delta^{13}C$ bivariate matrix

Source	df	MS	Pseudo-F	P(perm)
Species	3	5.68	1.17	0.37
Subarea (Species)	11	5.69	0.88	0.57
In vs Off (Subarea (Species))	8	4.86	3.27	0.005
Residuals	69	1.49		
Total	91			
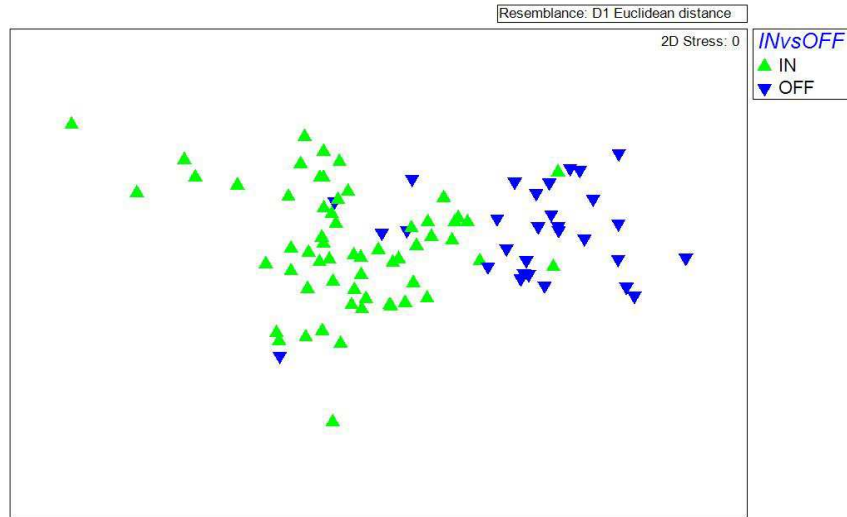
Fig. 3.48 Graphic representation of nMDS analysis for Inshore vs Offshore factor

The scatterplot that combined all $\delta^{15}N$ and $\delta^{13}C$ values of the four species highlighted a clear separation in the isotopic signatures, among genera and also between species of the same genus (Fig. 3.49)
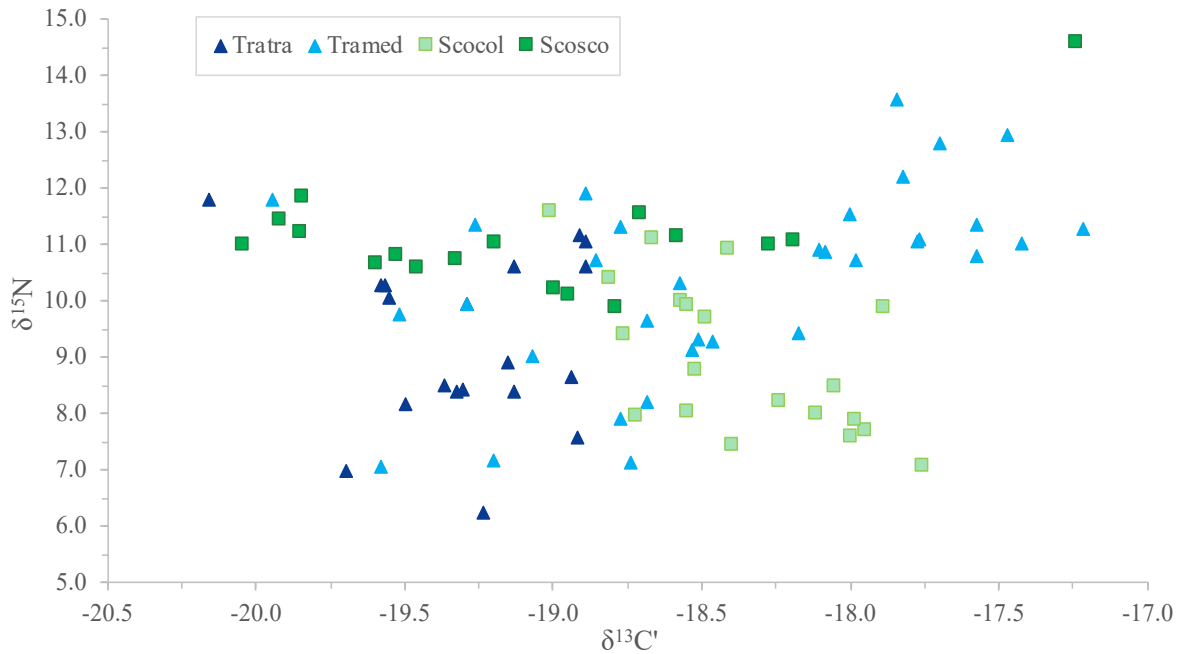
Fig. 3.49 Scatterplot of $\delta^{15}N$ and $\delta^{13}C$ data of the four species

