Ecological and functional role of small and medium pelagic fish in the northwestern Mediterranean Sea

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“*El mar es un espejo que nos devuelve a nuestra propia ignorancia*”

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Abstract

Mediterranean marine ecosystems are fundamentally driven by their pelagic communities and small and medium-size pelagic fish (SMPF) play crucial ecological roles. Although fluctuations of the populations of these organisms have been mainly attributed to environmental variability and overfishing, there is still a lack of understanding on specific mechanisms that drive their population changes. In recent decades a decline in landings, biomass and body condition of sardine and anchovy in the northwestern Mediterranean Sea has been observed and several hypotheses to explain these changes have been formulated. Therefore, accurate scientific data at species and community level is needed in order to understand the most likely causes of these declines. Within this context, this PhD thesis aims to improve the knowledge of the seasonal dynamics and functioning of the pelagic compartment focusing in the trophic relationships and energy dynamics of SMPF in the northwestern Mediterranean Sea.

I used different methods to advance on the knowledge about the ecological and functional role of several pelagic fish species in the western Mediterranean Sea, including clupeoids, horse mackerels and mackerels. Specifically, stable isotope and stomach content analyses in combination with direct and indirect body condition indeces, were used to study seasonal variation in energy content in relation to their breeding strategies, feeding preferences and trophic role in the pelagic food-web. Results showed stability in the trophic structure through the year with a trophic segregation of small pelagic fish depending on the ontogenetic stage. Seasonal differences in energy density were highly related with the spawning period and food availability. To integrate the new findings and to test the role of potential pressures on main pelagic species, I developed qualitative food-web models. Results showed that an increase of sea surface temperature in combination with an increase of explotation of sardine and anchovy and changes in zooplankton composition were the most plausible pressures to explain the observed changes in small pelagic fish populations. Overall, results provide pivotal information to assist the adaptive management of pelagic fish species in the region.

Resum

Els ecosistemes marins mediterranis estan fonamentalment dirigits per les seves comunitats pelàgiques on els peixos pelàgics petits i mitjans (SMPF) exerceixen funcions ecològiques crucials. Si bé les fluctuacions de les seves poblacions s'han atribuït principalment a la variabilitat ambiental i la sobrepesca, encara hi ha una manca de comprensió sobre els mecanismes específics que impulsen els canvis en la població. En les últimes dècades s'ha observat una disminució en la biomassa, en els desembarcaments i en la condició de la sardina i el seitó al nord-oest del mar Mediterràni, i s'han formulat diverses hipòtesis per explicar aquests canvis. Actualment, es necessiten dades científiques precises a nivell d'espècies i comunitats per comprendre les causes més probables d'aquestes disminucions. En aquest context, aquesta tesi doctoral pretén millorar el coneixement de la dinàmica estacional i el funcionament del compartiment pelàgic, centrant-se en les relacions tròfiques i la dinàmica energètica dels SMPF al nord-oest del Mar Mediterrani.

Es varen fer servir diferents tècniques per avançar en el coneixement del paper ecològic i funcional de diverses espècies de peixos pelàgics al mar Mediterrani occidental, incloent clupeoids, sorells i verats. En concret, es varen utilitzar anàlisi d'isòtops estables i de contingut estomacal en combinació amb índexs directes i indirectes de condició corporal, per estudiar la variació estacional del contingut d'energia en relació amb les seves estratègies de reproducció, preferències tròfiques i paper tròfic en la comunitat pelàgica. Els resultats van mostrar estabilitat en l'estructura tròfica al llarg de l'any amb una segregació tròfica dels peixos pelàgics petits en funció de l'etapa ontogenètica. Les diferències estacionals en la densitat energètica van estar altament relacionades amb la temporada de reproducció i la disponibilitat d'aliments. Per integrar tota aquesta informació i testar el paper de les pressions potencials en les principals espècies pelàgiques, es va desenvolupar un model qualitatiu. Els resultats van demostrar que l'augment de la temperatura superficial del mar en combinació amb un augment d'explotació de la sardina i el seitó i canvis en la composició del zooplancton, eren les pressions més pausibles per explicar els canvis observats en les poblacions de peixos pelàgics petits. En general, els resultats proporcionen informació fonamental per contribuir al maneig adaptatiu de les espècies de peixos pelàgics a la regió.

Resumen

Los ecosistemas marinos mediterráneos están fundamentalmente condicionados por sus comunidades pelágicas donde los peces pelágicos pequeños y medianos (SMPF) desempeñan funciones ecológicas cruciales. Si bien las fluctuaciones de sus poblaciones se han atribuido principalmente a la variabilidad ambiental y la sobrepesca, todavía hay una falta de comprensión sobre los mecanismos específicos que impulsan los cambios en las poblaciones pelágicas. En las últimas décadas se ha observado una disminución en la biomasa, en los desembarques y en la condición de la sardina y la anchoa del noroeste del mar Mediterráneo, y se han formulado varias hipótesis para explicar estos cambios. Actualmente, se necesitan datos científicos precisos a nivel de especies y comunidades para comprender las causas más probables de estas disminuciones. En este contexto, esta tesis doctoral pretende mejorar el conocimiento de la dinámica estacional y el funcionamiento del compartimento pelágico, centrándose en las relaciones tróficas y la dinámica energética de los SMPF en el noroeste del mar Mediterráneo.

Se usaron diferentes técnicas para avanzar en el conocimiento del papel ecológico y funcional de varias especies de peces pelágicos en el Mar Mediterráneo occidental, incluyendo clupeoides, jureles y caballas. En concreto, se utilizó el análisis de isótopos estables y de contenido estomacal en combinación con índices directos e indirectos de condición corporal, para estudiar la variación estacional del contenido de energía en relación con las estrategias de reproducción de los SMPF, las preferencias tróficas y el papel trófico en la comunidad pelágica. Los resultados mostraron estabilidad en la estructura trófica a lo largo del año con una segregación trófica de peces pelágicos pequeños en función del estadío ontogenético. Las diferencias estacionales en la densidad energética estuvieron altamente relacionadas con el periodo de reproducción y la disponibilidad de alimentos. Para integrar toda esta información y testar el rol de las presiones potenciales en las principales especies pelágicas, se desarrolló un modelo cualitativo. Los resultados mostraron que un aumento de la temperatura de la superficie del mar en combinación con el aumento de la explotación de la sardina y la anchoa y cambios en la composición del zooplancton fueron las presiones más plausibles para explicar los cambios observados en las poblaciones de peces pelágicos pequeños. En general, estos resultados proporcionan información fundamental para contribuir al manejo adaptativo de las especies de peces pelágicos en la región.

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General Introduction

Ecological importance of Small and Medium Pelagic Fish

Small and Medium sized Pelagic Fish (SMPF), also known as forage fish, are ecologically and economically important organisms in marine ecosystems worldwide, representing more than 26% of total marine fishery landings (FAO 2018a). In many countries, such as Peru and Namibia, Small-sized Pelagic Fish (SPF) and Medium-sized Pelagic Fish (MPF) are an essential source of animal protein (Tacon & Metian 2009). Moreover, they are key elements of the marine food web (Cury et al. 2000, 2011).

Several marine ecosystems show a configuration with numerous species at lower (such as plankton species) and higher (such as fish, seabirds and other predators) trophic levels and few species at intermediate levels that are highly abundant and thus very important ecologically (Cury et al. 2000, Bakun 2006). Such systems have been referred as *wasp-waist* ecosystems and in many of them the variability in the trophic dynamics is mediated by SPF. Due to their important biomass, production, and trophic relationships, SPF impact the dynamics of other organisms across the entire marine food web performing a top-down and bottom-up control simultaneously (Rice 1995; Figure 1). Therefore, changes in SPF can have ultimate ecologic and socio-economic wide consequences.

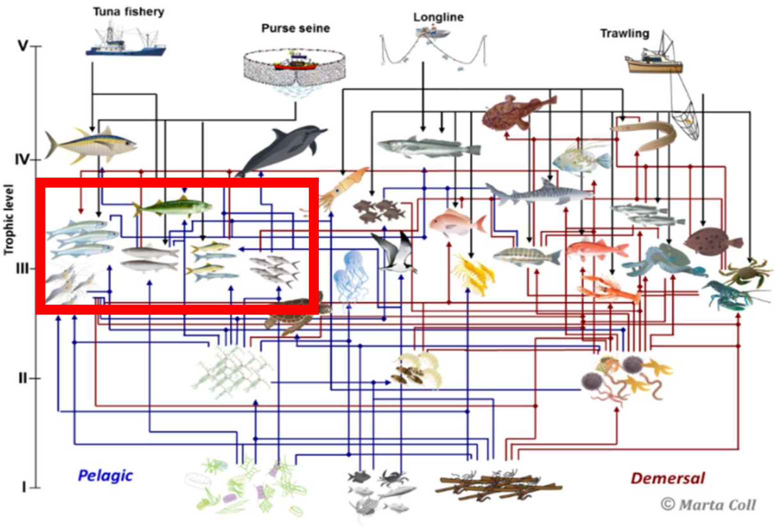

Figure 1. Trophic relationships of main functional groups of marine organisms in the northwestern Mediterranean Sea (adapted from Coll et al. 2006). Red box identifies small and medium pelagic fish.

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Fluctuations in SPF populations have been widely described as an effect of environmental changes even in the absence of fisheries (Baumgartner et al. 1992, Cury et al. 2000). These SPF species, including sardine (genera *Sardina*, *Sardinella* and *Sardinops*) and anchovy (genera *Engraulis*), are distributed worldwide. Their relatively short life-cycle, high mobility and their plankton-base feeding make these species highly sensitive to fluctuations in environmental factors, including those related to human-induced climate change (Agostini & Bakun 2002, Checkley et al. 2009).

Collapses of SPF have been observed in different ecosystems, such as the Pacific sardine (*Sardinops sagax*) in the California current and in the Benguela upwelling region, the Peruvian anchoveta (*Engraulis ringens*) in southeast Pacific Ocean or more recently the European anchovy (*Engraulis encrasicolus*) in the Bay of Biscay (Checkley et al. 2009, Roux et al. 2013, Taboada & Anadón 2016). The collapse of these SPF has been mainly attributed to a combination of fishing pressure and environmental-dependent recruitment success processes. Although examples exist of the depletion of SPF and the impact on marine ecosystems (Guénette et al. 2008, Roux et al. 2013), the role that fisheries play in those fluctuations remains difficult to disentangle from the environmental variability (Essington et al. 2015).

While much progress has been made in understanding the primary aspects of large-scale changes in small pelagic fish, still one of the grand challenges is to have the ability to anticipate fluctuations in their populations in order to manage the resource in a sustainable and adaptive way. SPF populations fluctuate at various time scales (i.e. seasonal, decadal) and ecological units (i.e. from species to community). Potential effects of future climate change create the necessity to understand ecosystem functioning as a whole in order to be able to manage it. The prediction of SPF responses is challenging and in many cases there are gaps in the understanding on how multiple stressors influence SPF and how they interact (Hodgson & Halpern 2019).

In order to overcome these limitations and fill several gaps of knowledge, a holistic understanding of the functioning of the pelagic ecosystem is needed. To integrate knowledge of specific physiological, biological and ecological processes from the individual to the ecosystem level and taking into account the trophic relationships and transfer of energy between species within the food web, considering environmental and anthropological pressures is a must in order to advance in the implementation of ecosystem-based fisheries management (Figure 2).

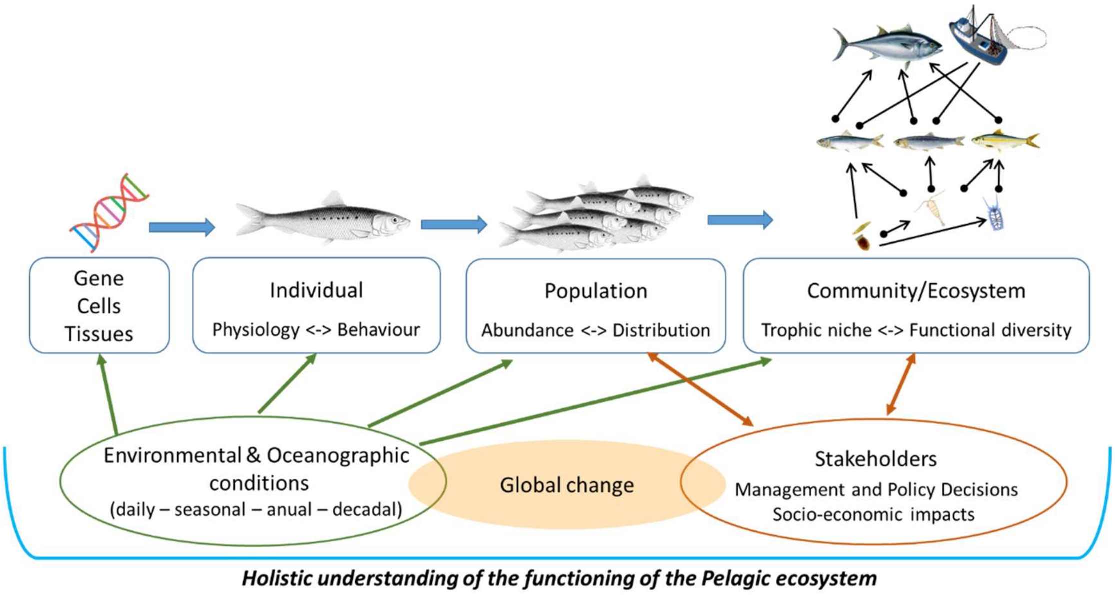

Figure 2. Scheme of hierarchical levels of biological organization and interaction with biotic and abiotic factors. Adapted from Metcalfe et al. 2012 and McKenzie et al. 2016.

Within this context, this PhD thesis aims to improve the knowledge of the seasonal dynamics and functioning of the pelagic compartment focusing in the trophic relationships and energy dynamics of SMPF in the Mediterranean Sea, and ultimately enhance the use of holistic approaches.

Small and Medium Pelagic fish in the Mediterranean Sea

SMPF are commercial species that are mainly caught by purse-seiners and mid-water trawlers. In the Mediterranean Sea, fishing catches are dominated by SPF representing nearly 49% of the catch (FAO 2018). The most abundant SPF are European sardine (*Sardina pilchardus*; Walbaum, 1792) and European anchovy (*Engraulis encrasicolus*; Linnaeus, 1758), representing 16% and 22%, respectively, of the total catches during 2014-2016 (FAO 2018). Additionally, European anchovy (hereafter anchovy) contributed to a 12% of the total landings value and European sardine (hereafter sardine) to the 9% during 2014-2016 (FAO 2018). Another SPF present in the Mediterranean Sea is round sardinella (*Sardinella aurita*; Valenciennes, 1487), more abundant in southern Mediterranean waters (Tsikliras et al. 2005).

SPF landings are important in all areas of the Mediterranean Sea. In the western Mediterranean basin, SPF represent 46% of the catches with sardine contributing 26%, anchovy 13%, and round sardinella 7% during 2014-2016 (Figure 3). Within this period, in terms of economic value, sardine and anchovy represented 22% of total landing value of commertial species in the Mediterranean Sea (FAO 2018).

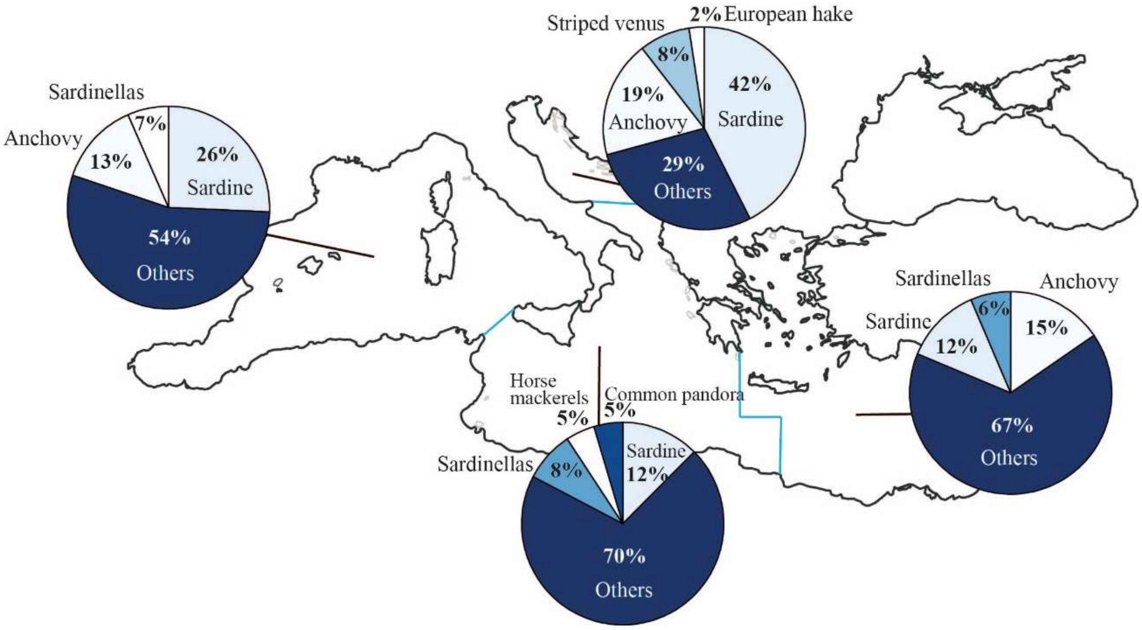

Figure 3. Percentage of landings by species in the different GFCM sub-regions (western Mediterranean, Adriatic Sea, central Mediterranean and eastern Mediterranean), average 2014-2016 period. Main species reported: anchovy (*Engraulis enrasicolus*), sardine (*Sardina pilchardus*), sardinellas (*Sardinella* spp., mainly *Sardinella aurita*), horse mackerels (*Trachurus* spp.), European hake (*Merluccius merluccius*), common pandora (*Pagellus erythrinus*) and striped venus (*Chamelea gallina*). Source: Modified from Figure 26 of SoMFi report (FAO 2018b).

Previous studies using ecosystem models have highlighted the ecological key role of SPF in all Mediterranean ecosystems (e.g. Coll et al. 2006, 2008, Tsagarakis et al. 2010, Piroddi et al. 2017). Different biotic factors such as environmental conditions, fishing activity and trophic interactions played a significant role in driving small pelagic fish historical dynamics in different ecosystem models of the Mediterranean Sea (Coll et al. 2008, 2009, 2012). Due to the high biomass of SPF in the Mediterranean Sea and their potential wasp-waist role, changes in their abundance can highly impact their prey and predators modifying ecosystem structure and functioning in the Mediterranean Sea.

Sardine and anchovy co-occur in Mediterranean waters. Their distribution is mainly located in coastal areas and continental shelves of the whole basin (Giannoulaki et al.

2011, 2014). The main spawning grounds are located in the western Mediterranean coast, Gulf of Lions, areas of the Gulf of Tunis and Gulf of Gabes and in the eastern Mediterranean Sea. Within the western Mediterranean Sea, the surroundings of the Ebro River Delta, the study area where this thesis is conducted, is considered an important spawning and nursery ground for sardine and anchovy (Giannoulaki et al. 2014).

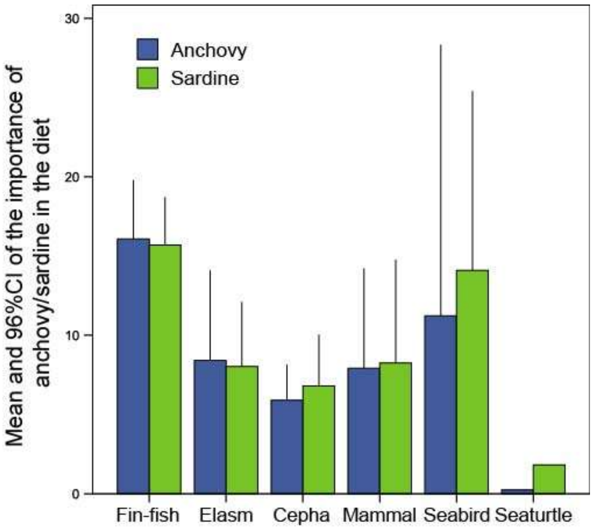

Figure 4. Importance in % weight of anchovy and sardine in the diet of main predators by type of predator in the Mediterranean Sea.
Source: Albo-Puigserver et al. 2019.

In the continental shelf associated to the Ebro River delta, sardine and anchovy have been the most important species in terms of biomass and commercial landings in historical times (Palomera et al. 2007). Moreover, different species of seabirds (i.e. Audouin's gull *Larus audouinii*), marine mammals (i.e. bottlenose dolphin *Tursiops truncatus*), fin-fish (i.e. bluefin tuna *Thunnus thynnus*) and sharks (i.e. blue shark *Prionace glauca*) prey on sardine and anchovy in the Mediterranean Sea (Figure 4; Stergiou & Karpouzi 2002, Albo-Puigserver et al. 2019). For some predators, sardine and anchovy are the main source of food and population variability of forage fish could influence their fitness (Piroddi et al. 2011). At the same time, sardine and anchovy have been shown historically to be able to exert a top-down control on their prey (Coll et al. 2008, 2009).

The other small pelagic fish present at lower biomasses in the continental-shelf of the Ebro river Delta (Western Mediterranean Sea) and with lower commercial interest is round sardinella. This is a more tropical species that prefers warmer waters and has been traditionally found in the mid-southern part of the Mediterranean Sea. However, in the lasts decades a northward expansion of round sardinella has been observed in the Mediterranean Sea and in Atlantic waters off Mauritania (Alheit et al. 2014). An expansion in the distribution of round sardinella has also been observed in the Aegean Sea (Eastern Mediterranean) as well as in the western Mediterranean coast (Tsikliras

2008). This northward expansion has been related to the increase of sea surface temperature (Sabatés et al. 2006, Maynou et al. 2014).

Apart from small-sized pelagic fish, medium-sized pelagic fish (MPF) also play an important role in marine ecosystems (Lleonart & Maynou 2003, Juan-Jordá et al. 2013). In the Mediterranean Sea, the MPF of higher commercial interest are the Atlantic horse-mackerel (*Trachurus trachurus,* Linnaeus 1758), Mediterranean horse-mackerel (*Trachurus mediterraneus,* Steindachner 1868), Atlantic mackerel (*Scomber scrombus,* Linnaeus 1758) and Atlantic chub mackerel (*Scomber colias,* Gmelin 1789) (Bas 1995). MPF represented 7% of the total catches in the Mediterranean Sea during 2014-2016 (Figure 5; Pauly & Zeller 2015, FAO 2018).

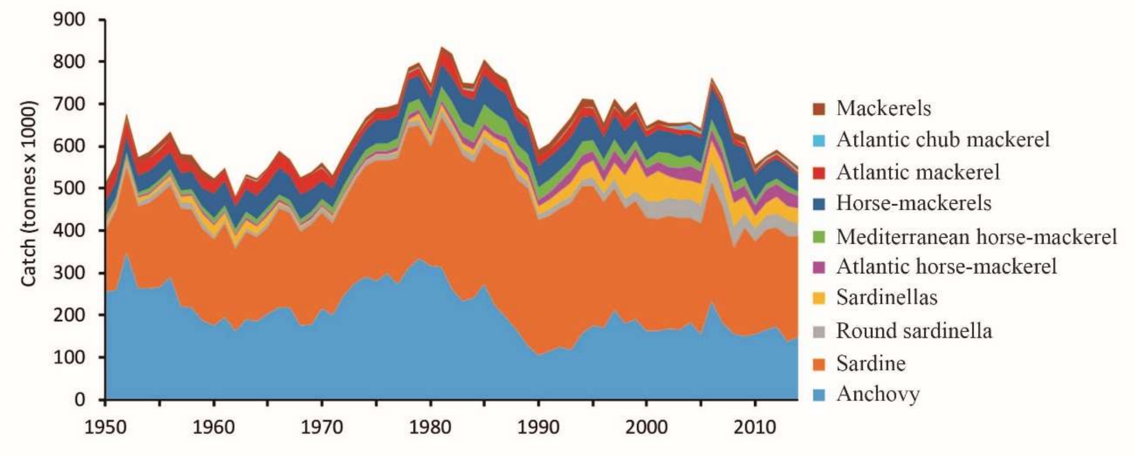

Figure 5. Catch reconstruction data from the Mediterranean Sea. Source: Pauly & Zeller (2015) Sea Around Us database.

MPF are pelagic migratory species with wide distributions that can form large shoals. Their economic value is low but they occupy a key position in the food web and are also essential elements of the diet of larger pelagic and demersal fin-fish, sharks, and marine mammals (Zardoya et al. 2004). At the same time, they prey on macrozooplankton and can prey on small pelagic fish such as sardine and anchovy, being potential competitors and predators of SPF (Karachle & Stergiou 2017).

Interannual variability in catches of MPF has been observed in the western Mediterranean (Coll et al. 2006). In the Adriatic Sea a sharp decline in those species was reported in the last three decades due to overfishing (Lotze et al. 2011). Despite the ecological importance of MPF, little attention has been given to the assessment of these species and limited biological data is available in the Mediterranean Sea (Lleonart 2008).

Biological characteristics of SPF: sardine, anchovy and round sardinella

Sardine, anchovy and round sardinella have a relatively short life span of 4 to 8 years. This short life cycle makes them more vulnerable to environmental variability and hence it is possible to observe rapid changes in the population dynamics of these species due to unfavourable environmental conditions.

SPF show important differences in biological features (Palomera et al. 2007). The three species have indeterminate fecundity (oocytes continue to be recruited during the spawning season) and are batch spawners (eggs are released in multiple events over a period of months). Sardine reproduces in winter, between October-November and March, with a peak of reproduction in January-February (Palomera et al. 2007). It spawns in cold waters between 12-14 ºC and 19ºC. Instead, anchovy spawns in late spring and summer, between April and September with a peak in May-June (Figure 6; and Palomera 1992). Round sardinella spawns also in summer from June to October with a peak of spawning in August (Figure 6; Palomera & Sabatés 1990). At the end of the first year of life (life stage 0 and 1), most of SPF have already spawned.

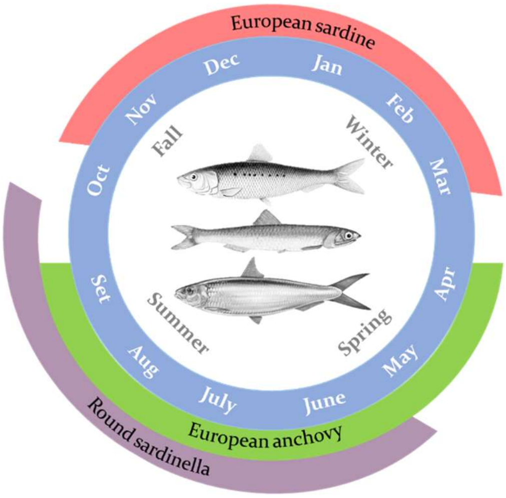

Figure 6. Reproduction period of European sardine (red), European anchovy (green) and Round sardinella (violet). Adapted from Quattrocchi et al 2017.

Size at first maturity was historically estimated at 13 cm for sardine (Abad & Giraldez 1983), 11 cm for anchovy (Palomera et al. 2003) and 15 cm for round sardinella (Tsikliras & Antonopoulou 2006). However, in the last decade a decrease in size at first maturity has been observed for sardine and anchovy in the northwestern Mediterranean Sea (Brosset, Lloret, et al. 2016, Albo-Puigserver et al. 2019), which is now 9.67 cm for sardine and 9.74 cm for anchovy. Minimum landing sizes are 11 cm for sardine and 9 cm for anchovy (Regulation (EC) No 1967/2006). Therefore, recruitment overfishing for both species has been suggested, because the minimum catch size was lower than the size at first maturity (Palomera et al. 2007).

The feeding habits of SPF have been mainly studied through stomach content analysis. There are many studies on the diet of sardine and anchovy in the

Mediterranean Sea and few studies on feeding habits of round sardinella (Box 1. Stomach content analysis of planktivorous fish). In the case of sardine and anchovy, recent studies in the Gulf of Lions (NW Mediterranean Sea) have found differences in the diet during the last two decades (Brosset, Le Bourg, et al. 2016). However, in the Catalan Sea (NW Mediterranean Sea) few studies are available (Tudela & Palomera 1995, 1997). The published diet information indicates that both species are planktivorous fish that mainly prey on copepods, cladocerans, decapod larvae and diatoms (Table 1). Seasonal variability in feeding preferences has been observed due to changes in the plankton composition and abundance throughout the year (Costalago & Palomera 2014). In the case of sardine, in the Gulf of Lions adults have been reported to feed on diatoms, with a more filter feeding behavior than juveniles (Costalago & Palomera 2014, Costalago et al. 2014, Le Bourg et al. 2015). Instead, anchovy is mainly zooplanktivorous (Tudela & Palomera 1997, Costalago et al. 2014, Brosset, Le Bourg, et al. 2016). Juvenile and larval stages of both species also prey on zooplankton and phytoplankton (Morote et al. 2010, Costalago et al. 2014) (Table 1).

Box 1: Stomach content analysis of planktivorous fish

Visual analysis of stomach contents is the most widely used method for the study of feeding habits of fishes (Figure 1). Advantages of Stomach Content Analysis (SCA) are the high taxonomic level obtained and the possibility to know the size size and life-stages of prey. Limitations include the short time-period that reflect the stomach content (i.e. 24h) and the over or under estimation of prey items identified depending on prey digestibility (i.e. otoliths *versus* gelatinous plankton; Nielsen et al. 2018).


Figure 1. Stomach of a small pelagic fish.

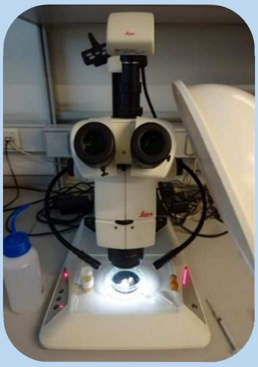
Figure 2. Stereomicroscope.

There are different indeces to quantify the relative contribution of each prey item to the total diet (Hyslop 1980). The most widely used are:

- ✓ % N – Numerical: number of a prey type as proportion of the total number of items in the whole stomach content.
- ✓ % FO –Frequency of occurrence: percentage of stomachs containing a particular prey type.
- ✓ % W or % V – Weight or Volume of a food type in relation to the weight or volume of the whole stomach content.

In the case of small pelagic planktivorous fish a stereomicroscope is used for the SCA (Figure 2). The %W can be calculated directly (weighing a group of prey items) or indirectly measuring the length of the prey and using published length-weight relationship to reconstruct the dry or wet weight of the preys (Borme et al. 2009; Figure 3).

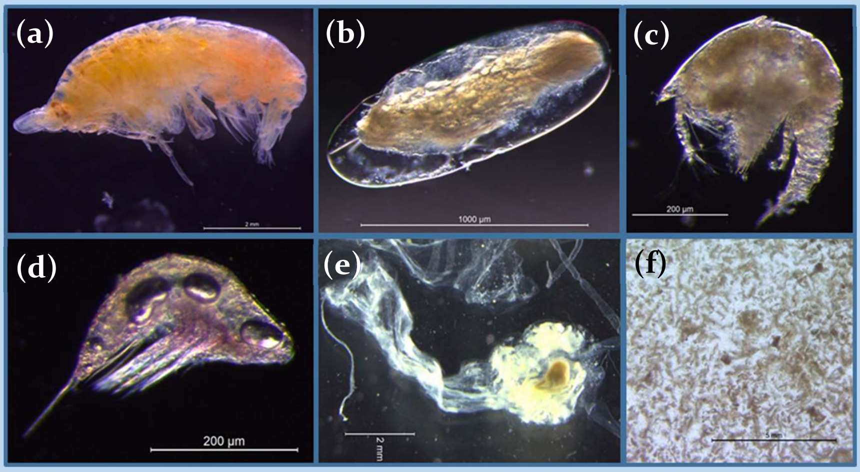

Figure 3. Examples of prey items found in the stomachs of round sardinella (a) Amphipoda Hyperiidea, (b) egg of anchovy, (c) *Euterpina acutifrons*, (d) *Microsetella* sp., (e) Salpida, (f) stomach content of round sardinella containing mainly Calanoid copepods of the genera *Acartia*.

In the western Mediterranean Sea, there is only information on feeding ecology of round sardinella at larval stage (Table 1; Morote et al. 2008). At larval stages, the diet of round sardinella was similar to the larvae of anchovy with preference for copepods at different stages and cladocera (Morote et al. 2008). For juvenile and adult stages in the central and eastern Mediterranean, round sardinella has been reported to feed mainly on zooplankton such as copepods and cladocera, and phytoplankton in productive areas (Lomiri et al. 2008, Madkour 2012).

Table 1. Available published data on stomach content analysis and the main prey groups reported for European anchovy (*Engraulis encrasicolus*), European sardine (*Sardina pilchardus*) and round sardinella (*Sardinella aurita*) in the NW Mediterranean Sea for Geographical Sub Areas 06 (Spanish coast) and GSA07 (French coast; Gulf of Lions). Seasons with available information for each life stage are indicated with an 'x' (1= winter; 2=spring; 3=summer; 4= fall). In grey cells with missing information for all the seasons.

SPF	**Life stages**	**Main prey groups**	**Season GSA06**				**Season GSA07**				**References**
			1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	
Anchovy	Larvae	Cladocera, copepod			x	x				x	Costalago et al. 2014; Le Bourg et al. 2015; Morote et al. 2010; Plounevez and Champalbert, 2000; Intxaustin et al. 2017; Tudela and Palomera, 1997; Brosset et al. 2016; Tudela et al. 2002
	Juvenile	Copepod, cladocera, appendicularia					x		x	x	
	Adult	Copepod, euphausiacea, decapod		x					x		
Sardine	Larvae	Tintinnid, copepod				x					Costalago et al. 2014; Costalago and Palomera 2014; Le Bourg et al. 2015; Morote et al. 2010; Brosset et al. 2016
	Juvenile	Copepod, cladocera, crustacea, mysids, diatom					x		x	x	
	Adult	Copepod, cladocera, diatom, appendicularia					x		x		
Sardinella	Larvae	Copepod, cladocera		x							Morote et al. 2008
	Juvenile	▨									
	Adult	▨									

In the last decades, other trophic markers such as the stable isotope analysis (SIA) have been widely used to study the trophic ecology and overlaps between species of pelagic fish (Box 2. Stable isotope analysis of $\delta^{15}N$ and $\delta^{13}C$). The analysis of the stable isotopes of $\delta^{15}N$ and $\delta^{13}C$ in combination with stomach content analysis has been used to analyze food web structures and trophic niche relationship (Layman et al. 2012). The use of both techniques is very useful to better understand the trophic ecology of organisms at different time-scales and resolutions and has been successfully used in the study of small pelagic fish (e.g. Costalago et al. 2014, Le Bourg et al. 2015).

Box 2: Stable Isotope Analysis of δ^{15}N and δ^{13}C

The use of stable isotopes of nitrogen (δ^{15}N) and carbon (δ^{13}C) has been increasingly used as an effective tool to study trophic segregation among species, complementing stomach content information (Hussey et al. 2011).

Carbon isotopic ratios, C^{13}/C^{12}, show little change with trophic transfers and are useful indicators of the dietary source of carbon (Kelly 2000). In nitrogen isotopic ratios, N^{15}/N^{14}, the lighter isotope is excreted in greater proportion than the heavier isotope, with a predictable increase throughout the trophic chain, allowing estimates of consumer trophic level (Figure 1; TL Eq. 1). The offset between the stable isotope ratio of the prey and the predators is the discrimination factor (**Δ** ^{15}N; Vander Zanden & Rasmussen 2001).

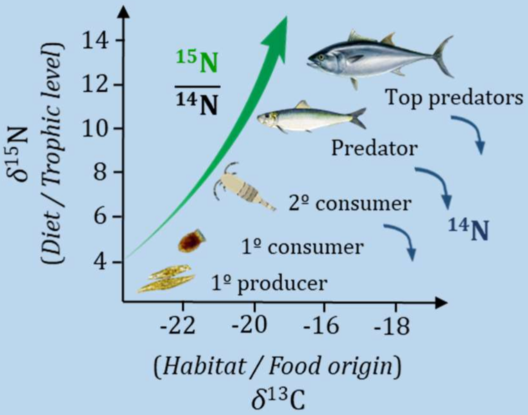

Figure 1. Representation of the pelagic trophic chain and stable isotope dynamics.

$$TL_{consumer} = TL_{basal} + (\delta^{15}N_{consumer} - \delta^{15}N_{basal})/\Delta\delta^{15}N \qquad \text{Eq. 1}$$

The bivariate space of δ^{15}N and δ^{13}C represents the habitat and resources used, comparable to the n-dimensional environmental space that defines the ecological niche (Newsome et al. 2007). To analyze the ecological niche, different community metrics are available (Layman et al. 2012). Two of them are presented below (Figure 2 and 3).

Ellipses → Trophic width

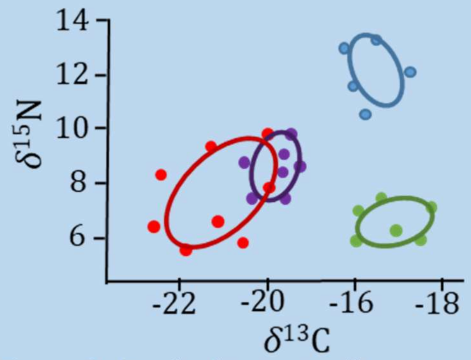

Figure 2. Standard isotopic ellipse area, a measure of trophic width. The overlap between two ellipses is the degree of isotopic niche overlap between species (Jackson et al. 2011).

Centroid → Isotopic niche position

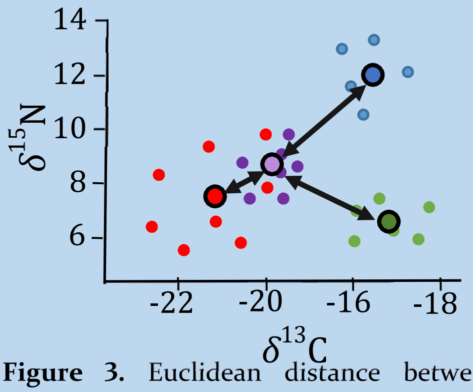

Figure 3. Euclidean distance between centroids are calculated to evaluate the niche position of each species related to the rest of the community (Turner et al. 2010).

Linked to the reproduction period and food availability, small pelagic fish have different seasonal patterns in the body condition. Condition and energy storage of species fluctuate during the year due to different biological processes (growth, maintenance and reproduction) and on external factors (food availability or temperature). As a consequence of the seasonal environmental variability, pelagic marine species adapt their energy allocation strategy (Figure 8). The energy obtained from feeding is dynamically allocated between growth and reproduction processes (Kooijman 2010). This allocation has a direct effect on the energy transfer through the ecosystem, as growth represents biomass available for upper trophic levels through predation, while reproduction is an investment in future generations, which provides recruitment and also energy at lower or intermediate trophic levels (through predation in the plankton communities or cannibalism; Leggett & Deblois 1994). Energy partition shows a seasonal pattern, which is species specific and depends on the seasonal and inter-annual ecosystem productivity cycles (Pecquerie et al. 2009). Reproductive strategies include a palette of seasonal cycles in energy allocation, from species that accumulate energy in specific tissues (“capital spawners”) *versus* those that rely on daily incoming energy (“income spawners”)(Bonnet et al. 1998). In sardine and anchovy, the energy allocation strategy differs. Anchovy has been described mainly as an income breeder, while sardine has been described mainly as a capital breeder (McBride et al. 2015, Brosset, Lloret, et al. 2016).