As a conclusion, the wider scatterplot with δ15N and δ13C mean values of taxa of several trophic levels, of a typical Mediterranean pelagic food web, was plotted, together with isotopic values of the four species (Fig. 3.50). The scatterplot evidenced an intermediate position of *Scomber* spp. and *Trachurus* spp., located between small pelagics (*i.e. Sardina pilchardus*, *Engraulis encrasicolus*) and typical apical predators like bonitos (*Sarda sarda*), tunas (*Euthynnus alletteratus* and *Thunnus thynnus*) and the bottlenose dolphin *Tursiops truncatus*.
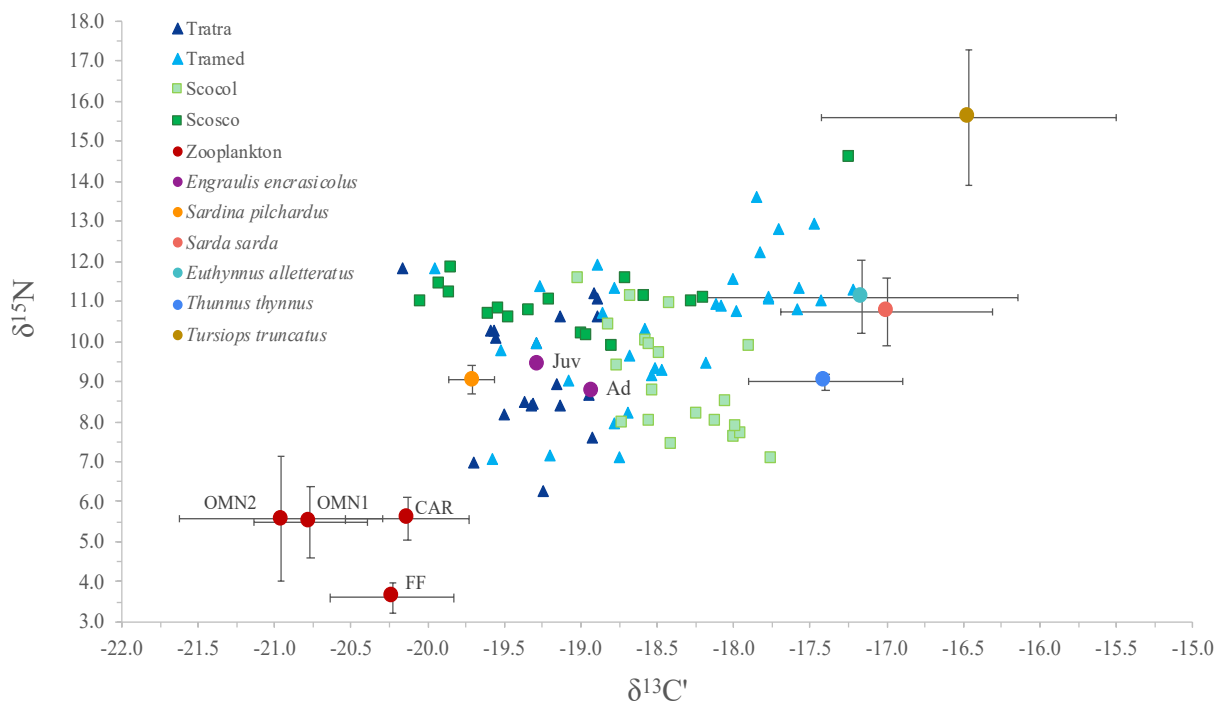
Fig. 3.50 Final scatterplot of isotopic mean values (±SD) for different taxa, present in the Mediterranean pelagic food web

Chapter Four

4. DISCUSSION

4.1 Overall diet composition of species based on SCA, SIA and

environmental drivers

4.1.1. Scomber colias

Overall *Scomber colias* resulted to feed mostly on fish and crustaceans mainly captured in the water column. Our findings agree with other studies carried out in different basins where the species is found to prey mostly on fish larvae (Sever et al., 2006), and crustaceans, like amphipods, copepods (Sever et al., 2006), euphausiids (Castro, 1995) and decapods' larvae (Čikeš Keč et al., 2012). While mysids were abundant prey in Canary islands (Castro, 1995) and in the eastern Adriatic (Čikeš Keč et al., 2012), this item was absent in *S. colias'* stomachs from this study. Most frequent preys were represented by salpids, in all the subareas. The importance of gelatinous plankton in the diet of this species was already highlighted by Sever *et al*. (2006) and Cardona *et al.* (2012). The frequent occurrence of thaliaceans and hydrozoans in almost all of the stomachs could be attributable to their blooms, generally observed in spring or at the beginning of summer (Menard et al., 1994), which are increasing in the Adriatic Sea (Boero et al., 2013; Palmieri et al., 2014). Fullness indexes were the highest in the GSA18 offshore. Trophic diversity was always higher

in offshore sectors and the highest number of taxa was found in stomachs of GSA 18. Probably, the greater depths found in the offshore and southern sectors guarantees access to a greater number of trophic resources. Indeed euphausiids, which are abundant prey in this area, are typically found in offshore waters, where they migrate at surface during diel vertical migrations (Andersen & Sardou, 1992).

Stable isotope analyses showed a decrease in $\delta^{15}N$ values with size. This could be related to the high consumption on euphausiids in offshore areas were large specimens preferentially lived. Euphausiids are mostly represented by primary consumers and omniviores of low trophic levels (Fanelli et al., 2009; Fanelli, Cartes, et al., 2011) and this may explain such trend. Accordingly, the increase in $\delta^{13}C$ values with size indicated a displacement of large specimens from inshore to offshore areas.

Our results confirm that *Scomber colias* feeds almost exclusively in the pelagic compartment and in general on low trophic level species, with a good intraspecific resource partitioning obtained by a different distribution of specimens according to size, with juveniles mostly inhabiting inshore waters while adults mainly living in offshore ones.

Environmental parameters like dissolved oxygen and chlorophyll-*a* recorded one month and two months before sampling seem to be the environmental

drivers that most influenced the diet of individuals of GSA17 C and GSA 17 S. This fully agree with the type of prey ingested by specimens in this area, which are mostly salpids and other species with high turnover rate thus responding quickly to the peak of primary production. Contrarily, diet of individuals of GSA 18 was related to chlorophyll-*a* four months before and temperature. This result pointed out to a more delayed response of *S. colias* prey to primary production inputs from this area. On the other hand, the diet of the species in the southern Adriatic seemed to be mostly driven by changes in water masses, i.e. temperature which fueled prey availability in offshore waters.

4.1.2 Scomber scombrus

Few specimens were analyzed for stomach contents for *Scomber scombrus* to allow an adequate description of the diet. Moreover, stomach were almost empty and full of Trematoda parasites, which are quite commons in *Scomber* species and can be used in stocks discrimination (Feki et al., 2018; Køie, 1991). In the GSA 17 S a higher number of taxa were found in stomach contents and the trophic diversity was slightly higher. According to other studies carried out in the Bay of Biscay (Bachiller & Irigoien, 2015; Olaso et al., 2005), in Greenland (Jansen et al., 2019) and in the Mediterranean Sea (Cardona et al.,

2012) bony fishes, thaliaceans, hyperiids, decapods' larvae and cephalopods were found.

Isotopic signatures of both $\delta^{15}N$ and $\delta^{13}C$ showed a slight increase with size, despite total lengths ranged only between 14 and 20 cm. Probably, larger specimens included in this range of sizes performed a dietary shift to prey of higher trophic levels. $\delta^{13}C$ increase with size could explain a displacement of large specimens from inshore to offshore areas, and/or a greater reliance on marine food sources more depending on vertical flux.