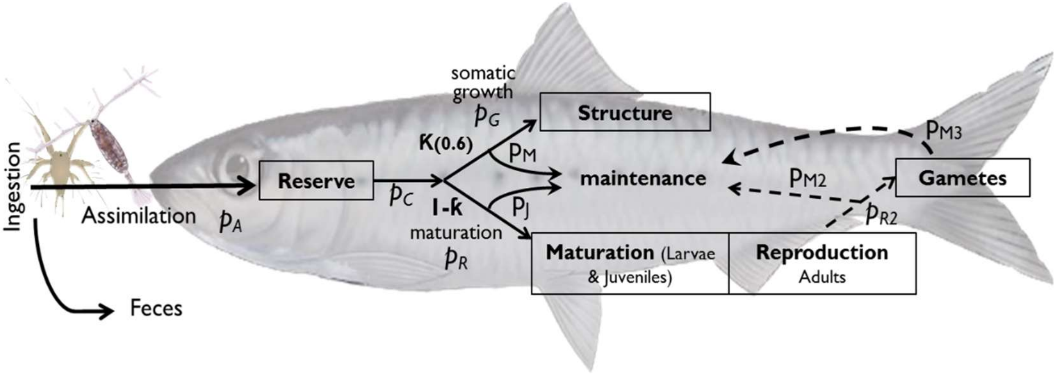

Figure 8. Conceptual diagram of the dynamic energy budget of an individual of European sardine (adapted from Pethybridge et al. 2013).

Studies have traditionally focused on morphological condition indexes and few studies have look at the energy allocation of sardine and anchovy with bioenergetic and biochemical indexes (Lloret et al. 2013, Brosset et al. 2015; Box 3. Methods to measure body condition and energy content). In the Gulf of Lions, seasonal variability of lipid content of sardine and anchovy adults has been described, with the lowest values in winter for sardine and in spring for anchovy and higher values in larger individuals (Pethybridge et al. 2014, Ferrer-maza et al. 2016). For larval stages of both SPF, an increase of energy density with the development of the larvae was described in the spawning area of the Ebro river delta (Barroeta et al. 2017).

The variability in body condition and energy storage in SPF has important implications for fish recruitment and ecosystem structure (Peck et al. 2013, Saraux et al. 2019). Moreover, changes in body condition or energy density of forage fish, such as SPF, have been observed to have important implications for higher trophic levels. For example, declines in top-predators such as the common guillemots (*Uria aalge*) have been observed in the North Sea and linked to a reduction in the quality (in terms of energy reduction per unit) of the main prey, sprat and lesser sandeels (*Sprattus sprattus* and *Ammodytes marinus,* respectively; Wanless et al. 2005). Then, it is important to understand the energy allocation at individual level in order to understand the ecological energetics of the ecosystem (how energy is acquired, retained and transferred from one trophic level to another in the food web; Wiegert 1988).

Box 3: Methods to measure body condition & energy content

Body condition is often used to describe the health and nutritional status of fish populations. This association is based on the premise that body condition is correlated with the lipids or energy stored (Schloesser and Fabrizio, 2017). The study of fish condition has been related to life-history and energy allocation strategies and other measures of fitness (Lloret et al 2013).

The principal form of energy storage are the lipids. Instead, proteins, that are the main compound of body structure, usually remain constant and are less energetic than lipids (Shulman and Love, 1999).

Several indirect and direct indices have been developed to assess fish condition. Indices used in this thesis are described below:

Indirect indices

✓ *Relative Condition Factor – Kn*
Kn is a length independent morphometric indicator (Le Cren 1951), where heavier fish (i) than the mean, for a given size, are supposed to be in better condition.

$$\mathrm{Kn} = \frac{\mathrm{Weight}_{(i)}}{\mathrm{W}_{\mathrm{predicted}}}$$

✓ *Water content – % DW*
Water content or the inverse, dry weight (%DW), is often related with total lipid and energy density.

$$\%\,\mathrm{DW} = \frac{\mathrm{DW}_{(i)}}{\mathrm{WW}_{(i)}} \cdot 100$$

✓ *Gonadosomatic Index – GSI*
GSI is used as a measure of reproductive investment and considers the weight of the gonad of an individual (i) in relation to the total weight of (i).

$$\mathrm{GSI} = \frac{\mathrm{Gonad}_{\mathrm{W}\,(i)}}{\mathrm{Weight}_{(i)}}$$

Direct indices

✓ *Biochemical analysis- Total Lipids*
One method to extract lipid from muscle of fish is the Folch method (Folch et al. 1957) that uses 2:1 chloroform-methanol mixture (Figure 1).

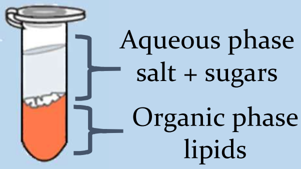

Figure 1. Folch's extraction.

✓ *Energy density – Direct Oxigen Bomb Calorimetry*
This method measures the heat of a rapid combustion transformed into energy units (kJ or kcal per gram). The ED measures all organic compounds; lipids, proteins and carbohidrates (Lamprecht 1999) (Figure 2).

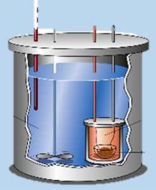

Figure 2. Oxigen bomb.

Biological characteristics of MPF: mackerels and horse-mackerels

Medium pelagic fish (MPF) have been described as species that have higher plasticity in the food spectrum, higher mobility (vertical and horizontal) and live longer than SPF. For these reasons, they are considered to be less influenced by environmental variability, and have been observed to increase when SPF decline in high productive areas (Bas et al. 1995). However, in the Mediterranean Sea information on the biology, ecology and stock assessment of these species is very limited (Giannoulaki et al. 2017). The need to generate additional information on the status of these four species has been recently highlighted (STECF 2016), while in the Adriatic Sea, a decline of Atlantic mackerel has been reported (Meneghesso et al. 2013).

Adult MPF prey mainly on macro-zooplankton, icthyoplankton and small fish or molluscs (Stergiou & Karpouzi 2002). Whereas larval and juvenile stages prey mainly on zooplankton. Therefore, depending on the ontogenetic stage of MPF, these species can compete or directly interact with SPF. Atlantic mackerel (*Scomber scombrus*) is mainly a winter spawner with a peak of spawning in January and a long reproduction period from November to April (Zardoya et al. 2004, Meneghesso et al. 2013). Instead, the reproduction period of Atlantic chub mackerel (*Scomber colias*) is mainly between May and August (Cengiz 2012). Atlantic horse-mackerel (*Trachurus trachurus*) spawns between November and May and Mediterranean horse-mackerel (*Trachurus mediterraneus*) spawns in spring and summer, between May and August (Planas & Vives 1953, Karlou-Riga 2000, Raya & Sabatés 2015) (Figure 9).

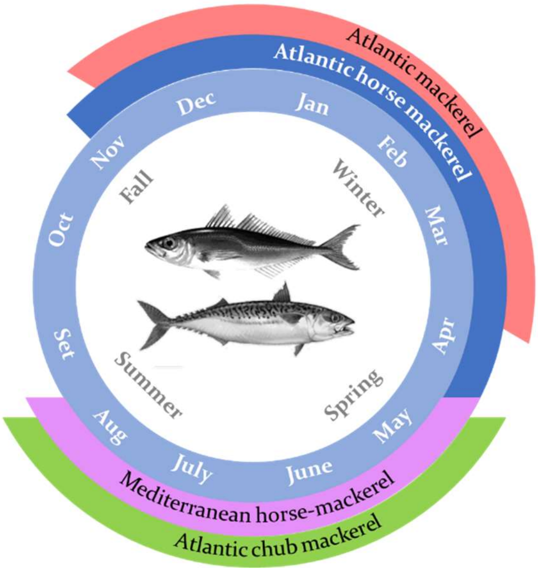

Figure 9. Reproduction period of Atlantic and Mediterranean horse-mackerel, Atlantic mackerel and Atlantic chub mackerel. Adapted from Quattrocchi et al 2017.

Current situation of SPF population

The last report on *The state of Mediterranean and Black Sea Fisheries*, has reported that the small pelagic fish stocks in the Mediterranean Sea show on average fishing mortality rates close to the target, except for sardine (FAO 2018b). In the northwestern Mediterranean Sea the most recently stock assessment of the geographical sub-area GSA 06 (Spanish coast) approved by the General Fisheries Comission of the Mediterranean (GFCM, FAO) indicated that the landings of sardine have been declining since the 90s and the stock of sardine is in overexplotation. In the case of anchovy, after a continuous decline in landings between 1994 and 2013, since 2014 the landings have been increasing in the GSA06 and the stock assessment approved by the GFCM indicated that the stock is sustainably exploited (Report SAC-WGSASP, 2018). However, in both species individuals of the older ages have disappeared from the population and body condition and growth have declined (Report SAC-WGSASP, 2018).

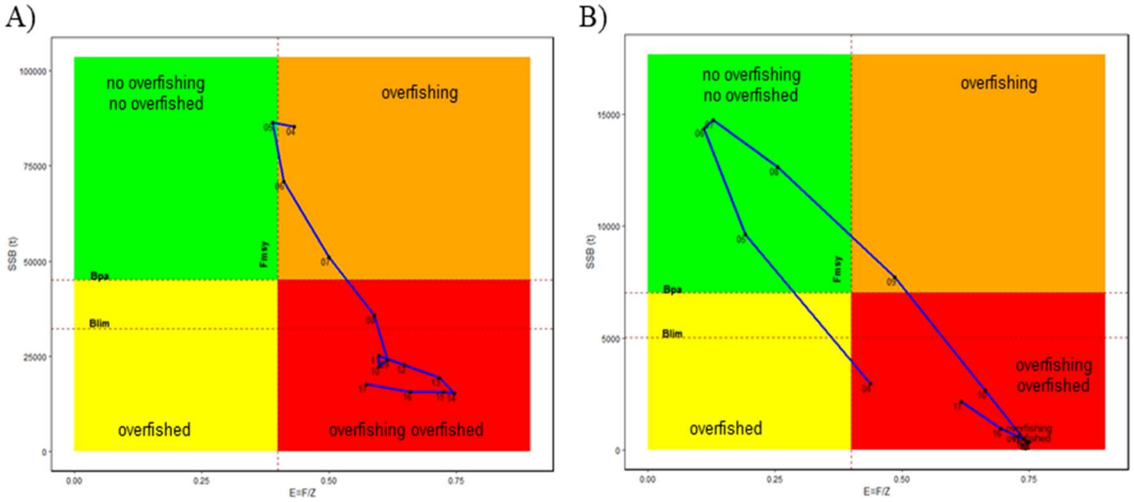

Figure 10. Kobe plot for stock assessment resuls of sardine (A) and anchovy (B) stock in GSA 06 using Blim, Bpa and E of Patterson as reference points (ICES 2017). With thhis methodology under this scenarios, both species are currently overexploited. Source: Ramírez et al. 2019.

Moreover, a recent study that used different stock assessment methods with different input parameter calculation, have suggested that the stocks of both species have been historically highly exploited or overexploited, and they are currently overfished (Ramirez et al. 2019, Coll and Bellido 2019; Figure 10).

Recent changes of SPF have been observed in the Mediterranean Sea, in parallel with an overall increase of fishing effort, changes in environmental variability and a decline of primary productivity (Piroddi et al. 2017). As mentioned above, fluctuations of SPF are common in nature in several part of the world. However, the present decline has lasted longer and has been extended not only to a decline in landings and biomass, but also a decline in body condition and growth with the disappearance of older age classes in the northwestern Mediterranean and in other areas of the Mediterranean Sea, in combination with a spatial expansion of round sardinella population (Van Beveren et al. 2014, Brosset et al. 2017, Coll & Bellido 2019, Saraux et al. 2019).

These declines in landings and the decrease in body condition and size have caused a social alarm and fishermen have been forced to stop or reduce their activity in Spain and France because the low economic viablility. In the Catalan coast several purse-seiners have stopped their activity and in the Gulf of Lions the fishing activity have almost stopped in the period 2016-2018 (Coll & Bellido 2019, Saraux et al. 2019; Figure 11).

PESCA

La captura de sardinas cae un 86% en siete años

Los pescadores califican la situación de dramática y reclaman medidas urgentes para evitar la desaparición del sector

Las capturas de sardina, las más bajas en 15 años

ECONOMIA ARENYS DE MAR - 29 març 2016 2.00 h

Poca sardina i seitó petit

- La flota d'encerclament del port d'Arenys pateix la crisi del peix blau amb la davalla captures de sardina i la reducció de la talla del seitó, que en devalua el preu
- El govern es compromet a encarregar un estudi per esbrinar-ne les causes

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El desplome de las capturas de sardinas pone en jaque a la flota de cerco

- Las cofradías catalanas han solicitado al Gobierno y la Generalitat medidas urgentes

Pescadores de la barca L'Avi Juanito seleccionan las capturas de pescado azul a su llegada al puerto de Tarragona.

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Viaje al fondo de la crisis pesquera

La sobreexplotación, el cambio climático y hasta la recuperación de los depredadores pueden estar det declive de sardinas y boquerones

ANTONIO MADRIDEJOS / BARCELONA
Miércoles, 09/03/2016 a las 22:01 CET

Embarcacion de cerco en el puerto de Tarragona. / JOAN REVILLAS

Figure 11. Information published in Spanish newspapers indicating the decline in landings of sardine and in body condition and size of anchovy in Catalonia and Valencia regions. Source: La Vanguardia, El Punt Avui, El Periodico Newspapers in 2016.

The decline in abundance, biomass and body condition has been linked to (i) environmental changes that can directly influence annual recruitment, growth and condition of organisms, and (ii) human pressures that can directly influence on recruitment overfishing or indirectly favoring or harming other species that compete or prey on SPF. Several hypotheses to explain these changes have been formulated (e.g. Sabatés et al. 2006, Maynou et al. 2014, Saraux et al. 2019; see Table 2).

The role of climate change in affecting the composition of plankton has been suggested to explain sardine and anchovy declines (Brosset et al. 2015, Brosset, Lloret, et al. 2016; Table 2). Moreover, competition between pelagic organisms that feed on zooplankton and can also prey on early life phases of SPF, such as the potential interspecific competition for food between the expanding round sardinella and the other SPF or the increase of jellyfish has also been proposed as a potential driver (Sabatés et al. 2006, Purcell et al. 2014, Tilves et al. 2016; Table 2).

Another hypothesis suggests that SPF species could be maturing at smaller body sizes, while allocating more energy to reproduction than growth in response to the impact of climate and fisheries. This could lead to individuals that spend more energy reproducing than growing and thus are smaller and are in a poorer condition (Silva et al. 2006, Pethybridge et al. 2013; Table 2).

Other potential causes of declines could be the recent recovery of some pelagic predators such as bluefin tuna, *Thunnus thynnus* (Van Beveren et al. 2017). Changes of anchovy and sardine landings and abundances have also been related with increases in fishing impact and the current high rates of exploitation (Palomera et al. 2007, STECF 2016, FAO 2018b). Lastly, the occurrence of pathogens, diseases or contamination has also been suggested as potential causes, although very limited information is available (Ferrer-Maza et al. 2016, Saraux et al. 2019; Table 2).

In order to understand how all environmental and human pressures can affect the dynamics of the system is essential to have a good knowledge on the (1) physiological ecology of the species to be able to predict changes in the life-history parameters and energy dynamics, (2) trophic interactions or “who eats who?”, (3) behavior ecology, and (4) the interconnectivity of the three of them. The need to combine all of this information and represent the complexity of marine ecosystems, requires the use of integrated models in order to understand the main patterns and trends (Box 4. Qualitative models of complex systems) (Fulton et al. 2003).

Table 2. Summary of the main (i) environmental and (ii) human driven hypotheses proposed in the literature to explain the observed current declines of sardine and anchovy populations in the northwestern Mediterranean Sea. Source: adapted from Saraux et al. 2019.

	Hypotheses	**Narratives of the hypotheses**
(i) Environmental	Bottom-up: Changes in zooplankton composition with lower quality of food	Due to the increase of SST and other environmental changes, plankton phenology may have changed in favour of less energetic food at crucial times of SPF life history. Lower energy prey for SPF could be translated in changes in the energy invested in reproduction, maintenance and growth.
	Bottom-up/competition: Increase of gelatinous zooplankton	The increase of gelatinous zooplankton could create a competition for food with SPF, or could be a low-energetic prey for SPF, changing the ecological energetics of the food web.
	Competition: Northward expansion of round sardinella	Due to the increase in SST, round sardinella abundance increased northwards and could compete for food with sardine and anchovy.
	Reduction of the reproduction rate of sardine	Due to the increase in SST the reproduction rate of sardine may have reduced since sardine prefers cold waters for reproduction (Palomera et al. 2007)
(ii) Human	Top-down: Recovery of predators	The management plans to recover some pelagic predators such as Bluefin tuna (*Thunnus thynnus*) could have increased predation of SPF
	Top-down: Overfishing of SPF	The stock of sardine and anchovy are being overfished removing older ages.
	Contamination	Pollutants from human activity might have changed sardine and anchovy physiology, increasing fish mortality.
(iii) Other	Parasitism and diseases	An increase of parasitism, viruses and bacterias could affect the reproduction, condition and mortality of sardine and anchovy.

Box 4: Qualitative models of complex systems

Quantitative predictions of food-web responses are not always needed to predict the direction of change. Instead, qualitative analysis of a system may help in predicting its general dynamics (Lassalle et al. 2014). Compared to other types of models, qualitative modeling sacrifices precision for realism and generality and is especially useful when the basic relationships between variables are understood, but when precise and detailed data are not available (Dambacher et al. 2009, Figure 1).

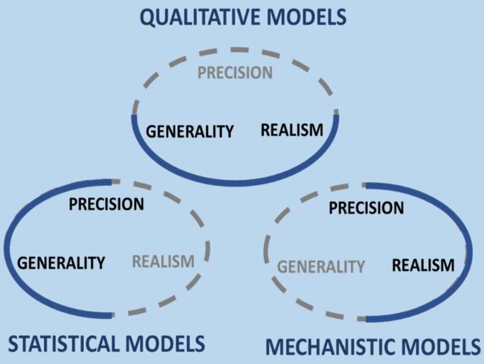

Figure 1. Scheme for ecological models. Adapted from Dambacher personal figure and Levins (1966).

Qualitative models are typically drawn as diagrams (signed digraphs) with circles representing the ecological variables and lines representing positive (pointed arrow), negative (solid circle) or absence (no line) relationship between model variables (i.e. Prey-predator interactions; Figure 2a). Then, these signed digraphs are converted into community matrices (Figure 2b).

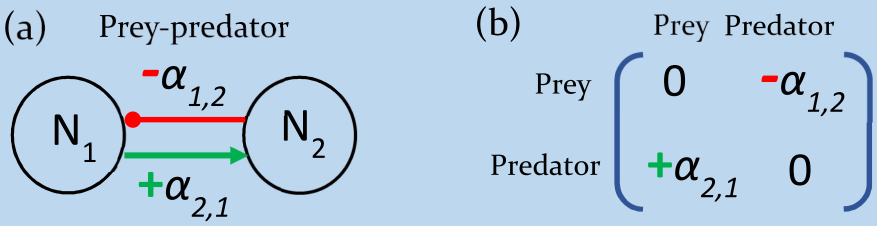

Figure 2. (a) Signed digraph model of a prey predator interaction. (b) Community matrix representing the prey predator interaction.

From the signed digraph the feedback properties of the system can be examined. The analysis of the matrix using algebra functions are mainly used to (1) test the stability of the system and (2) to predict the behaviour of the system response to a disturbance. Predictions of the direction of a perturbation (+, -, 0) are obtained by a summation of all direct and indirect effects. The summation of effects are obtained from the qualitative analysis of the inverse community matrix (Dambacher et al. 2002).

Study Area

The area of study where the present thesis was conducted is the continental shelf and upper slope associated with the Ebro River Delta (Figure 12), in the northwestern Mediterranean Sea. This is one of the most important spawning grounds of both anchovy and sardine (Bellido et al. 2008, Giannoulaki et al. 2011, 2013). Moreover, it is a hotspot of biodiversity and an essential area for the conservation of marine predators such as seabirds, turtles, cetaceans, tunas and others that prey on sardine and anchovy (Arcos et al. 2009, Coll et al. 2015, Tomas et al. 2001, Piante and Ody 2015). It is also an important area for the fishing activity, especially in relation with SPF (Palomera et al. 2007).

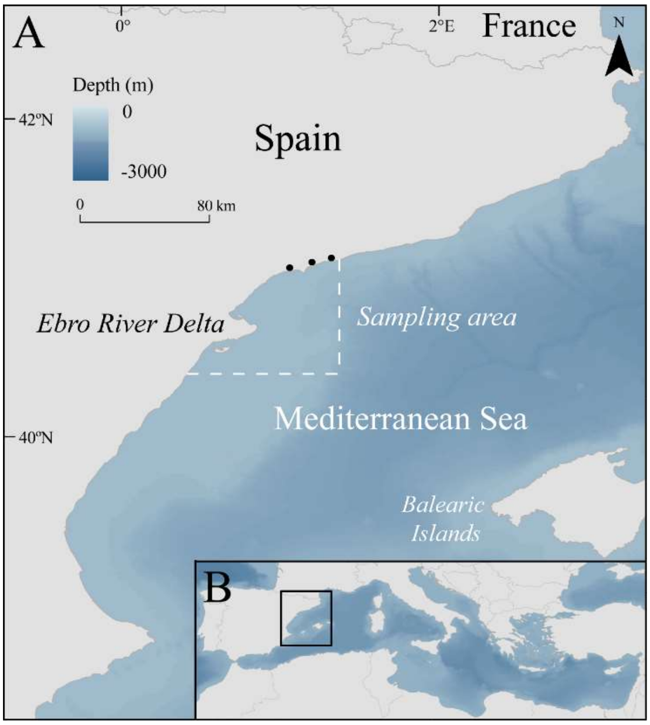

Figure 12. (A) Map of the study area where the individuals were collected. The sampling area (dashed line) and the fishing harbors, where most of the samples were landed, are indicated with (•). (B) Position of the study area in the Mediterranean Basin.

The Ebro delta continental shelf has a high environmental variability between seasons, with a period of stratification in summer and a mixing period in winter (Palomera et al. 2007). Although the Mediterranean Sea is considered an oligotrophic sea, in the Ebro Delta continental shelf the combination of the river runoff with wind mixing processes creates conditions similar to the upwelling zones (Lloret et al. 2004). The main current flow in the region is southwesterly along the edge of the continental shelf and is associated with a salinity gradient separating the low salinity waters on the continental shelf from the saltier water in the open sea (Font et al. 1988; Palomera, 1992). This current displays strong mesoescale activity (i.e. eddies) that helps to ensure better condition for egg and larval survival of SPF (Sabatés et al. 2013, Quattrocchi et al. 2017).

Sea surface temperature and primary production follow annual cycles characterized by strong seasonality. During winter, the sea surface temperature is at its lowest and the wind is stronger and more frequent, which induces an intense water mixing on the shelf leading to higher nutrient availability in the surface (Salat 1996, Lloret et al. 2004, Coll et al. 2010). As a consequence, in the late winter and spring there is a phytoplankton bloom. In late spring and summer the increase of the sea surface temperature produces a water column stratification and there is a reduction on nutrients in the photic zone (Figure 13). At this time, the inputs of nutrients to the surface are supplied mainly by the Ebro River outflow (Estrada 1996, Palomera et al. 2007). Then, in fall the sea surface temperature is higher than the air temperature and the surface mixed layer goes deeper by convection until the thermocline is destroyed in winter (Sabatés et al. 2007). In shelf areas where the bottom is shallower, such as the Ebro Delta continental shelf, convection is an important process that can sustain a high productivity during late winter. These contrasting environmental conditions with two types of nutrient supply into the surface, wind mixing in winter and river runoff in summer, in combination with the important mesoescale activity of the area, create optimal conditions of high productiviy for spawning of sardine and anchovy in the Ebro Delta continental shelf (Palomera 1992, Olivar et al. 2001).

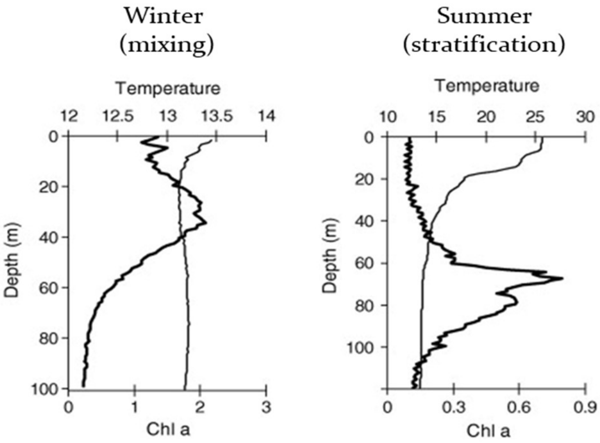

Figure. 13. Vertical profiles of temperature (thin line; C°) and Chlorophyll a (thick line; Chla, µg/l) during the mixed (left) and stratified periods (right). Source: Sabatés et al. 2007.

Seasonal changes in zooplankton composition and abundance have also been described in the NW Mediterranean Sea (Calbet 2001). Some studies have found higher zooplankton biomass after the late winter/ early spring phytoplankton bloom (Andreu & CM 1996, Fernández De Puelles et al. 2003). In general, copepods dominate the zooplankton community throughout the year, with a dominance of calanoid copepods during spring and winter (Calbet et al. 2001, Sabatés et al. 2007). In contrast, other groups present a clear pattern of seasonality. Cladocera and Appendicularia show high abundance in summer and Chaetognatha have a summer– autumn peak, whereas Cnidaria and Thaliacea are abundant in spring (Gili et al. 1988, Calbet 2001, Pascual 2016).

Although seasonal environmental variability is well documented and has been shown to affect the population dynamics of small pelagic fish, few studies have looked at potential changes in trophic interactions within the pelagic compartment (Lloret et al. 2001, 2004). To overcome this lack of knowledge, within this thesis a seasonal sampling in the study area was performed (Box 5: General sampling).

Box 5: General description of the sampling methodology

Data presented in this thesis were obtained with samples coming from monthly fisheries landings and from two oceanographic campaigns.

SPECIES SAMPLED

Small pelagic fish	Medium pelagic fish	Other competitors or predators of SMPF
European sardine	Atlantic horse-mackerel	Broadtail shortfin squid
European anchovy	Mediterranean horse-mackerel	European squid
Round sardinella	Atlantic mackerel	European hake
	Atlantic chub mackrel	Atlantic bonito

Individuals from fisheries were obtained mainly in the Tarragona harbour seasonally from Spring 2012 to Winter 2013, with exception of sardine and anchovy that were sampled monthly from April 2012 to March 2013.

50 individual per sampling were dissected in the lab and the following measures and tissues were collected:

- Total and gutted weight
- Total and standard length
- Sex, maturity stage and gonad weight
- Tissues: muscle, gonads, stomachs and individuals

In addition, two oceanographic campaigns of 15 days each were carried out in February 2013 and July 2013 in the research vessel Ángeles Alvariño. In these campaigns experimental bottom trawling and plankton nets were used.

Purse-seine landing SPF in the harbour of Cambrils (Tarragona)

Crew of the ECOTRANS oceanographic campaing in the RV Ángeles Alvariño

Objectives and Structure of the thesis

Despite all the scientific progress and knowledge that has been obtained in the last decades, there is still a need for an integrated and holistic understanding of the pelagic ecosystem in order to disentangle which of all hypotheses proposed or a combination of them is the most plausible explanation to the decline in biomass, landings, body condition and growth of sardine and anchovy in the northwestern Mediterranean Sea. Therefore, in this thesis I addressed different ecological aspects in order to investigate the ecological role of the main species in the pelagic compartment of the NW Mediterranean Sea, focusing on European anchovy and sardine, with the aim to contribute to the discussion of plausible causes of change. While the study of the ecological role of species comprises a wide array of components, in this thesis I focus in the study of trophodynamics, which is a fundamental aspect to understand the transfer of energy in the ecosystem (Figure 14; Bierwagen et al. 2018).

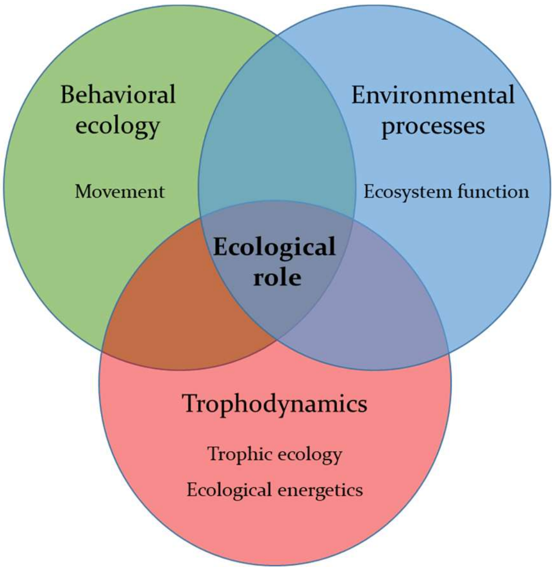

Figure 14. Diagram of areas of study that contribute to informing ecological roles of a species. Adapted from Bierwagen et al. (2018).

The general objective of this thesis is to improve the knowledge of the seasonal dynamics and functioning of the pelagic compartment focusing on the study of trophic relationships and energy dynamics of SMPF in the northwestern Mediterranean Sea.

The specific objectives of the thesis are:

1- To analyze the potential seasonal variability in the trophic relationships of the main species of SMPF within the pelagic compartment.

2- To evaluate ontogenetic changes in the trophic niche of SPF and potential competition between SPF.

3- To evaluate the energy content of SMPF that is available for higher trophic levels taking into account potential seasonal variability.

4- To characterize the energy allocation strategy of SPF related to the life-history traits in order to understand how environmental change could affect the populations of SPF.

5- To model alternative configurations of the pelagic food web and integrate all new results and the available knowledge to compare alternative hypotheses about how SPF species may have responded to different pressures.

To address these objectives, I combined analyses at species and community level. In the first part of the thesis (Part I: Chapter 1 and Chapter 2), I studied the trophic ecology of different species of the pelagic compartment using stable isotope and stomach content analysis. In a second part (Part II: Chapter 3 and Chapter 4), I studied the ecological energetics of SMPF using direct bomb calorimetry, morphological condition indexes and lipid content. These studies contributed to generate new key information about the biology and ecology of these marine resources of the northwestern Mediterranean Sea, that in combination with previous published study, I used to test main hypotheses of change in pelagic fish species of the northwestern Mediterranean Sea (Part III: Chapter 5) (Figure 15).

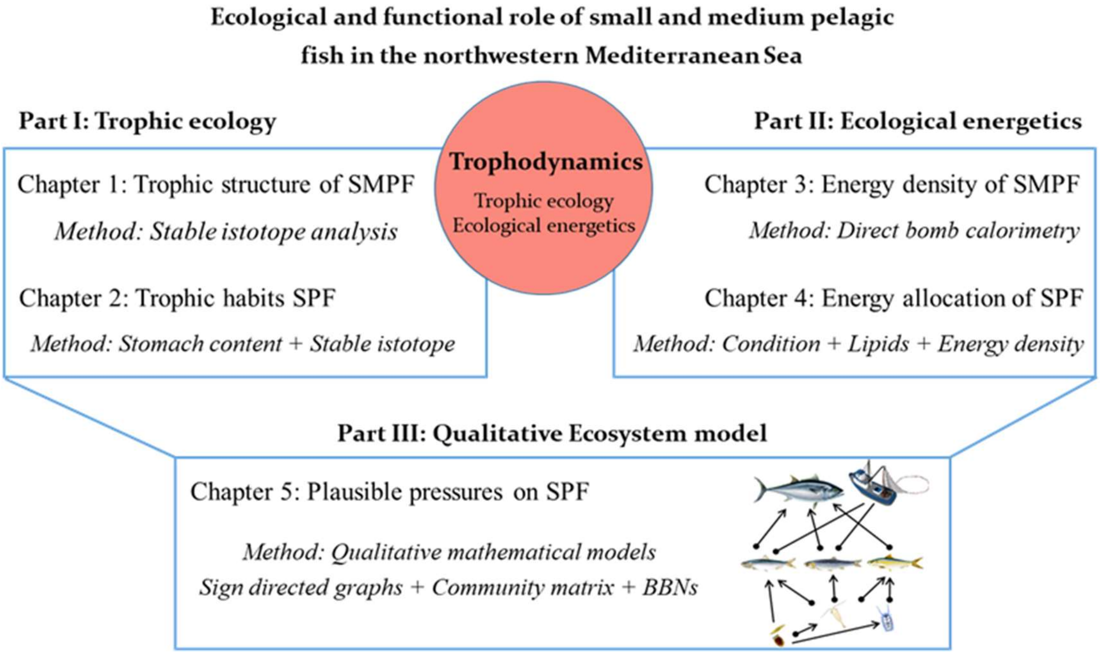

Figure 15. Structure of the present thesis. BBNs stand for Bayesian Beliefe Networks

Specifically, first I studied the seasonal variability of the ecological role of small and medium pelagic fish (**Chapter 1**) in order to investigate (1) which is the niche position of each group (SPF, MPF and Predators) with respect to each other, and (2) if there is a variation of the trophic relationships among SPF and MPF between seasons. I used stable isotope analysis and different trophic community metrics (Box 2). This chapter was published in a peer-reviewed journal: ***Albo-Puigserver, M.**, Navarro, J., Coll, M., Layman, C., Palomera, I., 2016. Trophic structure of pelagic species in the northwestern Mediterranean Sea. **Journal of Sea Research** 117: 27-35.*

Secondly, I investigated the potential competition between SPF at different ontogenetic stages. To do so I used stomach content analysis of round sardinella and stable isotope analysis of juveniles and adults of sardine, anchovy and round sardinella (**Chapter 2**) (Box 1 and Box 2). This chapter was published in a peer-reviewed journal: ***Albo-Puigserver, M.**, Borme, D., Coll, M., Tirelli, V., Palomera, I., Navarro, J., 2019. Trophic ecology of range-expanding round sardinella and resident sympatric species in the northwestern Mediterranean. **Marine Ecology Progress Series** 620: 139-154.*

From an energetic point of view, I assessed the seasonal energetic variability of pelagic species (**Chapter 3**) in order to investigate (1) which are the pelagic species with higher energy density and therefore could be considered higher prey quality for predators, and (2) if there is a variation of the seasonal energy density. To study the ecological energetics of the pelagic compartment, I used direct bomb calorimetry (Box 3). This chapter was published in a peer-reviewed journal: ***Albo-Puigserver, M.**, Muñoz, A., Navarro, J., Coll, M., Pethybridge, H., Sánchez, S., Palomera, I., 2017. Ecological energetics of forage fish from the Mediterranean Sea: seasonal dynamics and interspecific differences. **Deep Sea Research II - Topical Studies in Oceanography** 14:0 74-82.*

To better understand the energy allocation strategy of sardine and anchovy I used different measurements of condition (**Chapter 4**). I investigated seasonal variability in the condition of both species and energy allocated to reproduction maintenance and growth using morphometric measures (condition factor such as relative condition and gonadosomatic index), chemical tracers (lipids) and direct energetic measures (oxygen bomb calorimetry) (Box 3). Moreover, the suitability of each method to evaluate changes in body condition was assessed. This chapter is prepared to be submitted to a peer reviewed journal: ***Albo-Puigserver, M.***, *Sánchez, S., Coll, M., Bernal, M., Navarro, J., Sáez-liante, R., Palomera, I. Year-round energetic dynamics of anchovy and sardine in the northwestern Mediterranean Sea.*

Finally, in order to test the different main hypothesis of SPF population changes, I used qualitative mathematical ecological models to investigate the most plausible explanation(s) to the observed declines in sardine and anchovy and the expansion of round sardinella (**Chapter 5**). This study used the newly generated information on the energy dynamics and the trophic relationships between the main species in the pelagic compartment obtained during this thesis, in addition to information available from the literature. This chapter was published in a peer-reviewed journal: *Coll, M.,* ***Albo-Puigserver, M.****, Navarro, J., Palomera, I., Dambacher, J., 2019. Who is to blame? Plausible pressures on small pelagic fish population changes in the NW Mediterranean Sea.* ***Marine Ecology Progress Series*** *617-618: 277-294.*

PART I

Trophic Ecology

Chapter 1

Trophic structure of pelagic species in the northwestern Mediterranean Sea

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Abstract

Ecological knowledge of food web interactions within pelagic marine communities is often limited, impairing our capabilities to manage these ecologically and economically important marine fish species. Here we used stable isotope analyses to investigate trophic interactions in the pelagic ecosystem of the northwestern Mediterranean Sea during 2012 and 2013. Our results suggest that European sardine, *Sardina pilchardus*, and anchovy, *Engraulis encrasicolus*, are consumers located at relatively low levels of the pelagic food web. Unexpectedly, the round sardinella, *Sardinella aurita*, appeared to be located at a higher trophic level than the other small pelagic fish species, although previous studies found similarity in their diets. Isotope data suggested that trophic niches of species within the genera *Trachurus* spp. and *Scomber* spp., were distinct. Atlantic bonito *Sarda sarda*, European hake *Merluccius merluccius* and European squid *Loligo vulgaris*, appeared to feed at higher trophic levels than other species. Despite some intraspecific seasonal variability for some species, community trophic structure appeared relatively stable through the year. These data provide an important step for developing models of food web dynamics in the northwestern Mediterranean Sea.

Keywords: Stable isotopes; Pelagic fish; Isotopic niche; Food web; Community structure; Seasonal; Trophic segregation.

1.1 Introduction

Marine pelagic fisheries account for 26% of the world's total protein consumption (Tacon and Metian, 2009). With exploitation of these stocks increasing, there is a need to understand how the trophic structure may be shifting in pelagic systems (Pikitch et al., 2014). Yet, relative to coastal ecosystems, we have less knowledge of pelagic food webs and the trophic role of pelagic species (Miller et al., 2010). This is particularly true for small- and medium-sized pelagic fishes, which are ecologically and economically important species in marine ecosystems worldwide and represent >50% of the total landings in the Mediterranean Sea (Cury et al., 2000; Lleonart and Maynou, 2003).

Small pelagic fish species, such as *Sardina pilchardus* (European sardine) and *Engraulis encrasicolus* (European anchovy), account for significant biomass at intermediate trophic levels of Mediterranean Sea food webs and are the main prey resource for several marine predators (Coll et al., 2006; Cury et al., 2000; Pikitch et al., 2012, 2014). Medium pelagic fishes, such as mackerels and horse-mackerels, are also abundant in many pelagic food webs (Juan-Jordá et al., 2013; Lleonart and Maynou, 2003). These species can be prey species for higher trophic levels, as well as having top-down effects on small pelagic fish populations (Bayhan et al., 2013; Meneghesso et al., 2013; Trenkel et al., 2014).

Previous studies have described food web dynamics of the pelagic ecosystem in the Mediterranean Sea (see Bănaru et al., 2013; Coll et al., 2006; Corrales et al., 2015); however, little information about specific trophic interactions between small- and medium-sized pelagic fishes has been published. Likewise, although seasonal environmental variability has been shown to affect the population dynamics of small pelagic fishes, few studies have taken into account how this could affect trophic interactions (França et al., 2011; Lloret et al., 2001, 2004; Palomera et al., 2007). In this study we used stable isotope analysis to describe the overall community structure and examine the potential seasonal shifts in trophic interactions of 11 abundant pelagic species in the northwestern Mediterranean Sea. The main objectives were to analyze the specific trophic relationships between species and to compare the relative niche positions among seasons.

1.2 Material and methods

Study area

The study was conducted in the continental-shelf and upper slope area associated with the Ebro River Delta, from Cape Salou to Castelló de la Plana (Fig. 1; northwestern Mediterranean Sea). As a consequence of particular oceanographic conditions, including vertical mixing and river discharges, this area is an important fishing ground in the Mediterranean Sea (Coll et al., 2006; Lloret et al., 2004; Navarro et al., 2016). Moreover, it is an important area for threatened animals, including Balearic shearwater, *Puffinus mauretanicus*, Audouin's gull, *Larus audouinii* and loggerhead turtle *Caretta caretta* as well as other predators that also prey on small pelagic fishes (Arcos et al., 2009; Coll et al., 2015; Tomas et al., 2001). From May to October the ecosystem is characterized by a distinct thermocline and stratification of the water column, resulting in a reduction of nutrients in the photic zone (Salat, 1996). During the stratified season, riverine inputs are the main source of nutrients at the surface (Palomera et al., 2007; Salat et al., 2002). In contrast, from November to April, the water temperature is lower and the water column mixed, leading to higher nutrient availability at the surface (Salat et al., 2002).