These results confirm that also *Scomber scombrus* feeds almost exclusively in the pelagic compartment and most likely on higher trophic level prey with increase size. Intraspecific resource partitioning based on different spatial distribution according to specimens' size cannot be confirmed, because of the low samples' size obtained for this species.

The temperature was the environmental parameter that most influenced the diet of GSA 17 S individuals, in agreement with previous observations that showed a correlation of some preferred prey of *S. scombrus* here found, *i.e.* hyperiids, with water mass condition (Cartes et al., 2010).

4.1.3 Trachurus mediterraneus

Our results on the diet of *Trachurus mediterraneus* agree with previous studies already performed in the Adriatic Sea, with copepods, amphipods, decapods, mysids and fish larvae as most representative preys (Šantić et al., 2003, 2013). Differently from Šantić *et al.* (2003), who found abundant euphausiids and polychaetes in horse mackerels' diet, these preys were rare or absent in our samples. Conversely, we frequently found several benthic prey such as gammarid amphipods, benthic decapods, bivalves and gastropods in stomach contents. Despite some authors define *Trachurus* species as pelagic carnivores, our findings mainly agree with a study from the Black Sea that classified *T. mediterraneus* as a fish with a mixed diet of zooplankton, benthos and fish (Georgieva et al., 2019) that could vary with seasons. An interesting aspect was represented by the fact that benthic preys were absent in the GSA 18, probably because the high depths of this subarea limit the search for food to the upper levels of the water column. Copepods were the most important prey, in terms of %IRI, in the GSA 17 N and in the GSA 18, but the species composition of copepods was totally different, according to mesoscale variations in the zooplanktonic communities (Menicucci, 2021). Gut parasites like *Anisakis* sp. and digenean trematodes were found frequently, especially in the northern subareas, accordingly to Costa *et al*. (2017).

δ^{15}N trend significantly with size, showing that adults certainly preferred prey of higher trophic levels than those of juveniles. δ^{13}C values markedly increased with size, although this could not be related to a displacement of larger specimens in offshore areas (as observed for *S. colias*). This carbon enrichment was likely due to a remarkable shift of predation of larger specimens on benthic and suprabenthic prey, as in fishes carbon enrichment tend to be associate with benthic food chains (Pinnegar & Polunin, 2000).

Therefore, *Trachurus mediterraneus* feeds in both pelagic and benthic compartment, with a good intraspecific resource partitioning, linked to ontogenetic shift in diet. Moreover, an intraspecific spatial distribution occurs, with adults mainly inhabiting offshore areas and juveniles the offshore ones.

The low variance explained by the DISTLM model for this species could be due to the mix trophic behavior previously described, pointing to feeding on both the water column and close to the bottoms, while for the model only variables linked to the water column were used. Thus, other variables, representing also the benthic compartments need to be used for further implementation of the model in this sense.

4.1.3 Trachurus trachurus

Our results showed that the most representative food item, in terms of %IRI, were copepods, euphausiids, amphipods and fish larvae, accordingly to other studies carried out on the diet of this species from the Adriatic Sea (Jardas et al., 2004; Šantić et al., 2005) and other areas of the Mediterranean Sea (Rumolo et al., 2017). Those authors highlighted a frequent occurrence of polychaetes, mysids, cephalopods and decapods in *T. trachurus* stomach contents, while they were rare or absent in our samples. Conversely to *T. mediterraneus,* and also to previous study from the Sicily channel (Rumolo *et al*., 2017), here we did not find benthic or suprabenthic prey. The highest fullness indexes, as well as the highest number of taxa in stomachs, were registered in southern subareas (*i.e.* GSA17 S and GSA18), where *Nyctiphanes couchii* euphausiid was the most frequent prey as also observed by Šantić et al. (2005). The greater depths of the southern Adriatic Sea, as already seen in *S. colias*, likely favor the presence of species that undertake diel vertical migrations of hundreds of meters, as euphausiids (Andersen & Sardou, 1992). Copepoda were very abundant in individuals of GSA 17 N and GSA 18 but with large differences in species composition, as observed for *T. mediterraneus*.

δ^{15}N values slightly increased with size, however, only juveniles were available for this species (TL range: 4-12 cm). This likely points out to a more

pronounced consumption of prey of higher trophic level in specimens of bigger size. Otherwise $\delta^{13}C$ values showed an almost constant trend with size, suggesting that at least specimens of the size range explored mostly remain in the same area without great inshore-offshore displacement. And/or change in the food source (pelagic *vs*. benthic).

Our results showed that *Trachurus trachurus* juveniles feeds most exclusively in the pelagic compartment. Conversely to *T. mediterraneus*, an intraspecific resource partitioning linked to ontogenetic shift in diet or spatial segregation cannot be confirmed, as larger specimens were not available from this study.

Chlorophyll-*a* concentration was the most important driving factor of the diet of this species from GSA 17 N, likely due to the high reliance of the species on zooplankton in this subarea. In the GSA 17 S, dissolved oxygen and temperature seem to be the environmental drivers that most influenced the individuals' diet, mostly driven by changes in the water masses dynamics.

Except for *S. colias*, fish scales were, in terms of %IRI, a relevant stomach content in the other species, especially in the GSA 17 subareas. The presence of this items in stomachs could be related to fisheries discard, which quantities are relatively high in the Adriatic Sea (Santojanni et al., 2005), and could represent an easily accessible food. However, it is more likely that the scales

came from the rubbing between fishes in the sampling net, occurred mainly when they were sailed on board.