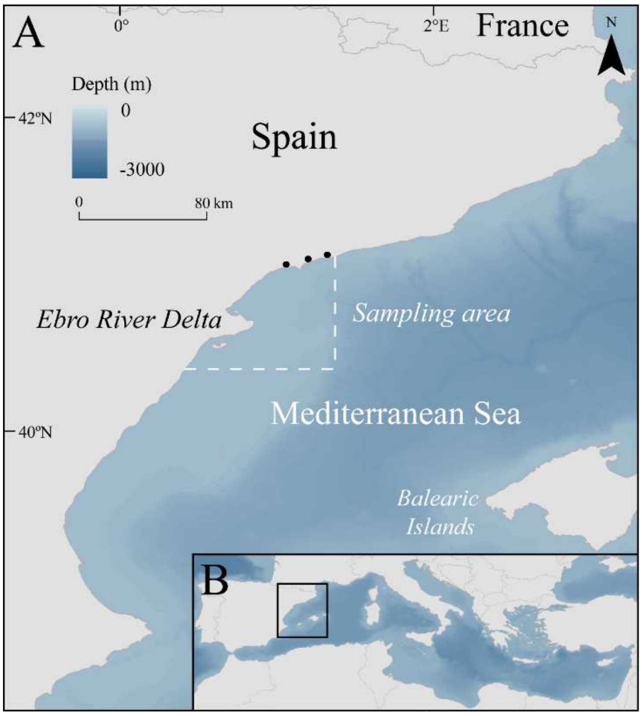

Fig. 1. (A) Map of the study area where the individuals were collected on the Ebro Delta continental shelf, northwestern Mediterranean. The sampling area (dashed line) and the fishing harbors where most of the samples were landed are indicated with (●). (B) Position of the study area in the Mediterranean Basin.

Sampling

We sampled eleven species of small and medium pelagic fishes, squids and potential predators of small pelagic fishes (see Table 1). We only sampled adult individuals to avoid potential ontogenetic differences in the isotopic values, since fish species often have ontogenetic niche shifts (Bode et al., 2004; Chouvelon et al., 2012). A total of 443 individuals were collected (spring 2012, summer 2012, fall 2012 and winter 2013) from commercial vessels of the harbors of Tarragona, Torredembarra and Cambrils (Fig. 1), as well as from an experimental oceanographic cruise in winter 2013 (ECOTRANS Project, Institut de Ciències del Mar, CSIC). All individuals were immediately frozen after capture and stored at –20 °C and then the morphological measurements (total body weight and total body length) and tissue collection were conducted in the laboratory. During the experimental oceanographic cruise in winter 2013, samples of microplankton were collected with a calVET net (53–200 µm) and frozen and stored at –20 °C.

Table 1. Main prey of focal species as based on previous reports in the Mediterranean Sea.

Species name	**Main prey**	**References**
Engraulis encrasicolus	Copepods, cladocerans	Costalago et al., 2012; Tudela and Palomera, 1997
Sardina pilchardus	Copepods, cladocerans, diatoms	Costalago and Palomera, 2014; Nikolioudakis et al., 2012
Sardinella aurita	Copepods, decapods larvae, fish larvae	Karachle and Stergiou, 2014; Lomiri et al., 2008
Trachurus mediterraneus	Copepods, euphasiids, fish	Bayhan et al., 2013; Yankova et al., 2008
Trachurus trachurus	Copepods, euphasiids, fish	Jardas et al., 2004; Šantić et al., 2005
Scomber scombrus	Euphasiids, decapod larvae, fish	Olaso et al., 2005
Scomber colias	Copepods, mysids, decapod larvae, fish	Castro, 1993; Keč et al., 2012
Illex coindetii	Fish, crustaceans	Martínez et al., n.d.; Rosas-Luis et al., 2014
Loligo vulgaris	Fish, crustaceans, cephalopods	Coelho et al., 1997; Valls et al., 2015
Merluccius merluccius	Benthopelagic and pelagic fish, decapods, euphausiids	Bozzano et al., 1997; Cartes et al., 2004
Sarda sarda	Small pelagic fish	Campo et al., 2006; Navarro et al., in press

Stable isotope analyses

Over the last few decades, stable isotope analyses have been broadly used to study the structure of food webs and trace energy and mass flows in ecosystems Layman et al., 2012). Particularly, ^{13}C and ^{15}N are stable isotopes commonly used to study trophic pathways. $\delta^{13}C$ may vary substantially among primary producers, but shows little change from prey to consumers; alternatively, $\delta^{15}N$ reflects stepwise enrichment with each trophic level. Therefore, $\delta^{13}C$ is often used as a proxy of the original source of dietary carbon and $\delta^{15}N$ as a proxy of relative trophic position (Layman et al., 2012).

A small portion of the dorsal muscle from fish species, and of the mantle from squid species, was dissected from each individual. All samples were freeze-dried, powdered and 0.28–0.33 mg of each sample was packed into tin capsules. Isotopic analyses were performed at the Laboratory of Stable Isotopes of the Estación Biológica de Doñana (www.ebd.csic.es/lie/index.html). Samples were combusted at 1020 °C using a continuous flow isotope-ratio mass spectrometry system (Thermo Electron) by means of a Flash HT Plus elemental analyser interfaced with a Delta V Advantage mass spectrometer. Stable isotope ratios were expressed in the standard δ-notation (‰) where $\delta^{13}C$ or $\delta^{15}N = ([R_{sample} / R_{standard}] - 1) \cdot 1000$ where R is ^{13}C:^{12}C or ^{15}N:^{14}N relative to Vienna Pee Dee Belemnite ($\delta^{13}C$) and atmospheric N^2 ($\delta^{15}N$). Based on laboratory standards, the measurement error was ±0.1 and ±0.2 for $\delta^{13}C$ and $\delta^{15}N$, respectively. Following Post et al. (2007), a correction to the $\delta^{13}C$ values was made to account for the presence of lipids on individuals with a C:N ratio higher than 3.5. Three species of the family Scombridae required this correction (5 individuals of S. scombrus, $\Delta\delta^{13}C = 1.11 \pm 0.34$; 4 of *S. colias*, $\Delta\delta^{13}C = 1.64 \pm 0.85$; and 8 of *S. sarda*, $\Delta\delta^{13}C = 1.56 \pm 0.19$).

Estimated trophic level

To estimate the trophic level (TL) of each individual based on isotopic values we used the equation proposed by Vander Zanden and Rasmussen (2001):

$$TL_{consumers} = TL_{basal} + (\delta^{15}N_{consumer} - \delta^{15}N_{basal}) / \Delta\delta^{15}N$$

where $\delta^{15}N_{consumer}$ and $\delta^{15}N_{basal}$ were, respectively, the $\delta^{15}N$ values of each individual sampled and the $\delta^{15}N$ values of microplankton sampled in the oceanographic cruise in winter 2013 (ECOTRANS Project, Institut de Ciències del Mar, CSIC) ($\delta^{15}N_{basal} = 3.25 \pm$

0.62). Microplankton samples for other seasons were not available. We applied a basal trophic level (TL_{basal}) of 1.5 assuming that the microplankton is mostly composed by phytoplankton (primary producers; TL = 1) and micro- and mesozooplankton (typically primary consumers; TL = 2) (Costalago et al., 2012). Due to the lack of specific diet tissue discrimination factors associated with trophic transfers ($\Delta\delta^{15}N$) for the species studied in the present study, we used a conventional $\Delta\delta^{15}N$ of 3.4 (Post, 2002) to keep our results comparable with previous works in the northwestern Mediterranean Sea (e.g., Polunin et al., 2001; Valls et al., 2014; Costalago et al., 2012). We are aware that using a single isotopic baseline and $\Delta\delta^{15}N$ induces sources of potential bias into our data set. Yet, we are not attempting herein to make definitive estimates of trophic positions. We instead are interested in relative comparisons among species and to identify apparent general patterns in trophic structure (see Layman et al., 2005 for a similar general approach). We hope that these preliminary data will help generate new hypotheses that can be more rigorously tested with refined baseline values and species-specific tissue turnover data.

Seasonal variability

Differences in $\delta^{15}N$ and $\delta^{13}C$ values among seasons for each species were tested using one-way semi-parametric permutation multivariate analyses of variance tests (PERMANOVA test) on the Euclidean distance matrix (Anderson et al., 2008). PERMANOVA allows for the analysis of complex designs (multiple factors and their interaction) without the constraints of multivariate normality, homoscedasticity, and when there are a greater number of variables than in traditional ANOVA tests. The method calculates a pseudo-F statistic directly analogous to the traditional F-statistic for multifactorial univariate ANOVA models, using permutation procedures to obtain P-values for each term in the model (Anderson et al., 2008). In the case of a significant result, pairwise tests were performed. PERMANOVA tests were carried out with PRIMER-E 6 software (Clarke and Gorley, 2006). The significance level for all tests was adopted at $P<0.05$.

Trophic niche width

To provide insight into species' trophic niche width, and to estimate the degree of isotopic niche overlap between species, we calculated Bayesian isotopic ellipse areas corrected by sample size (SEA_B) (Jackson et al., 2011). This metric represents a measure of the core isotopic niche with higher values indicating broader trophic niche breadth (Layman et al., 2012). Isotopic standard ellipse areas and their overlap were calculated using the routine Stable Isotope Bayesian Ellipses in the SIAR library (SIBER; Jackson et al., 2011).

The isotopic niche position (centroid location) of the species was evaluated to determine whether isotopic niche position differed between species. To obtain measures of central tendency of each species we used nested linear models and residual permutation procedures. Two species can be assumed to occupy a different isotopic niche position if the Euclidian distance is significantly greater than zero (for additional details and R code for the test see Turner et al., 2010).

We also used the isotope data to explore potential predators on the anchovy and sardine. All $\delta^{15}N$ and $\delta^{13}C$ values for each sardine and anchovy species were corrected by diet tissue discrimination factors of +3.4 and +1.3 for $\delta^{15}N$ and $\delta^{13}C$ values, respectively (Post, 2002). The convex hull polygon containing all the corrected $\delta^{15}N$ and $\delta^{13}C$ isotopic values of sardine and anchovy was then plotted. Predator species that had an ellipse overlap with a convex hull polygon were assumed to be potential predators of sardine and anchovy.

1.3 Results

Trophic structure

Across sampling periods, the relative position of species appeared fairly consistent; there were no major shifts in the relative position of species across seasons. *S. pilchardus* and *E. encrasicolus* had the lowest estimated trophic levels with the lowest $\delta^{15}N$ and $\delta^{13}C$ values and mean estimated trophic levels in both species of 2.9 ± 0.1 (Table 2; Fig. 2). *S. aurita* had higher $\delta^{15}N$ and $\delta^{13}C$ values than the other two small pelagic fishes (a mean estimated trophic level of 3.2 ± 0.1; Table 2; Fig. 2). Mackerels and horse mackerels, *T. trachurus* and *S. colias* both had a mean estimated trophic level of 3.3 ± 0.2, while *T. mediterraneus* and *S. scombrus* had an estimated trophic level of 3.6 ± 0.2 and 3.6 ± 0.1, respectively. The two squid species seemed to have different isotopic niches, with *I. coindetii* having lower values of δ^{15}N and δ^{13}C than *L. vulgaris* (estimated TL = 3.2 ± 0.3 for *I. coindetii* and TL = 3.8 ± 0.2 for *L. vulgaris*; Fig. 2; Table 2). *M. merluccius*, *S. sarda* and *L. vulgaris* had the highest δ^{15}N values of the species studied, with estimated trophic levels close to 4 (Table 2; Fig. 2).

Table 2. Mean and standard deviation of δ^{15}N and δ^{13}C values, total body length values (BL; cm) and estimated trophic level (TL) of small pelagic fish, medium pelagic fish, squids and large pelagic and demersal fish species during spring, summer, fall, and winter, along with the number of specimens sampled (n). Cells corresponding to *T. trachurus* not sampled in summer are indicated with nd (no data).

	Spring 2012					Summer 2012				
Species	n	δ^{15}N(‰)	δ^{13}C(‰)	BL (cm)	TL	n	δ^{15}N(‰)	δ^{13}C(‰)	BL (cm)	TL
E. encrasicolus	14	8.0±0.4	-19.3±0.3	13.9±1.9	2.9±0.1	10	8.1±0.3	-19.0±0.2	14.5±1.2	2.9±0.1
S. pilchardus	10	7.8±0.6	-19.6±0.6	17.9±0.6	2.8±0.2	11	8.1±0.3	-20.0±0.4	15.7±1.2	2.9±0.1
S. aurita	10	8.7±0.4	-18.7±0.2	23.6±1.5	3.1±0.1	14	9.0±0.4	-18.7±0.1	20.7±3.5	3.2±0.1
T. trachurus	10	9.0±0.4	-19.0±0.3	24.5±1.9	3.2±0.1	nd	nd	nd	nd	nd
T. mediterraneus	17	10.0±0.6	-18.4±0.3	26.3±4.4	3.5±0.2	10	10.5±0.3	-18.2±0.3	33.4±2.0	3.6±0.1
S. scombrus	7	9.9±0.4	-18.6±0.3	31.5±5.3	3.5±0.1	19	10.6±0.4	-19.2±1.4^{a}	28.4±3.5	3.7±0.1
S. colias	11	9.0±0.3	-19.1±0.6	33.4±3.6	3.2±0.1	10	10.0±0.2	-18.4±0.3^{a}	35.1±1.6	3.5±0.0
I. coindetii	11	8.0±0.5	-19.2±0.3	17.1±1.7	2.9±0.1	4	9.6±0.3	-18.4±0.1	13.3±0.4	3.4±0.1
L. vulgaris	12	11.4±0.6	-18.0±0.3	20.3±3.6	3.9±0.2	6	11.3±0.4	-17.9±0.4	17.2±1.4	3.9±0.1
M. merluccius	7	10.7±0.6	-18.5±0.3	49.3±2.8	3.7±0.2	6	10.4±0.5	-18.3±0.5	43.2±6.2	3.6±0.2
S. sarda	13	10.8±1.0	-17.7±0.4	47.3±3.5	3.7±0.3	9	11.4±0.5	-17.9±0.8^{a}	47.7±7.5	3.9±0.1

	Fall 2012					Winter 2013				
Species	n	δ^{15}N(‰)	δ^{13}C(‰)	BL (cm)	TL	n	δ^{15}N(‰)	δ^{13}C(‰)	BL (cm)	TL
E. encrasicolus	10	8.1±0.2	-18.8±0.2	14.0±1.3	2.9±0.1	11	8.2±0.2	-19.3±0.2	14.0±0.8	3.0±0.1
S. pilchardus	8	8.2±0.3	-19.4±0.6	17.7±1.0	3.0±0.1	9	8.1±0.4	-19.2±0.4	12.9±0.5	2.9±0.1
S. aurita	10	9.2±0.2	-18.2±0.2	24.0±1.8	3.3±0.1	10	9.2±0.5	-18.5±0.1	23.2±1.1	3.2±0.1
T. trachurus	10	9.3±0.2	-18.6±0.1	24.4±1.9	3.3±0.1	10	9.4±0.8	-19.0±0.4	28.9±4.3	3.3±0.2
T. mediterraneus	19	10.3±0.6	-18.1±0.5	27.6±5.9	3.6±0.2	11	10.6±0.5	-18.4±0.5	29.6±4.5	3.7±0.1
S. scombrus	12	10.3±0.3	-18.6±0.5	31.2±3.1	3.6±0.1	12	9.9±0.5	-18.4±0.3	27.7±1.6	3.5±0.2
S. colias	7	9.9±0.3	-18.0±0.2	35.9±3.2	3.5±0.1	10	9.2±0.3	-18.3±0.5	33.9±3.0	3.2±0.1
I. coindetii	9	9.3±0.4	-18.2±0.3	15.2±1.4	3.3±0.1	12	9.7±0.7	-18.6±0.6	18.2±2.1	3.4±0.2
L. vulgaris	9	10.9±1.0	-17.3±0.6	20.5±2.0	3.8±0.3	12	11.2±0.8	-17.6±0.5	20.1±3.2	3.8±0.2
M. merluccius	8	10.4±1.0	-18.4±0.4	52.0±15.4	3.6±0.3	9	10.4±0.8	-18.4±0.4	39.1±6.3	3.6±0.2
S. sarda	5	10.8±1.3	-18.1±0.9^{a}	46.2±9.2	3.7±0.4	9	11.4±0.3	-18.0±0.7^{a}	44.5±1.0	3.9±0.1

[a] Indicates species with lipid corrected δ^{13}C values in the corresponding season.

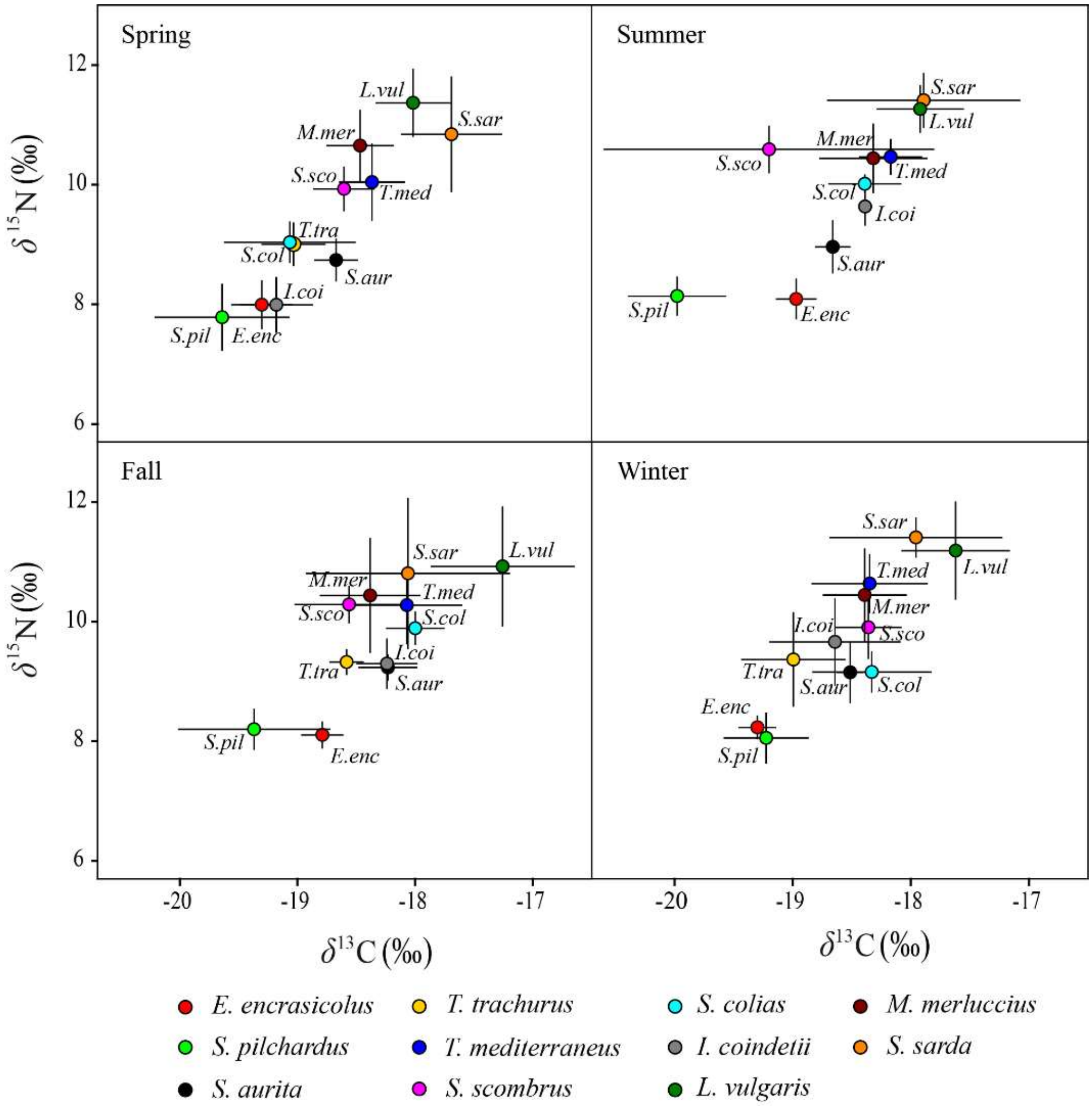

Fig. 2. Mean and standard deviation of δ¹³C and δ¹⁵N values of each species during spring, summer, fall, and winter.

Species seasonal variability

Only the predators *L. vulgaris*, *M. merluccius* and *S. sarda* did not have distinct seasonal differences in δ^{15}N and δ^{13}C values (Table 3). Differences for other species were small suggesting no major seasonal shifts in trophic structure. For each species, the range of seasonal variation was 0.2 to 0.7 for δ^{15}N and of 0.2 to 0.8 for δ^{13}C, with the exception of *S. colias* ($\Delta\delta^{15}$N = 1.0; $\Delta\delta^{13}$C = 1.1) and *I. coindetii* ($\Delta\delta^{15}$N = 1.7; $\Delta\delta^{13}$C = 1.0) (Fig. 3).

In most species the mean seasonal variability in the estimated trophic level was not >0.2. *I. coindetii* had the highest change, a mean difference of 0.5 between spring and the rest of the seasons (Fig. 3). Data for δ^{15}N, δ^{13}C, body length and estimated trophic level are reported in Table 2.

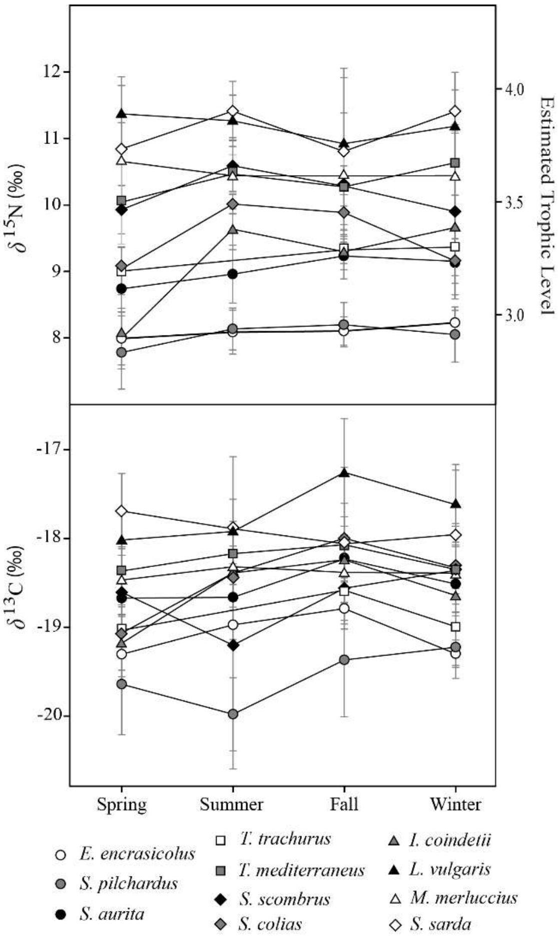

Fig. 3. Seasonal variation of the mean and standard deviation of δ^{15}N, estimated trophic level and δ^{13}C values by season of each focal species.

Table 3. PERMANOVA test results (Pseudo-F values) of significant differences for δ^{15}N and δ^{13}C values between seasons for each species. Pairs of means differing significantly ($P < 0.05$) by pairwise tests are indicated by the letters - seasons with the same letter were not significantly different. Cells corresponding to *T. trachurus* not sampled in summer are indicated with nd (no data). There were no differences among seasons for *L. vulgaris*, *M. merluccius* and *S. sarda*.

Species	Pseudo-F (p-value)	Spring	Summer	Fall	Winter
E .encrasicolus	6.32 (<0.01)	a	b	b	a
S. pilchardus	3.37 (<0.01)	a,b,c	a	b	c
S. aurita	5.07 (<0.01)	a	a,b	c	b,c
T. trachurus	2.90 (0.02)	a	nd	b	a,b
T. mediterraneus	2.58 (0.04)	a	a,b	a,b	b
S. scombrus	3.56 (<0.01)	a,b	a	a	b
S. colias	15.82 (<0.01)	a	b	c	d
I. coindetii	17.72 (<0.01)	a	b	b	b
L. vulgaris	1.97 (0.09)				
M. merluccius	0.15 (0.96)				
S. sarda	1.14 (0.35)				

Isotopic niche overlap

Isotopic niche width seemed larger for species positioned higher in the food web (Figure 4). The epipelagic fish *S. sarda* had the largest isotopic niche width, while *E. encrasicolus* and *S. aurita* had relatively small ones (Figure 4). The four medium pelagic fish species and *M. merluccius* had similar isotopic niche widths across seasons, with the exception of *T. trachurus* and *S. scombrus* which had a larger isotopic niche width in winter and in summer, respectively (Figure 4). For squid, the niche width of *L. vulgaris* was larger than that of *I. coindetii* (Figure 4).

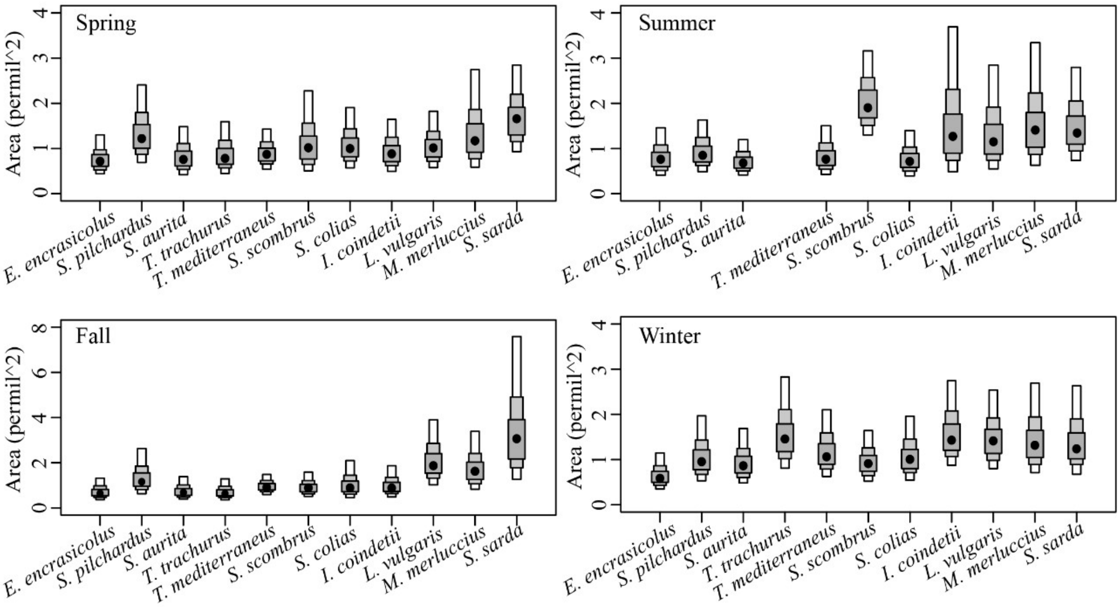

Fig. 4. Density plot of the standard ellipse areas of each species and for each season. Black points correspond to the mean standard ellipse area, while boxes show 50%, 75% and 95% credible intervals for mean estimates.

Most species had some trophic niche overlap with at least one other species (Table 4; Fig. 5). However, the isotopic niche position between species overlapping was significantly different, suggesting distinct trophic roles within this food web (Table 4). In general, summer had the least, and winter the most, niche overlap between species pairs. In spring and winter, *E. encrasicolus* and *S. pilchardus* had a similar isotopic niche position, whereas they appeared rather different during summer. *S. aurita* was distinct from the other small pelagic fish species in all seasons (Table 4; Fig. 5).

Among the four species of medium pelagic fishes, *T. trachurus* had a high isotopic niche overlap with *S. colias* in spring, while *T. mediterraneus* overlapped substantially with *S. scombrus* in all seasons (Table 4; Fig. 5). The two squid species had distinct isotopic niches across all seasons. *I. coindetii* had high isotopic niche overlap with several species of small and medium pelagic fishes, depending on the season, whereas *L. vulgaris* had amore similar niche position to *S. sarda* (Table 4; Fig. 5).

Table 4. Percentage values of overlap of the Bayesian isotopic ellipses between each pair of species for each season. Overlaps of 0 percent are indicated with a dash. Pairs with isotopic niche positions that were not significant differently (see Methods; P>0.05) are indicated with a (*). Cells corresponding to T. trachurus not sampled in summer are indicated with nd (no data).

	S.pilchardus	*S.aurita*	*T.trachurus*	*T.mediterraneus*	*S.scombrus*	*S.colias*	*I.coindetii*	*L.vulgaris*	*M.merluccius*	*S.sarda*	*S.pilchardus*	*S.aurita*	*T.trachurus*	*T.mediterraneus*	*S.scombrus*	*S.colias*	*I.coindetii*	*L.vulgaris*	*M.merluccius*	*S.sarda*
E.encrasicolus	52*	-	-	-	-	-	63*	-	-	-	-	-	nd	-	-	-	-	-	-	-
S.pilchardus		-	-	-	-	-	33*	-	-	-		-	nd	-	-	-	-	-	-	-
S.aurita			5	-	-	23	-	-	-	-			nd	-	-	-	-	-	-	-
T.trachurus				-	-	65*	-	-	-	-				nd	nd	nd	nd	nd	nd	nd
T.mediterraneus					42*	-	-	-	-	-					14	-	-	-	46*	-
S.scombrus						-	-	-	17	-						-	-	2	15	-
S.colias							-	-	-	-							9	-	18	-
I.coindetii	Spring							-	-	-	Summer							-	-	-
L.vulgaris									13	33									8	43*
M.merluccius										-										-
E.encrasicolus	20	-	-	-	-	-	-	-	-	-	24*	-	-	-	-	-	-	-	-	-
S.pilchardus		-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-
S.aurita			6	-	-	-	63*	-	-	-			-	-	13	31*	14	-	-	-
T.trachurus				-	-	-	7	-	-	-				-	7	10	57*	-	11	-
T.mediterraneus					15	-	-	10	4	17					14	-	12	15	61*	3
S.scombrus						-	-	-	41*	14						5	47*	-	32*	-
S.colias							5	1	-	3							26	-	-	-
I.coindetii	Fall							-	-	1	Winter							-	31	-
L.vulgaris									-	30*									2	40*
M.merluccius										20										6

Following correction for diet-tissue discrimination factors, the convex hull of anchovy and sardine partially overlapped the isotopic niche of *S. sarda*, *L. vulgaris*, and *M. merluccius* in almost all seasons, suggesting that these may be the most likely predators on sardine and anchovy (Figure 5).

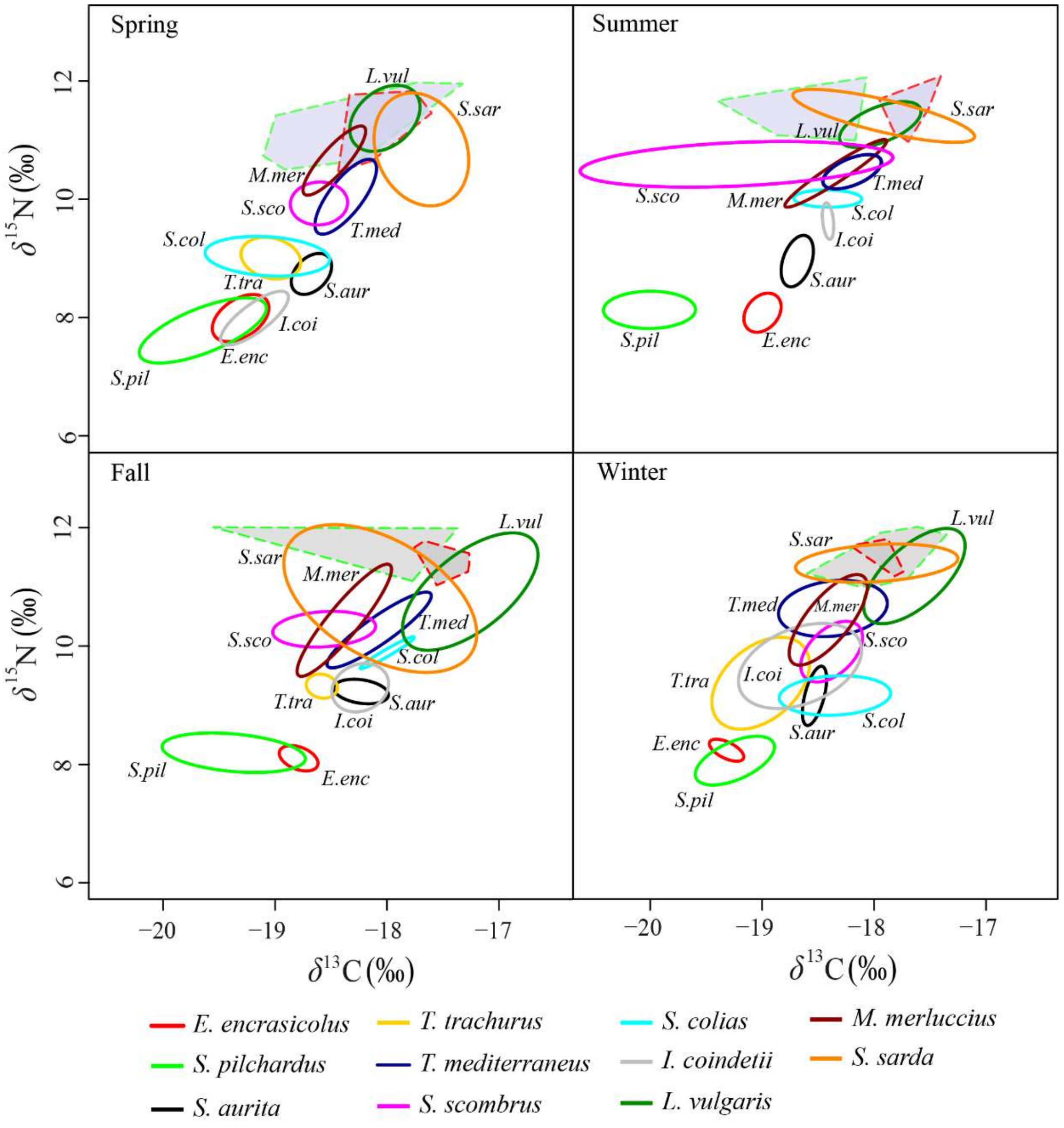

Fig. 5. Standard ellipses for each species during spring, summer, fall, andwinter. The grey convex-hull represents the total isotopic area of potential predators of *Engraulis encrasicolus* (red dashed line) and *Sardina pilchardus* (green dashed line), accounting for prey–predator isotopic fractionation of 3.2‰ and 1.3‰ for δ^{15}N and δ^{13}C values, respectively.

1.4 Discussion

Consistent with the fact that *S. pilchardus* and *E. encrasicolus* are plankton feeders (Costalago and Palomera, 2014; Costalago et al., 2012; Tudela and Palomera, 1997), our results indicated that they had the lowest trophic position of all the species studied. Similar to previous studies (Cardona et al., 2015; Costalago et al., 2012; Le Bourg et al., 2015), *S. pilchardus* and *E. encrasicolus* showed a similar trophic niche position with a large overlap of their core isotopic niches. However, during summer, the species seemed to partition resources, occupying different positions along the $\delta^{13}C$ axis. This is consistent with previous studies in the northwestern Mediterranean that have found that in summer *S. pilchardus* preyed more on cladocerans and appendicularians (with depleted values of $\delta^{13}C$), while *E. encrasicolus* prey more on copepods (Costalago et al., 2012, 2015; Tudela and Palomera, 1997). Moreover, lower dietary overlap between sardine and anchovy has been observed in areas and periods of high biological productivity (Chouvelon et al., 2015; Jemaa et al., 2015). Thus, the higher trophic niche segregation observed between sardine and anchovy in summer may be explained by greater variety of food resources, largely as a consequence of the Ebro River discharges that contribute to the early spring phytoplankton bloom.

The larger isotopic niche width of *S. pilchardus* suggests that it may feed on a broader range of prey. Previous studies showed that *S. pilchardus* is an opportunistic feeder with a greater filtering capacity than *E. encrasicolus* due to a higher number of gill-rakers that allow *S. pilchardus* to prey on diatoms, whereas diatoms have not been reported in the diet of *E. encrasicolus* at adult stages (Costalago and Palomera, 2014; Costalago et al., 2014; Pethybridge et al., 2014). *S. aurita* is a species that has been described as a plankton feeder similar to *S. pilchardus* and *E. encrasicolus* (Karachle and Stergiou, 2014; Lomiri et al., 2008). However, the clear trophic segregation and higher trophic position of *S. aurita* from *S. pilchardus* and *E. encrasicolus*, suggests that *S. aurita* may prey on larger zooplankton or larvae of other small pelagic fishes (Lomiri et al., 2008).

Contrary to what we expected, there were differences in the isotopic niches between species within the genera *Trachurus* and *Scomber*. This segregation between congeneric species could be explained by either differences in the feeding habits or differences in spatial distribution. A dietary difference may be more likely for Scomber species, as it has been described that *S. scombrus* in the Atlantic preyed mainly on euphausiids and fishes, whereas for *S. colias* one of the most important prey in the Mediterranean was mysids (Keč et al., 2012; Olaso et al., 2005). If in the Mediterranean Sea *S. scombrus* has also a higher predation rate on fish than *S. colias*, then, this could explain the higher trophic level of *S. scombrus* (Polunin et al., 2001). Alternatively, for the *Trachurus* species, Lloris and Moreno (1995) suggested that *T. mediterraneus* is located more commonly in shallow coastal areas, while *T. trachurus* has a wider distribution and often a more benthic behavior. Thus, the isotopic segregation between *Trachurus* spp. may be due to higher δ^{15}N of primary producers in coastal areas as a consequence of the dissolved nutrients and particulate organic matter (POM) in river plumes (Radabaugh et al., 2013). Further dietary studies for both genera are needed to clarify the most plausible explanation(s).

Our results are consistent with previous studies in showing that squids have a broad trophic width in marine food webs (Coll et al., 2013; Navarro et al., 2013). I. *coindetii* and *L. vulgaris* seem to have different trophic roles, with the former occupying lower positions in the food web with isotopic values closer to small pelagic fishes, and the latter apparently positioned higher in the food web and being a potential predator of anchovy and sardine. These differences are in accordance with the dietary habits described for both species. *I. coindetii* has been reported to prey mainly on crustaceans, whereas fish have been described as the main prey of *L. vulgaris* (Martínez-Baena et al., 2016; Rosas-Luis et al., 2014; Valls et al., 2015).

High δ^{15}N values and trophic niche overlap for *M. merluccius*, *L. vulgaris* and *S. sarda* suggested that they feed at higher trophic levels than the other species. This is consistent with the fact that *M.merluccius* and *S. sarda* have been described primarily as piscivorous (Cresson et al., 2015; Harmelin-Vivien et al., 2012; Navarro et al. 2017). Based on the discrimination corrected values, it is reasonable to infer that *L. vulgaris*, *M. merluccius* and *S. sarda* prey on small pelagic fishes. The three species had a large isotopic niche width probably due to their opportunistic feeding tendencies.

Despite intraspecific seasonal variability for some species, overall community trophic structure appeared relatively stable through the year. That is, *E. encrasicolus* and *S. pilchardus* were positioned at lower trophic levels than the other species studied, mackerels and horse mackerels occupy intermediate positions, and the large pelagic and demersal fish were highest in the web. This study is one of the first steps in understanding the seasonal food web dynamics of the pelagic species in the northwestern Mediterranean Sea, including novel data sets, e.g., some of the first isotope data on pelagic fishes and squids in the Mediterranean Sea that cover all seasons. More research is needed in this area, as many questions remained unanswered, especially according to how spatial variability affects the inferred trophic structure of this system. In particular, stronger isotopic baselines are needed, migratory behavior needs to be more well-documented, and abiotic drivers of dietary patterns need to be developed. Such data are of upmost importance to better manage stocks of these species.