4.2 Resource partitioning of small pelagic fishes based on SCA and SIA

A good resource partitioning was evidenced among these four small pelagic species from the Adriatic Sea, accordingly to both SIA and SCA results. Food partitioning was particularly evident between *Scomber colias* and the two species of *Trachurus*, while this was less evident between the two *Scomber* species. Such apparent niche overlap between the two *Scomber* species was actually avoided thanks to spatial segregation. Indeed, *S. colias* was absent in the GSA 17 N and its abundances were higher in southern sectors of the Adriatic while *S. scombrus* occurred almost exclusively in the northern ones, in agree with a pattern of spatial distribution observed for these species along the Portuguese coasts (Baptista et al., 2019), driven by water temperatures. A less evident food partitioning and similar isotopic trends were observed also between juveniles of *Trachurus* species, in agreement with Šantić et al., (2013) study, that reports similar exploitations of food resources and types of prey between juveniles of these two species, in the Adriatic Sea. However, a spatial segregation between species occurred, to minimize trophic niche overlap, with *T. mediterraneus* individuals located in the northern and central sectors while

T. trachurus individuals were relegated to deeper southern sectors. This is in total agreement with a different range of spatial distribution of both horse mackerels species along a bathymetric pattern, seen in southern Sicilian waters, were the spatial niche occupied by Mediterranean horse mackerel was displayed in more shallow waters than those of the Atlantic horse mackerel (Milisenda et al., 2018).

Diet generalism *vs*. specialization can be interpreted as a resource partitioning indicator (Fanelli et al., 2011). A more generalized diet was noticeable for *Trachurus mediterraneus* and *Scomber colias* when compared with their congeneric species. *T. trachurus* was already found to be a more specialist predator than *T. mediterraneus*, with a narrow size of the trophic niche (Deudero, 2001). *Scomber scombrus* resulted the species with the most specialized diet, although results can be biased by the poor sample size. $\delta^{15}N$ values of this species were quite higher compared with those of the other species for the same lengths, confirming a diet specialization on prey of higher trophic level since small sizes (Olaso et al., 2005).

Environmental drivers as salinity and chlorophyll-*a* one month before sampling, seemed to influence diet of *Trachurus* species, which are more related to this kind of parameters in water masses. On the contrary, *Scomber colias* diet seemed influenced by other drivers as temperature and chlorophyll-

a several months before sampling, which could provide totally different kind of prey. Therefore, environmental drivers can play an important role in resource partitioning among these species.

$\delta^{15}N$ values showed how these fishes have a similar trophic position, as values were not significantly different among species. Results of $\delta^{13}C$ showed separations in $\delta^{13}C$ values between *Trachurus* species and between *T. trachurus vs. S. colias*, due to the fact that these species distribute their abundances inshore or offshore, trying to avoid niche overlaps.

PERMANOVA results of combined $\delta^{13}C$ and $\delta^{15}N$ data showed significant differences between values obtained in inshore *vs.* offshore individuals of the same species. Other studies already showed how spatial variations in the distributions of individuals within the same fish species can provide significant differences in isotopic signatures (Fanelli et al., 2018; Rumolo et al., 2016; Rumolo et al., 2017), because of the different trophic dynamics between costal and oceanic food webs. These differences, related to spatial segregation of individuals of the same species, could be performed in order to avoid niche overlapping.

4.3 Role of Scomber spp. and Trachurus spp. in the pelagic food web

An expected scenario was obtained from the combined scatterplot of $\delta^{15}N$ and $\delta^{13}C$ values of several trophic groups, that could occur in a typical pelagic food web in the Adriatic Sea. $\delta^{15}N$ and $\delta^{13}C$ values increased at each trophic level, from zooplankton to top predators. The four species of this study were located at an intermediate trophic level, between low trophic level species, such as zooplankton, but greater than the level occupied by other small pelagics such as sardines and anchovies, and high trophic level species, such as bonitos, tunas and at the top of the food web, dolphins. This location highlights the crucial role of *Scomber* and *Trachurus* species in pelagic food webs (Trenkel et al., 2014), as wasp-waist controllers, linking the transfer of energy from the lowest to the highest levels of the marine food web (Rice, 1995).

Our result also agree with isotope data obtained for these species from the western Mediterranean Sea, which suggested that trophic niches of *Trachurus* spp. and *Scomber* spp., were distinct (Albo-Puigserver et al., 2016). Actually, Mediterranean horse mackerel values were very close to those of top predators and by far higher than those of the Atlantic horse mackerel. Furthermore, Atlantic mackerel seemed to occupy a higher trophic level when compared with the Atlantic chub mackerel. However, samples of *T. trachurus* and *S. scombrus* of bigger sizes should have been necessary to better clarify these aspects.

Chapter Five

5. CONCLUSIONS

This is the first study that applied the integration of stomach content and stable isotope analyses, at the same time for these four species, in the Adriatic Sea. The results proved:

- intraspecific ontogenetic and spatial resource partitioning, more remarkable in *Scomber colias* and *Trachurus mediterraneus,*

- interspecific resource partitioning based mainly on different spatial distributions and diet generalism *vs.* specialism, among species,

- resource partitioning within and between species driven by environmental conditions of water masses,

- the crucial role of *Scomber* and *Trachurus* species in pelagic trophic webs.

All the information acquired improve our knowledges on these species, that play a fundamental role in pelagic food webs and of high commercial interest in the Adriatic Sea. Moreover, this study can establish a reference baseline for long-term comparisons, to asses if, with the ongoing climate changes and the consequent general warming of Mediterranean waters, together with increasing fishing pressures, the dynamics of resource partitioning can change.

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ANNEXES

Annex 1

List of %W, %N, %F and %IRI values of stomach contents found in *Scomber colias,* in each subarea