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Chapter 2

Trophic ecology of range-expanding round sardinella and resident sympatric species in the NW Mediterranean

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Abstract

The recent northward expansion of the round sardinella *Sardinella aurita* in the Mediterranean Sea has been documented as a consequence of rising sea temperature. At the same time, declines in sardine and anchovy biomass have been observed in the NW Mediterranean Sea, necessitating an assessment of whether the expansion of round sardinella may affect sardine and anchovy populations. Here, we combined stomach content and isotopic analyses to describe the trophic habits of round sardinella in the NW Mediterranean Sea and its trophic relationships with 2 sympatric small pelagic fish, European anchovy *Engraulis encrasicolus* and European pilchard *Sardina pilchardus*. Results revealed changes in the diet of round sardinella during the year. In summer, the most important prey were copepods (*Acartia* spp.) and cladocerans (*Penilia avirostris*). During winter, the diet was composed mainly of copepods and tunicates (mainly appendicularians), but microplankton was also numerically important in adult diets. In contrast to previous studies, during spring, round sardinella principally fed on salps (Thaliacea). To our knowledge, this is the first time that salps have been identified as an important prey for round sardinella. When compared to coexisting small pelagic fish, we found that round sardinella adults had a different trophic niche than anchovy and sardine. In contrast, round sardinella juveniles partially overlapped the trophic niche with the juveniles of the other 2 species. Therefore, the range expansion of round sardinella probably would not affect sardine and anchovy populations. Only in a situation of food limitation could juveniles of round sardinella compete with and affect both sympatric species. Our results provide new insights into the ecological role of this range-expanding species in the NW Mediterranean Sea, and highlight the importance of gelatinous zooplankton as prey.

Keywords: Gelatinous zooplankton; Sardine; Anchovy; Small pelagic fish; Trophic segregation; Stable isotopes; Stomach contents; Trophic pathways.

2.1 Introduction

Climate change has biological effects on physiological, phenotypical and distributional patterns of marine species that can affect biological interactions (Hughes & Grand 2000). The expansion of certain species outside of their native range may have important effects on ecosystems (Sorte et al. 2010, Last et al. 2011). Although shifts or expansions in the geographic distribution of non-native or range-expanding species (i.e. species that enter a habitat in which they do not currently exist) have been well documented, the potential ecological and socioeconomic consequences of these expansions are not well understood, due to the amount of ecological information required at different levels of organization (species, community and ecosystem; Madin et al. 2012). The basic knowledge of how these species use trophic resources is crucial information to evaluate their impact on the new community (Sunday et al. 2015). Overall, 2 main trophic pathways have been suggested to explain resource acquisition by non-native or range-expanding species: (1) they may exploit novel niche opportunities that most native species are unable to use (opportunism hypothesis), or (2) they may exploit the resources used by native species, displacing the native species from their preferred niches (competition hypothesis) (Tilman 2004, San Sebastián et al. 2015).

Traditionally, the round sardinella *Sardinella aurita* has been widely distributed in the southern part of the Mediterranean Sea and subtropical waters of the Atlantic Ocean, due to its preference for spawning in warm waters (Ben-Tuvia 1960, Sabatés et al. 2006, 2009). The northward expansion of round sardinella in the Mediterranean Sea has been documented over the last decade as a consequence of the increase in sea surface temperatures associated with global warming (Sabatés et al. 2006, Tsikliras 2008). Similarly, in other areas of the Atlantic Ocean such as the coast of Mauritania, Morocco and the Canary Archipelago, higher abundances of this species have been recorded in recent years due to high surface temperatures (Zeeberg et al. 2008, Alheit et al. 2014).

As a consequence of the high feeding intensity and the wide plasticity and adaptability to environmental fluctuations and food availability of round sardinella (Cury & Fontana 1988, Tsikliras et al. 2005, Morote et al. 2008, 2010), this species may have an impact via trophic competition or direct predation on closely-related

Clupeiformes, such as the European pilchard *Sardina pilchardus* (hereafter referred to as sardine) and the European anchovy *Engraulis encrasicolus* (hereafter referred to as anchovy), 2 well-established small pelagic fish species in the northern Mediterranean Sea. The 3 species have been described as planktivorous fish that mainly prey on copepods, cladocerans and diatoms (Table 1).

Table 1 - Summary of the available published data on diet, included the results of the present study, based on stomach content analysis and the main prey groups reported for round sardinella (*Sardinella aurita*), anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) in the NW Mediterranean Sea. Seasons with available information are indicated with an 'x' (1= winter; 2=spring; 3=summer; 4= fall). New information from present study is indicated in grey.

SPF	Life stages	Main prey groups	Season				References
			1	2	3	4	
Round sardinella	Larvae	copepods naulplii and postnauplii, cladocera		x			Morote et al. 2008; present study
	Juvenile	copepods, cladocera, appendicularia, salps	x	x	x		
	Adult	copepod, cladocera, appendicularia, salps, diatoms, tintinnids	x	x	x		
Anchovy	Larvae	copepods eggs, nauplii and postnauplii, cladocera		x	x		Costalago et al. 2014; Le Bourg et al. 2015; Morote et al. 2010; Plounevez and Champalbert, 2000; Intxausti et al. 2017; Tudela and Palomera et al. 1997; Brosset et al. 2016
	Juvenile	copepods, cladocera, appendicularia	x		x	x	
	Adult	copepod, cladocera, decapods, appendicularia		x	x		
Sardine	Larvae	tintinnids, copepods nauplii and postnaupli				x	Costalago et al. 2014; Costalago and Palomera 2014; Le Bourg et al. 2015; Morote et al. 2010; Brosset et al. 2016
	Juvenile	copepods, cladocera, appendiculari, mysids, diatoms	x	x	x	x	
	Adult	copepods, cladocera, appendicularia, diatoms	x		x		

Previous trophic studies conducted in the eastern Mediterranean Sea revealed that round sardinella, sardine and anchovy exploit similar food resources throughout the year but with differences between seasons (e.g. for round sardinella, copepods were described as the main prey in fall, while for sardine, copepods were the main prey in winter; Karachle & Stergiou 2014). On the contrary, an isotopic niche analysis of these 3 clupeids in the NW Mediterranean Sea found different trophic niches for adult stages (Albo-Puigserver et al. 2016). Although the trophic habits of sardine and anchovy in the western Mediterranean Sea have been well studied (e.g. Costalago et al. 2012, Le Bourg et al. 2015, Brosset et al. 2016, and see references in Table 1), to our knowledge, only 1 study conducted in this area found high dietary overlap between round sardinella and anchovy at larval stages (Morote et al. 2008). No information on the diet composition of juveniles and adults of round sardinella in the western

Mediterranean is available. This impairs our current capability to assess the ecological consequences that the northward expansion of this species has on other pelagic species.

In addition to the northward expansion of round sardinella, in recent decades important declines in biomass, landings and body condition of sardine and anchovy have been observed in the NW Mediterranean Sea (Van Beveren et al. 2014, Brosset et al. 2017). The stocks of both species are overfished in this area (Coll & Bellido 2019). In contrast, round sardinella landings have increased, with large fluctuations due to its low commercial value (Palomera et al. 2007, Coll et al. 2019). At present, the potential factors controlling the population of small pelagic fish in the NW Mediterranean are still unclear and there is an urgent need to understand the mechanisms that are driving these fluctuations and to assess whether the expansion of round sardinella affects sardine and anchovy populations (competition hypothesis) and the whole pelagic marine food web (Coll et al. 2019). The 3 species mainly occur from the coastal area to the edge of the continental shelf. Sardine is found in waters up to 200 m depth, although it is more common in shallower areas, and anchovy is distributed in a larger area from the coast to off-shore waters, to areas where the maximal depth is around 400 m (Palomera et al. 2007). Round sardinella has a preference for depths of 40–60 m (Zgozi et al. 2018), and spawning of round sardinella takes place in shallower waters than anchovy, although both species spatially overlap at the larval stages (Sabatés et al. 2008, Schismenou et al. 2008).

Accordingly, the aim of the present study was to investigate the diet of round sardinella on a seasonal basis, and compare it with trophic information on sardine and anchovy from the same area, through complementary methodologies: stomach content analysis and stable isotope analysis (δ^{15}N and δ^{13}C values). Isotopic information provides a long-term, integrated measure of the assimilated food to explore the trophic niche relationships between the 3 sympatric species on a wide temporal scale, while stomach content analysis provides more detail about the prey (Boecklen et al. 2011). Although outcomes of stomach content and isotopic analysis need to be interpreted with caution (Nielsen et al. 2018), their combination is very useful to better understand the trophic ecology of organisms at different time scales and resolutions, and has previously been successfully used in the study of small pelagic fishes (e.g. Costalago et al. 2014, Le Bourg et al. 2015).

2.2 Materials and methods

Study area and sampling procedure

The study area was located on the continental shelf of the Ebro River Delta (NW Mediterranean Sea; Fig. 1). This area is an important fishing ground and spawning area for small pelagic fishes and has been identified as a priority area of conservation (Palomera et al. 2007, Coll et al. 2012, 2015, Piante & Ody 2015). In the NW Mediterranean Sea, sea surface temperatures and primary production follow annual cycles characterized by strong seasonality. Although the coastal zones of the area are considered oligo trophic, inputs from the Ebro outflow and episodes of strong winds increase the availability of nutrients. During fall and winter, the water temperature is at its lowest and water column mixing is induced by strong winds, leading to higher nutrient availability at the surface, with a peak of phytoplankton in late winter and spring (Salat et al. 2002). On the other hand, in late spring and summer, during the period of water column stratification, there is a reduction in nutrients in the photic zone. At this time, the inputs of nutrients to the surface are supplied by the Ebro River outflow (Estrada 1996, Palomera et al. 2007). Zooplankton abundance and composition show high spatial and temporal variability in the NW Mediterranean Sea (Calbet et al. 2001). However, some studies have found higher zooplankton biomass after the late winter/ early spring phytoplankton bloom (Andreu & Duarte 1996, Fernández de Puelles et al. 2003). In general, copepods dominate the

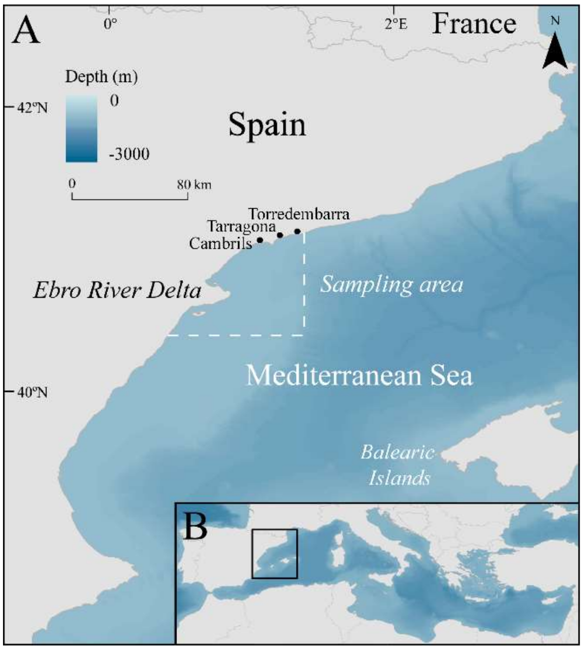

Fig. 1. (A) Sampling area (dashed line) in the NW Mediterranean Sea where round sardinella *Sardinella aurita*, sardine *Sardina pilchardus* and anchovy *Engraulis encrasicolus* were collected. The harbours where samples were landed (Torredembarra, Tarragona and Cambrils) are indicated. (B) Location of the study area in the Mediterranean Sea.

zooplankton community throughout the year, with a dominance of calanoid copepods during spring and winter (Calbet et al. 2001, Sabatés et al. 2007). In contrast, other groups present a clear pattern of seasonality. Cladocera and Appendicularia show high abundance in summer and Chaetognatha have a summer– autumn peak, whereas Cnidaria and Thaliacea are abundant in spring (Gili et al. 1988, Calbet et al. 2001, Pascual 2016).

For stomach content analysis, samples of adults and juveniles of round sardinella were obtained from commercial vessels working in the study area (spring 2012 [April and May], winter 2013 [March]) and from an experimental oceanographic cruise carried out in the same study area in summer 2013 (July) (ECOTRANS Project, Institut de Ciències del Mar–CSIC; Fig. 1, Table 2). Samples obtained from commercial vessels, corresponding to spring 2012 and winter 2013, were also used for stable isotope analysis (see Section 2.3). Samples from the experimental oceanographic cruise in summer 2013 were only used for stomach content analysis. For stable isotope analysis, samples of adults and juveniles of round sardinella, sardine and anchovy were obtained from commercial vessels working in the study area in spring, summer and fall 2012 and winter 2013. All individuals were immediately frozen after capture and stored at −20°C until morphological measurements, tissue and stomach collection were conducted. Total body length ranges considered to classify the individuals as adults or juveniles were based on the size at first maturity (when 50% of individuals at that size are mature), which was defined as 15 cm for round sardinella (Tsikliras & Antonopoulou 2006), 11 cm for anchovy (Palomera et al. 2003) and 13 cm for sardine (Abad & Giraldez 1983).

Several factors can contribute to variation in isotopic signatures and stomach content analysis. It is important to take into account the limitations of both techniques (Boecklen et al. 2011, Nielsen et al. 2017). Stomach content analysis provides information on diet over a short period of time (e.g. 1 d), but at a high taxonomic resolution. On the other hand, stable isotopes of muscle tissue integrate the diet of a consumer over a long period (from several weeks to some months in marine fish; Vander Zanden et al. 2015). Values of stable isotope are mostly affected when changes in environmental conditions are persistent in time (seasonal differences) or space (spatial differences) (Boecklen et al. 2011). Therefore, in this study, stable isotope values of summer 2012 are assumed to be representative of summer season for other years (e.g. summer 2013) in the same sampling area.

Table 2. Sampling information of round sardinella (*Sardinella aurita*) collected for dietary analysis. Vacuity index (V%) is the percentage of empty stomachs. Time fished is the usual fishing time range per vessel according to fishing gear.

Season	Size class range	Pool	Stomachs analysed	V %	Sampling date	Time fished GMT (h)	Fishing gear
Spring 2012	9.6-13.6	No	20	40	13/04/12	00:00-06:00	purse seine
	19.8-25.2	No	10	20	23/04/12	10:00-04:00	gillnet
	19-26	No	20	0	04/05/12	00:00-06:00	purse seine
Winter 2013	10.8-11.9	Yes	10	0	26/03/13	10:00-04:00	gillnet
	12.0-13.0	Yes	10	0	26/03/13	10:00-04:00	gillnet
	13.2-14.7	Yes	10	0	26/03/13	10:00-04:00	gillnet
	22.2-24.0	Yes	5	0	20/03/13	00:00-06:00	purse seine
Summer 2013	12-16.2	Yes	13	0	10/07/13	06:00-07:00	bottom trawling
	16.8-23.6	Yes	13	7.7	10/07/13	06:00-07:00	bottom trawling
	21.5-29.5	Yes	7	28.6	07/07/13	06:00-07:00	bottom trawling
	17.5-24.6	Yes	9	0	07/07/13	09:00-10:00	bottom trawling
	16.7-20.1	Yes	10	0	08/07/13	11:00-12:00	bottom trawling

Stomach content analysis of round sardinella

In total, 137 stomachs of round sardinella caught in spring 2012, winter 2013 and summer 2013 were analysed. The stomach contents were extracted under a stereomicroscope (Leica M205 C) and preserved individually in a buffered 5% formaldehyde– seawater solution. No regurgitation was detected in the oesophagus, and the contents of the intestine were discarded. After disaggregation of stomach contents, pools of 5 to 13 stomachs were diluted to a known volume of filtered seawater, and homogenized aliquots of each pool were examined under a stereomicroscope at 100× magnification until no new prey items were found. Pooling of the stomach contents of fish from the same haul in dietary studies of small pelagic fish is a common practice, since individuals from the same haul present similar prey items in the stomachs (Van Der Lingen 2002, Nikolioudakis et al. 2012). In this study, to pool the stomachs, factors such as size, haul and fishing day were taken into account. Aliquots of 4–7 ml of the total volume of the pools (140 ml) were analysed. Stomach contents obtained in spring 2012 were not pooled since it was not possible to homogenize the predominant food items; therefore, all stomach contents were individually analysed (Table 2). All prey in the aliquots were identified to the lowest taxonomical level possible (generally up to species or genus level) and counted. Only prey items that could be identified were recorded, and the numbers of identified prey in the pool were standardized to numbers of prey per stomach. Whenever possible, the length of each prey was measured using an ocular micrometer. Dry weight of prey was mathematically reconstructed from the literature using length–weight relationships for specific species, genera or groups (Borme et al. 2009, Costalago et al. 2012, Brosset et al. 2016; Table S1 in the Supplement at www. int-res. com/articles/ suppl/ m620 p139 supp. pdf). The contribution in terms of weight of dinoflagellates, tintinnids and diatoms was not considered because of their low weight compared to metazoan prey. Numerical size-frequency histograms of the prey were constructed for each season and each ontogenetic group. To describe the contribution of each prey group to the diet, the numerical percentage (%N*i* = contribution by number of food type *i* in relation to the number of items in the whole contents) and the weight percentage (%W*i* = dry weight of food type *i* in re lation to the weight of the whole stomach contents) were calculated. All of these trophic metrics were based on the number of non-empty stomachs.

Stable isotope analysis

Stable isotopes of δ^{15}N and δ^{13}C have been broadly used to study trophic ecology of consumers (Layman et al. 2012). Particularly, δ^{13}C is often used as a proxy of the primary source of dietary carbon and δ^{15}N as a proxy of trophic position (Layman et al. 2012). In the present study, we analysed δ^{15}N and δ^{13}C values in muscle samples of round sardinella, anchovy and sardine caught in spring, summer and fall of 2012 and winter 2013. Specifically, a small portion of the dorsal muscle without skin from each fish was sampled, freeze-dried and powdered, and 0.28–0.33 mg of powdered muscle was packed into tin capsules. Isotopic analyses were performed at the Laboratory of Stable Isotopes of the Estación Biológica de Doñana (www.ebd.csic.es/lie/index.html). Samples were combusted at 1020°C using a continuous flow isotope-ratio mass spectrometry system (Thermo Electron) by means of a Flash HT Plus elemental analyser interfaced with a Delta V Advantage mass spectrometer. Based on laboratory standards, the measurement error was ±0.1 and ±0.2 for δ^{13}C and δ^{15}N, respectively. The standards used were EBD-23 (cow horn, internal standard), LIE-BB (whale baleen, internal standard) and LIE-PA (feathers of razorbill, internal standard). These laboratory standards were previously calibrated with international standards supplied by the International Atomic Energy Agency (Vienna). Correction for lipids was not conducted, since the C:N ratio was < 3.5 for all samples (Post et al. 2007). To provide insight into species' trophic niche widths, and to estimate the degree of isotopic niche overlap between species, we calculated Bayesian isotopic standard ellipse areas corrected for sample size (SEAc), firstly for each season and then for all seasons together (Jackson et al. 2011). Higher values of this metric represent a broader trophic niche width (Layman et al. 2012). Isotopic SEAs and their overlap were calculated using the routine Stable Isotope Bayesian Ellipses in the SIAR library (SIBER; Jackson et al. 2011).

Statistical analyses

Differences in stomach contents of round sardinella between seasons and ontogenetic stages (juveniles and adults) were statistically compared using 2-way semi-parametric permutational multivariate analyses of variance (PERMANOVAs) based on a Bray-Curtis distance matrix (Anderson et al. 2008). Prior to these analyses, data were square-root transformed to minimize the impact of outliers. In the case of significant results, pairwise tests were performed. To analyse the average dissimilarity between seasons and to identify which prey made the greatest contribution to the observed differences in diet composition of adults and juveniles of round sardinella, the SIMPER analysis was applied (Clarke & Gorley 2006). Differences in $\delta^{15}N$ and $\delta^{13}C$ values between round sardinella, anchovy and sardine were tested using a 1-way semi-parametric PERMANOVA based on a Euclidean distance matrix (Anderson et al. 2008). Analyses were run using PRIMER-E 6 software (Clarke & Gorley 2006).

Potential ontogenetic changes in each species were explored by analysing the relationship between iso isotopic values and body size (total length) using linear regressions and adopting a significance level of $\alpha = 0.05$. Linear regression analyses were performed with R version 3.3.2. (R Core Team, 2018).