	GSA17 C				GSA17 S				GSA18			
	%W	%N	%F	%IRI	%W	%N	%F	%IRI	%W	%N	%F	%IRI
CNIDARIA												
Calycophorae	0.71	0.35	25.00	0.29	2.22	2.04	36.84	1.74	1.06	0.47	22.86	0.31
Total Cnidaria	**0.71**	**0.35**	**25.00**	**0.29**	**2.22**	**2.04**	**36.84**	**1.74**	**1.06**	**0.47**	**22.86**	**0.31**
MOLLUSCA												
Creseis acicula	-	-	-	-	-	-	-	-	0.02	0.18	17.14	0.03
Sepiolidae	3.25	0.09	12.50	0.46	-	-	-	-	10.80	0.56	20.00	2.02
Bivalvia larvae	-	-	-	-	-	-	-	-	0.18	0.35	11.43	0.05
Total Mollusca	**3.25**	**0.09**	**12.50**	**0.46**	**-**	**-**	**-**	**-**	**11.01**	**1.09**	**48.57**	**2.11**
CRUSTACEA												
Copepoda												
Calanus -like	0.00	0.09	12.50	0.01	0.01	0.26	10.53	0.03	0.00	0.03	2.86	0.00
Centropages sp.	0.04	1.86	50.00	1.05	0.02	1.02	21.05	0.24	0.00	0.15	2.86	0.00
Eucheta sp.	0.01	0.09	12.50	0.01	0.07	1.02	21.05	0.25	0.03	0.47	28.57	0.13
Oncea sp.	0.00	0.09	12.50	0.01	-	-	-	-	0.00	0.03	2.86	0.00
Sapphirina sp.	-	-	-	-	-	-	-	-	0.01	0.32	22.86	0.07
Temora stylifera	-	-	-	-	-	-	-	-	0.00	0.03	2.86	0.00
Unid. Copepoda	0.00	0.09	12.50	0.01	0.02	1.02	26.32	0.30	0.01	0.47	37.14	0.16
Total Copepoda	**0.05**	**2.21**	**100.00**	**1.10**	**0.12**	**3.32**	**78.95**	**0.83**	**0.05**	**1.50**	**100.00**	**0.36**
Stomatopoda												
Squillidae larvae	0.14	0.09	12.50	0.03	-	-	-	-	1.36	0.94	31.43	0.64
Total Stomatopoda	**0.14**	**0.09**	**12.50**	**0.03**	**-**	**-**	**-**	**-**	**1.36**	**0.94**	**31.43**	**0.64**
Euphausiacea												
Nematoscelis megalops	-	-	-	-	-	-	-	-	0.86	0.50	8.57	0.10
Nyctiphanes couchii	-	-	-	-	-	-	-	-	18.86	11.15	28.57	7.62
Euphausiidae larvae	-	-	-	-	-	-	-	-	1.05	7.13	20.00	1.46
Euphausiidae	-	-	-	-	-	-	-	-	0.20	0.23	8.57	0.03
Total Euphausiacea	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**20.97**	**19.02**	**65.71**	**9.22**
Decapoda												
Megalopa Brachiura	0.01	0.18	12.50	0.03	0.03	0.77	5.26	0.05	1.17	3.14	31.43	1.20
Zoea Brachiura	-	-	-	-	-	-	-	-	0.05	0.21	8.57	0.02
Zoea Penaeidae	-	-	-	-	0.62	0.13	5.26	0.04	0.03	0.12	2.86	0.00
Zoea Palaemonidae	-	-	-	-	-	-	-	-	0.02	0.09	8.57	0.01
Total Decapoda	**0.01**	**0.18**	**12.50**	**0.03**	**0.65**	**0.89**	**10.53**	**0.09**	**1.27**	**3.55**	**51.43**	**1.24**

Annex 1

	GSA17 C				GSA17 S				GSA18			
	%W	%N	%F	%IRI	%W	%N	%F	%IRI	%W	%N	%F	%IRI
Amphipoda												
Anchilomera blossevillei	-	-	-	-	-	-	-	-	0.14	0.23	14.29	0.05
Brachiscelus crusculum	-	-	-	-	-	-	-	-	0.05	0.03	2.86	0.00
Hyperia schizogeneios	-	-	-	-	-	-	-	-	0.07	0.26	2.86	0.01
Phronima atlantica	1.44	0.53	50.00	1.09	1.88	0.77	10.53	0.31	0.41	0.50	37.14	0.30
Phronima stebbingi	-	-	-	-	-	-	-	-	0.02	0.03	2.86	0.00
Phronimella elongata	-	-	-	-	-	-	-	-	0.01	0.06	5.71	0.00
Phrosina semilunata	-	-	-	-	-	-	-	-	0.32	0.26	11.43	0.06
Platiscelus sp.	-	-	-	-	-	-	-	-	0.01	0.03	2.86	0.00
Primno macropa	-	-	-	-	-	-	-	-	0.03	0.06	5.71	0.00
Themisto abissorum	-	-	-	-	0.06	0.64	15.79	0.12	2.11	4.43	42.86	2.49
Vibilia sp.	0.00	0.09	12.50	0.01	-	-	-	-	0.07	0.15	11.43	0.02
Hyperiidae	0.70	8.59	50.00	5.14	0.80	3.44	26.32	1.24	0.02	1.44	17.14	0.22
Hyperiidea	0.00	0.09	12.50	0.01	-	-	-	-	0.02	0.53	14.29	0.07
Total Amphipoda	**2.15**	**9.30**	**125.00**	**6.26**	**2.74**	**4.85**	**52.63**	**1.67**	**3.29**	**8.01**	**171.43**	**3.24**
Total Crustacea	**2.36**	**11.78**	**250.00**	**7.42**	**3.51**	**9.06**	**142.11**	**2.59**	**5.98**	**14.00**	**354.29**	**5.48**
THALIACEA												
Pyrosoma sp.	-	-	-	-	-	-	-	-	1.66	0.06	5.71	0.09
Salpidae	42.91	80.69	50.00	68.37	40.25	53.06	63.16	65.26	24.45	58.17	100.00	73.45
Total Thaliacea	**42.91**	**80.69**	**50.00**	**68.37**	**40.25**	**53.06**	**63.16**	**65.26**	**26.11**	**58.23**	**105.71**	**73.54**
OSTEICHTHYES												
Conger conger	-	-	-	-	-	-	-	-	0.51	0.03	2.86	0.01
Engraulis encrasicolus	-	-	-	-	-	-	-	-	3.10	0.12	11.43	0.33
E. encrasic. larvae	1.11	0.62	12.50	0.24	2.46	0.26	5.26	0.16	23.87	4.08	31.43	7.81
Serranus sp. larvae	-	-	-	-	1.22	0.13	5.26	0.08	2.39	0.59	17.14	0.45
Scombrus sp.	-	-	-	-	-	-	-	-	1.14	0.03	2.86	0.03
Scombrus sp. larvae	-	-	-	-	-	-	-	-	0.82	0.15	5.71	0.05
Trachurus mediterraneus	-	-	-	-	-	-	-	-	1.85	0.03	2.86	0.05
Uranoscopus scaber	0.63	0.09	12.50	0.10	-	-	-	-	-	-	-	-
Fish skeleton	4.45	0.80	25.00	1.45	38.86	4.46	52.63	25.25	0.95	0.76	20.00	0.30
Total Osteichthyes	**6.19**	**1.51**	**50.00**	**1.79**	**42.54**	**4.85**	**63.16**	**25.49**	**34.63**	**5.78**	**94.29**	**9.04**
OTHERS												
Unid.digestive pulp	43.08	0.27	37.50	17.98	-	-	-	-	-	-	-	-
Scales	1.39	4.78	50.00	3.41	11.49	30.74	10.53	4.92	0.08	0.59	20.00	0.12
Anisakis sp.	-	-	-	-	-	-	-	-	0.07	0.06	5.71	0.01
Trematoda parasites	0.11	0.53	37.50	0.27	-	-	-	-	0.08	0.76	25.71	0.19
Total Others	**44.58**	**5.58**	**125.00**	**21.66**	**11.49**	**30.74**	**10.53**	**4.92**	**0.22**	**1.41**	**51.43**	**0.32**

Annex 2

List of %W, %N, %F and %IRI values of stomach contents found in *Scomber scombrus,* in each subarea

	GSA17 N				GSA17 S			
	%W	%N	%F	%IRI	%W	%N	%F	%IRI
MOLLUSCA								
Sepiolidae	-	-	-	-	1.06	0.67	20.00	0.35
Total Mollusca	**-**	**-**	**-**	**-**	**1.06**	**0.67**	**20.00**	**0.35**
CRUSTACEA								
Decapoda								
Zoea Galatheidea	-	-	-	-	0.79	1.33	20.00	0.43
Total Decapoda	**-**	**-**	**-**	**-**	**0.79**	**1.33**	**20.00**	**0.43**
Amphipoda								
Anchilomera blossevillei	-	-	-	-	0.71	0.67	20.00	0.28
Hyperiidea	-	-	-	-	0.23	0.67	20.00	0.18
Total Amphipoda	**-**	**-**	**-**	**-**	**0.94**	**1.33**	**40.00**	**0.46**
Total Crustacea	**-**	**-**	**-**	**-**	**1.72**	**2.67**	**60.00**	**0.89**
THALIACEA								
Pyrosoma sp.	37.74	3.33	18.18	5.67	-	-	-	-
Salpidae	-	-	-	-	1.70	8.00	20.00	1.97
Total Thaliacea	**37.74**	**3.33**	**18.18**	**5.67**	**1.70**	**8.00**	**20.00**	**1.97**
OSTEICHTHYES								
Clupeiformes	-	-	-	-	75.73	0.67	20.00	15.50
Fish skeleton	-	-	-	-	9.43	0.67	20.00	2.05
Total Osteichthyes	**-**	**-**	**-**	**-**	**85.17**	**1.33**	**40.00**	**17.54**
OTHERS								
Scales	26.282	38.333	72.727	35.702	9.56	77.33	80.00	70.49
Trematoda parasites	35.978	58.333	81.818	58.624	0.80	10.00	80.00	8.76
Total Others	**62.26**	**96.667**	**154.55**	**94.326**	**10.35**	**87.33**	**160.00**	**79.25**

Annex 3

List of %W, %N, %F and %IRI values of stomach contents found in *Trachurus mediterraneus,* in each subarea

	GSA17 N				GSA17 C				GSA17 S				GSA18			
	%W	%N	%F	%IRI	%W	%N	%F	%IRI	%W	%N	%F	%IRI	%W	%N	%F	%IRI
MOLLUSCA																
Creseis acicula	-	-	-	-	0.01	0.08	5.00	0.01	-	-	-	-	0.19	2.76	25.00	0.65
Gasteropoda unid.	0.75	0.11	13.64	0.25	0.09	0.26	7.50	0.07	0.01	0.71	9.09	0.13	-	-	-	-
Bivalvia unid.	0.75	0.34	13.64	0.33	0.33	0.36	20.00	0.35	0.01	1.42	9.09	0.26	-	-	-	-
Illex coindetii	-	-	-	-	-	-	-	-	71.95	0.71	9.09	13.22	-	-	-	-
Total Mollusca	**1.50**	**0.45**	**27.27**	**0.58**	**0.43**	**0.70**	**32.50**	**0.42**	**71.98**	**2.84**	**27.27**	**13.61**	**0.19**	**2.76**	**25.00**	**0.65**
CRUSTACEA																
Cladocera																
Unid. Cladocera	0.01	0.03	4.55	0.00	-	-	-	-	-	-	-	-	0.07	0.46	25.00	0.12
Total Cladocera	**0.01**	**0.03**	**4.55**	**0.00**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.07**	**0.46**	**25.00**	**0.12**
Copepoda																
Acartia sp.	13.20	91.06	36.36	82.71	1.62	37.92	15.00	14.81	-	-	-	-	-	-	-	-
Aetidaeus sp.	0.01	0.03	4.55	0.00	0.01	0.05	5.00	0.01	-	-	-	-	-	-	-	-
Calanus -like	-	-	-	-	0.13	0.62	5.00	0.09	-	-	-	-	10.35	40.55	100.00	44.96
Centropages sp.	-	-	-	-	-	-	-	-	-	-	-	-	0.37	3.23	100.00	3.18
Eucheta sp.	-	-	-	-	-	-	-	-	0.21	9.22	9.09	1.72	11.14	13.36	50.00	10.82
Euterpina acutifrons	0.01	0.03	4.55	0.00	0.03	0.47	10.00	0.12	-	-	-	-	-	-	-	-
Oncea meditteranea	-	-	-	-	-	-	-	-	-	-	-	-	0.07	0.46	25.00	0.12
Sapphirina sp.	-	-	-	-	-	-	-	-	-	-	-	-	0.33	0.46	25.00	0.17
Temora stylifera	-	-	-	-	0.02	0.13	10.00	0.04	-	-	-	-	-	-	-	-
Total Copepoda	**13.22**	**91.11**	**45.45**	**82.71**	**1.80**	**39.19**	**45.00**	**15.07**	**0.21**	**9.22**	**9.09**	**1.72**	**22.25**	**58.06**	**300.00**	**59.26**
Ostracoda																
Unid. Ostracoda	0.02	0.05	9.09	0.01	0.08	0.70	20.00	0.39	-	-	-	-	-	-	-	-
Total Ostracoda	**0.02**	**0.05**	**9.09**	**0.01**	**0.08**	**0.70**	**20.00**	**0.39**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Stomatopoda																
Squillidae larvae	-	-	-	-	-	-	-	-	-	-	-	-	21.20	1.84	25.00	5.09
Total Stomatopoda	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**21.20**	**1.84**	**25.00**	**5.09**
Euphausiacea																
Euphausiidae larvae	-	-	-	-	-	-	-	-	-	-	-	-	1.59	2.30	25.00	0.86
Total Euphausiacea	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1.59**	**2.30**	**25.00**	**0.86**
Decapoda																
Alpheus glaber	-	-	-	-	12.72	0.08	5.00	1.60	12.07	1.42	18.18	4.91	-	-	-	-
Philoceras bispinosus	0.99	0.05	4.55	0.10	1.52	0.21	10.00	0.43	-	-	-	-	-	-	-	-
Leocarcinus depurator	48.70	0.03	4.55	4.83	-	-	-	-	-	-	-	-	-	-	-	-
Processa sp.	-	-	-	-	-	-	-	-	4.85	2.84	9.09	1.40	-	-	-	-
Penaeidae	-	-	-	-	13.02	0.08	7.50	2.45	-	-	-	-	-	-	-	-

Annex 3

	GSA17 N				GSA17 C				GSA17 S				GSA18			
	%W	%N	%F	%IRI	%W	%N	%F	%IRI	%W	%N	%F	%IRI	%W	%N	%F	%IRI
Brachiura	3.06	0.08	9.09	0.62	-	-	-	-	-	-	-	-	-	-	-	-
Megalopa Brachiura	0.01	0.03	4.55	0.00	0.34	0.31	10.00	0.16	0.00	0.71	9.09	0.13	-	-	-	-
Zoea Brachiura	0.01	0.03	4.55	0.00	0.00	0.08	2.50	0.01	-	-	-	-	-	-	-	-
Zoea Caridea	-	-	-	-	-	-	-	-	-	-	-	-	18.74	20.28	50.00	17.23
Total Decapoda	**52.77**	**0.21**	**27.27**	**5.57**	**27.60**	**0.75**	**35.00**	**4.65**	**16.92**	**4.96**	**36.36**	**6.44**	**18.74**	**20.28**	**50.00**	**17.23**
Mysidacea																
Siriella sp.	5.77	1.29	9.09	1.40	18.58	6.81	17.50	11.09	1.04	3.55	9.09	0.83	-	-	-	-
Mysidae	0.65	0.11	4.55	0.08	-	-	-	-	0.48	2.13	18.18	0.95	-	-	-	-
Total Mysidacea	**6.43**	**1.40**	**13.64**	**1.48**	**18.58**	**6.81**	**17.50**	**11.09**	**1.52**	**5.67**	**27.27**	**1.78**	**-**	**-**	**-**	**-**
Cumacea																
Cumella pigmea	0.02	0.03	4.55	0.00	0.01	0.03	2.50	0.00	-	-	-	-	-	-	-	-
Iphinoe sp.	0.09	0.08	9.09	0.03	-	-	-	-	-	-	-	-	-	-	-	-
Total Cumacea	**0.11**	**0.11**	**13.64**	**0.04**	**0.01**	**0.03**	**2.50**	**0.00**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Isopoda																
Anilocra frontalis	-	-	-	-	0.87	0.03	2.50	0.06	-	-	-	-	-	-	-	-
Sphaeroma sp.	0.14	0.05	4.55	0.02	-	-	-	-	-	-	-	-	-	-	-	-
Total Isopoda	**0.14**	**0.05**	**4.55**	**0.02**	**0.87**	**0.03**	**2.50**	**0.06**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Amphipoda																
Phistica marina	0.01	0.03	4.55	0.00	-	-	-	-	-	-	-	-	-	-	-	-
Westwoodilla rectirostris	-	-	-	-	1.61	0.49	5.00	0.26	-	-	-	-	-	-	-	-
Ampelisca sp.	15.92	2.90	22.73	9.33	0.11	0.05	5.00	0.02	-	-	-	-	-	-	-	-
Monoculodes sp.	-	-	-	-	0.08	0.13	5.00	0.03	-	-	-	-	-	-	-	-
Gammaridea	1.20	0.08	9.09	0.25	0.05	0.05	5.00	0.01	-	-	-	-	-	-	-	-
Anchilomera blossevillei	-	-	-	-	-	-	-	-	-	-	-	-	5.28	11.52	25.00	3.71
Phronima atlantica	-	-	-	-	-	-	-	-	0.03	0.71	9.09	0.14	-	-	-	-
Hyperiidae	-	-	-	-	-	-	-	-	-	-	-	-	0.14	0.46	25.00	0.13
Hyperiidea	-	-	-	-	-	-	-	-	0.24	4.96	9.09	0.95	-	-	-	-
Total Amphipoda	**17.13**	**3.01**	**36.36**	**9.59**	**1.85**	**0.72**	**20.00**	**0.32**	**0.28**	**5.67**	**18.18**	**1.08**	**5.43**	**11.98**	**50.00**	**3.84**
Total Crustacea	**89.83**	**95.96**	**154.55**	**99.42**	**50.80**	**48.23**	**142.50**	**31.58**	**18.93**	**25.53**	**90.91**	**11.02**	**69.28**	**94.93**	**475.00**	**86.40**
OSTEICHTHYES																
E. encrasic. larvae	-	-	-	-	16.68	0.67	2.50	1.08	-	-	-	-	-	-	-	-
Fish skeleton	2.42	0.08	4.55	0.25	4.53	0.47	12.50	1.56	7.76	5.67	54.55	14.67	23.95	1.84	50.00	11.39
Total Osteichthyes	**2.42**	**0.08**	**4.55**	**0.25**	**21.22**	**1.14**	**15.00**	**2.64**	**7.76**	**5.67**	**54.55**	**14.67**	**23.95**	**1.84**	**50.00**	**11.39**
OTHERS																
Unid.digestive pulp	3.21	0.05	9.09	0.65	9.14	0.21	20.00	4.67	-	-	-	-	6.58	0.46	25.00	1.56
Scales	1.41	1.32	13.64	0.81	17.70	43.46	37.50	57.27	1.32	65.25	45.45	60.56	-	-	-	-
Anisakis sp.	0.01	0.03	4.55	0.00	0.03	0.34	12.50	0.11	-	-	-	-	-	-	-	-
Trematoda parasites	0.33	0.45	36.36	0.62	0.04	0.23	20.00	0.14	0.01	0.71	9.09	0.13	-	-	-	-
Foraminifera	1.44	1.66	27.27	1.85	0.63	5.70	20.00	3.16	-	-	-	-	-	-	-	-
Total Others	**6.41**	**3.51**	**90.91**	**3.93**	**27.55**	**49.94**	**110.00**	**65.35**	**1.32**	**65.96**	**54.55**	**60.69**	**6.58**	**0.46**	**25.00**	**1.56**

Annex 4

List of %W, %N, %F and %IRI values of stomach contents found in *Trachurus trachurus,* in each subarea

	GSA17 N				GSA17 C				GSA17 S				GSA18			
	%W	%N	%F	%IRI	%W	%N	%F	%IRI	%W	%N	%F	%IRI	%W	%N	%F	%IRI
MOLLUSCA																
Creseis acicula	-	-	-	-	-	-	-	-	-	-	-	-	0.05	3.36	12.50	0.89
Total Mollusca	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.05**	**3.36**	**12.50**	**0.89**
CRUSTACEA																
Cladocera																
Unid. Cladocera	-	-	-	-	-	-	-	-	-	-	-	-	0.03	0.67	12.50	0.18
Total Cladocera	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.03**	**0.67**	**12.50**	**0.18**
Copepoda																
Acartia sp.	98.86	99.77	50.00	99.31	-	-	-	-	-	-	-	-	-	-	-	-
Calanus -like	-	-	-	-	-	-	-	-	0.14	3.39	9.09	0.22	0.40	16.11	25.00	8.66
Centropages sp.	-	-	-	-	-	-	-	-	0.05	1.51	4.55	0.05	0.08	4.70	37.50	3.76
Eucheta hebes	-	-	-	-	-	-	-	-	0.59	1.92	22.73	0.39	0.56	6.71	50.00	7.63
Eucheta marina	-	-	-	-	-	-	-	-	-	-	-	-	0.64	7.38	25.00	4.21
Sapphirina sp.	-	-	-	-	-	-	-	-	-	-	-	-	0.02	0.67	12.50	0.18
Euterpina acutifrons	-	-	-	-	-	-	-	-	-	-	-	-	0.07	1.34	12.50	0.37
Unid. Copepoda	-	-	-	-	-	-	-	-	0.68	12.55	13.64	1.24	-	-	-	-
Total Copepoda	**98.86**	**99.77**	**50.00**	**99.31**	**-**	**-**	**-**	**-**	**1.46**	**19.37**	**50.00**	**1.90**	**1.77**	**36.91**	**162.50**	**24.81**
Ostracoda																
Unid. Ostracoda	-	-	-	-	-	-	-	-	-	-	-	-	0.10	0.67	12.50	0.20
Total Ostracoda	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.10**	**0.67**	**12.50**	**0.20**
Euphausiacea																
Nematoscelis sp.	-	-	-	-	-	-	-	-	-	-	-	-	0.97	2.01	12.50	0.78
Nyctiphanes couchii	-	-	-	-	-	-	-	-	94.28	79.13	81.82	97.59	18.84	10.74	37.50	23.27
Euphausiidae larvae	-	-	-	-	-	-	-	-	-	-	-	-	0.30	6.71	25.00	3.68
Euphausiidae	-	-	-	-	-	-	-	-	2.54	3.47	4.55	0.19	6.88	3.36	12.50	2.69
Total Euphausiacea	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**96.83**	**82.59**	**86.36**	**97.78**	**27.00**	**22.82**	**87.50**	**30.42**
Decapoda																
Megalopa Brachiura	-	-	-	-	-	-	-	-	0.00	0.04	4.55	0.00	-	-	-	-
Zoea Penaeidae	-	-	-	-	-	-	-	-	-	-	-	-	0.27	0.67	12.50	0.25
Total Decapoda	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.00**	**0.04**	**4.55**	**0.00**	**0.27**	**0.67**	**12.50**	**0.25**
Amphipoda																
Parathemisto gaudichaudii	-	-	-	-	40.00	25.00	50.00	32.50	-	-	-	-	-	-	-	-
Themisto abissorum	-	-	-	-	-	-	-	-	-	-	-	-	0.82	2.01	12.50	0.74
Hyperiidae	-	-	-	-	-	-	-	-	0.33	1.17	18.18	0.19	7.35	26.17	25.00	17.59
Total Amphipoda	**-**	**-**	**-**	**-**	**40.00**	**25.00**	**50.00**	**32.50**	**0.33**	**1.17**	**18.18**	**0.19**	**8.18**	**28.19**	**37.50**	**18.33**
Total Crustacea	98.86	99.77	50.00	99.31	40.00	25.00	50.00	32.50	98.63	103.17	159.09	99.87	37.35	89.93	325.00	74.19
OSTEICHTHYES																
E. encrasic. larvae	-	-	-	-	-	-	-	-	-	-	-	-	36.01	3.36	12.50	10.32
Serranus sp. larvae	-	-	-	-	-	-	-	-	-	-	-	-	2.08	0.67	12.50	0.72
Total Osteichthyes	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**38.09**	**4.03**	**25.00**	**11.05**
OTHERS																
Unid.digestive pulp	-	-	-	-	-	-	-	-	1.14	0.11	13.64	0.12	24.36	1.34	25.00	13.48
Scales	-	-	-	-	40.00	25.00	50.00	32.50	0.23	0.19	4.55	0.01	-	-	-	-
Anisakis sp.	-	-	-	-	-	-	-	-	-	-	-	-	0.15	1.34	12.50	0.39
Trematoda parasites	1.14	0.23	50.00	0.69	20.00	50.00	50.00	35.00	-	-	-	-	-	-	-	-
Total Others	**1.14**	**0.23**	**50.00**	**0.69**	**60.00**	**75.00**	**100.00**	**67.50**	**1.37**	**0.30**	**18.18**	**0.13**	**24.51**	**2.68**	**37.50**	**13.87**