2.3 Results

Stomach contents of round sardinella

Overall, the stomach contents of round sardinella were composed of a wide variety of planktonic organisms (58 different prey categories were identified), composed mainly of zooplanktonic organisms (Figure 2, Table S2). We found significant differences in stomach content composition between seasons and between juvenile and adult stages (Fig. 2; seasons, pseudo-*F*2,118 = 70.30, p < 0.0001; ontogenetic, pseudo-*F*1,118 = 22.74, p < 0.0001; all pairwise tests, p < 0.001). In spring 2012, salps dominated the diet of round sardinella (N% = 25.16 and 85.11%, for juveniles and adults, respectively; W% = 70.96 and 99.20% for juveniles and adults, respectively; Fig. 3A,B). In addition, amphipods of the suborder Hyperiidea (Fig. 3C) were present in 68% of the stomachs of adults, although they were never abundant (N% = 0.92; Fig. 2). In spring, in juveniles of round sardinella, copepods were numerically the most important group (N = 53.77%; Table S2), with *Centropages* spp. and *Euterpina acutifrons* representing 12% of total prey number.

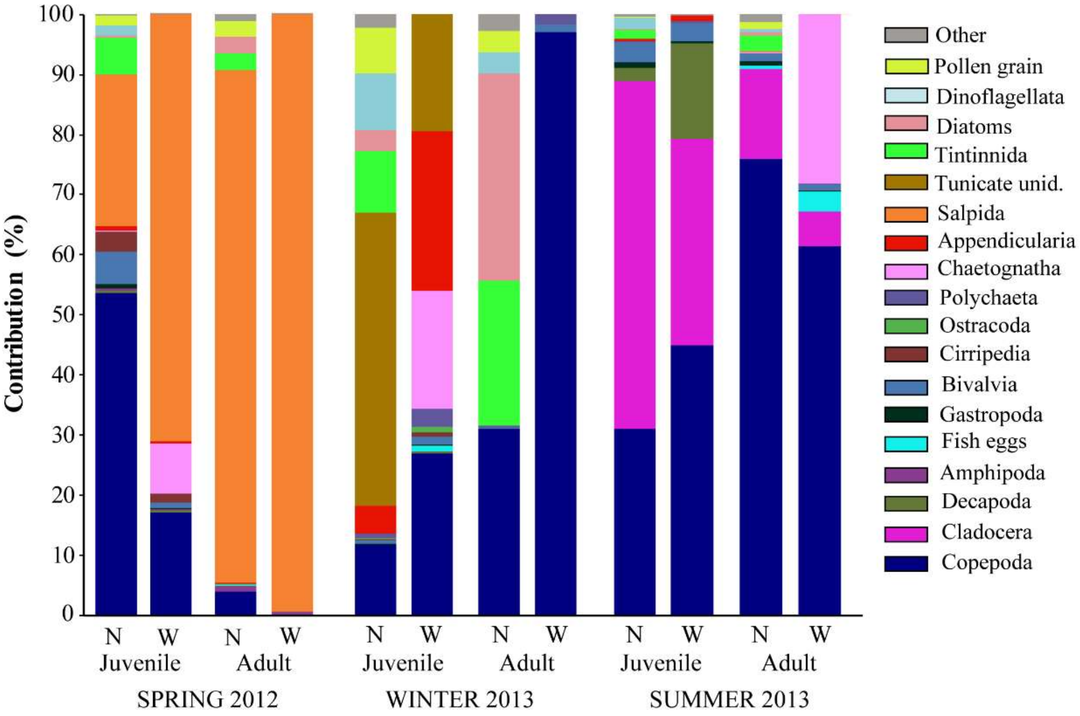

Fig. 2. Percentages of prey categories found in the stomach contents of round sardinella *Sardinella aurita* juveniles and adults by season (spring 2012, winter 2013 and summer 2013) in terms of number of prey items (N) and weight of prey items (W).

During winter 2013, the diet of juveniles and adults was also significantly different (pairwise test, p < 0.001). The diet of juveniles in winter was mainly composed of appendicularians and other unidentified tunicates that numerically represented 26.65% of the total contents and 45.91% in dry weight. Moreover, microplankton (diatoms, dinoflagellates and tintinnids) and copepods also made an important contribution to the total diet (N% = 31.88 and 21.79%, respectively). On the other hand, adults of round sardinella preyed mainly on copepods, tintinnids and diatoms (N% = 31.08, 4.81 and 34.59%, respectively), but in terms of weight, copepods dominated the diet by far (W% = 97.13%). Specifically, *Acartia* spp. and *E. acutifrons* were the most important copepods.

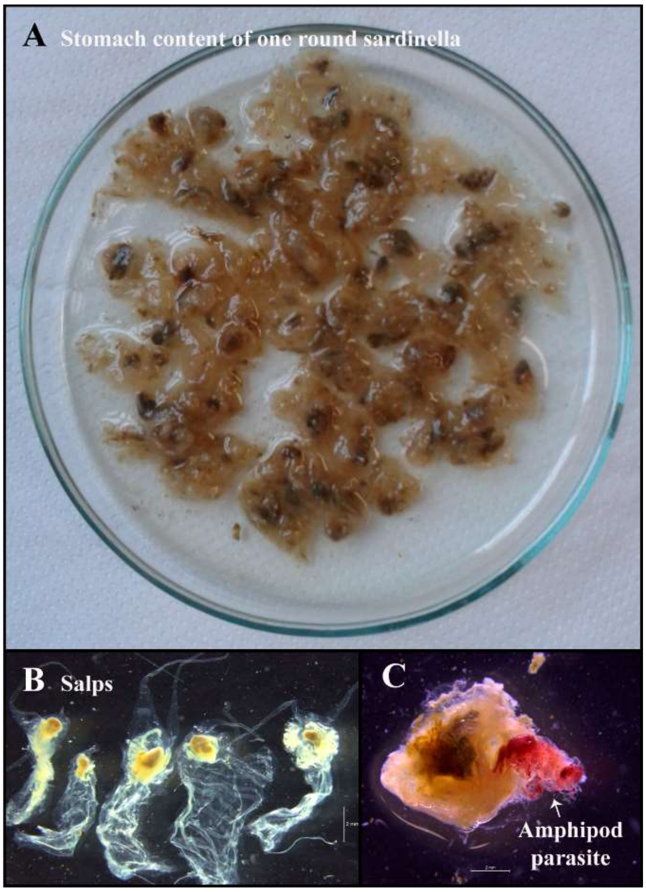

Fig. 3. Prey found in stomachs of round sardinella *Sardinella aurita* during spring: (A) Petri dish with the contents of 1 stomach, (B) salps isolated from the gut content, (C) specimen of salp with an amphipod parasite. Scale bars are 2 mm.

In summer 2013, the most important prey were copepods (N% = 53.56%, W% = 53.26%), mainly calanoids, with a higher numerical presence of *Acartia* spp. in adults and *Centropages* spp. in juveniles. Cladocerans (mainly *Penilia avirostris*) were abundant in both ontogenetic stages (Fig. 2). Decapods and chaetognaths were not numerically abundant but were important in terms of dry weight; decapods contributed to 15.92% of the total diet of juveniles and chaetognaths to 28.13% of the total diet of adults (Table S2).

Round sardinella consumed prey within a wide size spectrum throughout the year (Fig. 4). In general, juveniles consumed prey of smaller size than adults. In spring 2012, due to the consumption of salps, the range of prey length was very wide, with individuals from 0.1 mm to 20 mm (Fig. 4A,B). Prey of size classes 0.1–0.2, 0.7–0.8

and 2.6–2.7 mm were the most frequently consumed by juveniles, while adults showed preferences for larger prey within the 2.7–2.8, 3.3– 3.4 and 6–7 mm size class ranges. In winter 2013, due to the high presence of phytoplankton and tintinnids in the stomachs, a high proportion of the prey had a size range <0.1 mm (46.56% of the total prey measured) (Fig. 4C), while 20.3% of prey were within 0.4–0.5 mm). In summer 2013, there was a clear difference in size range between adults and juveniles (Fig. 4D). Prey items of 0.2–0.4 mm contributed 27.12% to the total measured prey in the diet of round sardinella juveniles, whereas in adults, prey items within the 0.6–0.9 mm size class contributed 43.60% to the total measured prey.

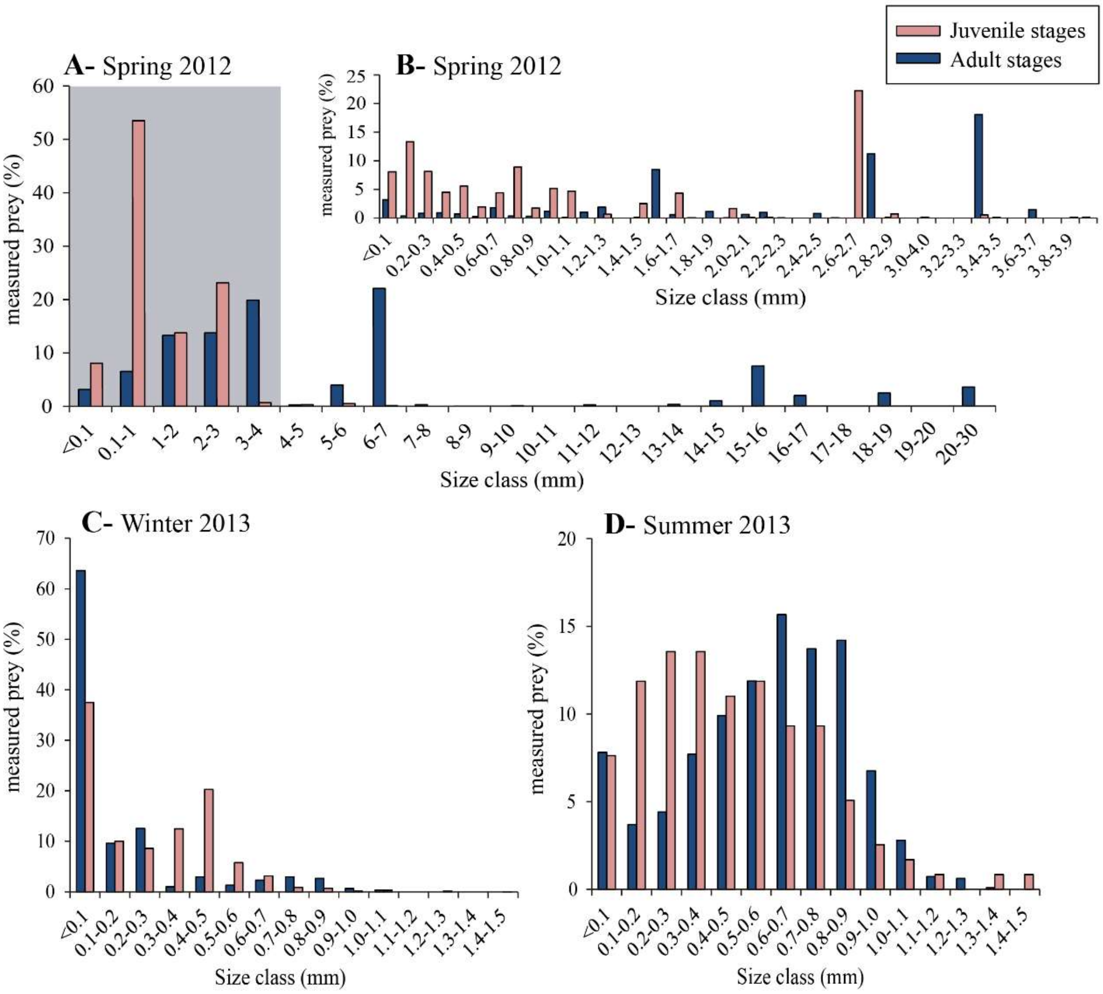

Figure 4. Proportion of the prey size classes in the stomach contents of round sardinella by season: (A,B) spring 2012, (C) winter 2013, (D) summer 2013. The shaded range from <0.1 to 4 mm in panel (A) is amplified to a higher resolution in panel (B).

Based on numerical composition, SIMPER analysis revealed that the average dissimilarity was high between seasons. The group that contributed more than 10% to the dissimilarity of the diet between spring 2012 and winter 2013 were diatoms, followed by tintinnids and dinoflagellates (9.49 and 8.73%, respectively) and copepods (7.41%; Table S3), which presented high abundances in winter, while salps that were the most abundant group in spring 2012 made a small contribution to the dissimilarity of diets due to their lower abundance in absolute terms. Comparing spring 2012 and summer 2013 diets, copepods and cladocerans (15.74 and 10.49%, res pectively) were the prey groups that contributed most to the dissimilarity between seasons. Cladocerans also highly contributed to the dissimilarity of diets between winter and summer 2013 (13.10%), followed by diatoms (9.37%) and tintinnids (7.55%; Table S3).

Isotopic comparisons among species and sizes

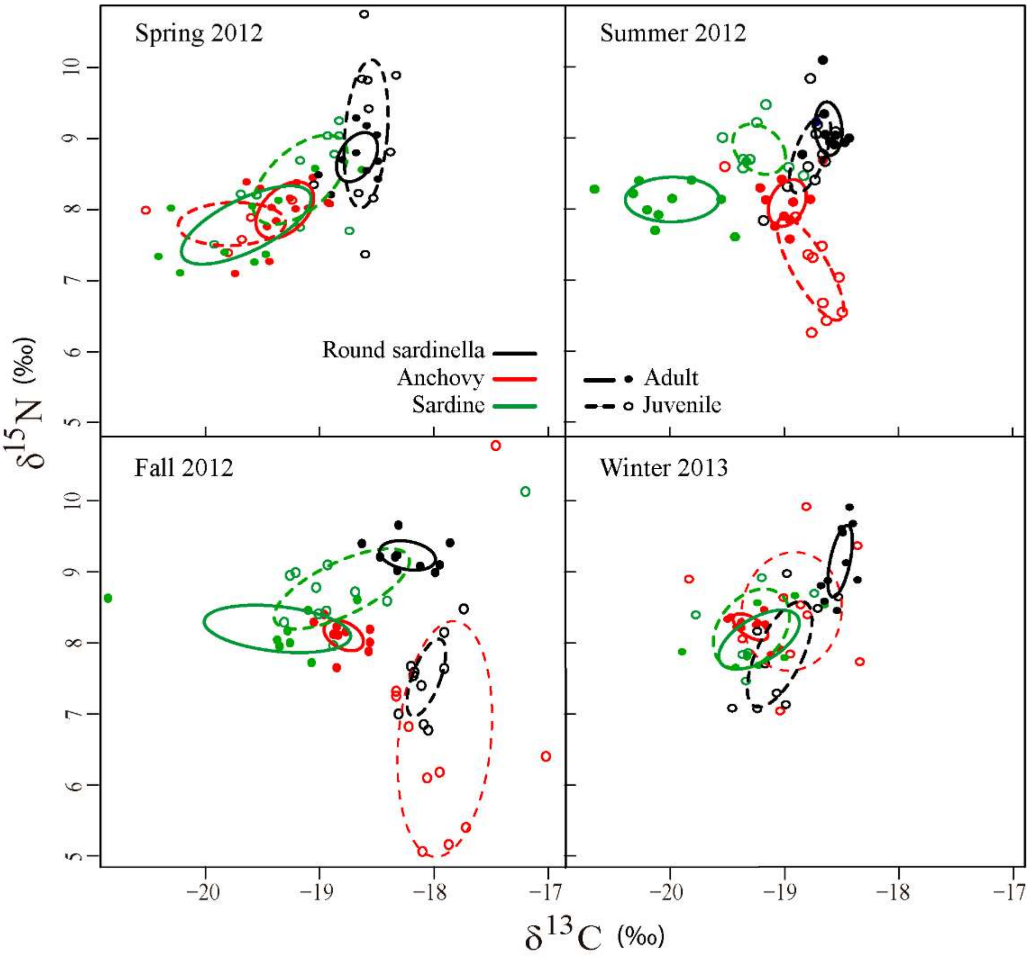

Figure 5. Standard ellipses for adults (solid lines) and juveniles (dashed lines) of round sardinella *Sardinella aurita*, anchovy *Engraulis encrasicolus* and sardine *Sardina pilchardus* during spring, summer, fall 2012 and winter 2013. Individual δ^{15}N and δ^{13}C values of adults (filled dots) and juveniles (open dots) are also graphed.

Stable isotopic values of round sardinella differed significantly from those of anchovy and sardine (Fig. 5; pseudo-F2,231= 23.19, $p < 0.001$). Pairwise tests indicated that, depending on the season, δ^{15}N and δ^{13}C values of round sardinella juveniles were similar to anchovy and sardine juveniles. Specifically, when comparing only between juvenile stages of round sardinella and the other 2 species, round sardinella had similar δ^{15}N and δ^{13}C with sardine in summer 2012 and anchovy in fall 2012 (Table 3). In winter 2013, round sardinella juveniles had δ^{15}N and δ^{13}C values similar to both sardine and anchovy juveniles. In contrast, comparison between adult stages revealed that round sardinella adults had significantly higher δ^{15}N and δ^{13}C values than anchovy and sardine adults during the different seasons (pairwise comparison $p < 0.001$; Table 3).

Table 3. Number of analysed individuals (n), mean (± standard deviation) of δ^{15}N and δ^{13}C values (in ‰), total body length (TL ± sd; in cm) of juveniles and adults of round sardinella (*Sardinella aurita*), anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) during spring, summer, fall and winter.

	Spring 2012		Summer 2012		Fall 2012		Winter 2013	
	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult
Round sardinella								
n	10	10	6	14	10	10	9	10
δ^{15}N	9.1±1.0	8.7±0.3	8.9±0.7	9.0±0.4	7.5±0.5	9.2±0.2	7.9±0.7	9.2±0.5
δ^{13}C	-18.6±0.2	-18.7±0.2	-18.8±0.2	-18.7±0.1	-18.1±0.2	-18.2±0.2	-19.0±0.3	-18.5±0.1
TL	11.0±0.7	23.6±1.5	13.2±0.6	20.7±3.5	10.3±1.4	24.0±1.8	11.8±0.8	23.2±1.1
Anchovy								
n	5	14	10	10	10	10	10	11
δ^{15}N	7.8±0.3	8.0±0.4	7.2±0.7	8.1±0.3	6.6±1.7	8.1±0.2	8.4±0.8	8.2±0.2
δ^{13}C	-19.8±0.5	-19.3±0.3	-18.8±0.3	-19.0±0.2	-17.9±0.4	-18.8±0.2	-18.9±0.4	-19.3±0.2
TL	10.3±0.2	13.9±1.9	8.2±0.7	14.5±1.2	8.4±0.9	14.0±1.3	9.6±1.1	14.0±0.8
Sardine								
n	10	10	8	11	11	8	6	9
δ^{15}N	8.4±0.6	7.8±0.6	8.8±0.4	8.1±0.3	8.8±0.6	8.2±0.3	8.2±0.6	8.1±0.4
δ^{13}C	-19.2±0.4	-19.6±0.6	-19.2±0.2	-20.0±0.4	-18.8±0.6	-19.4±0.6	-19.3±0.3	-19.2±0.4
TL	11.3±0.2	17.9±0.6	9.5±0.3	15.7±1.2	10.6±0.6	17.7±1.0	11.5±0.2	12.9±0.5

SEAc values (a proxy of trophic niche) of round sardinella overlapped differently with anchovy and sardine depending on the ontogenetic stage and season. In spring 2012, round sardinella juveniles showed little overlap with sardine juveniles (3.47%). Round sardinella adults also overlapped with sardine juveniles (9.84%; Fig. 5). In summer 2012, round sardinella juveniles overlapped with anchovy adults (17.02%), while round sardinella adults segregated completely from the other 2 species. In fall 2012 and winter 2013, there was greater SEAc overlap between round sardinella and the other 2 small pelagic fish. In fall 2012, the overlap of round sardinella juveniles with anchovy juveniles was 18.87%, while round sardinella adults overlapped with sardine juveniles (14.85%; Fig. 5). In winter 2013, round sardinella juveniles had an overlap of 26.74 and 39.19% with sardine juveniles and adults, respectively, and an overlap between round sardinella juveniles and anchovy adults and juveniles of 41.29 and 2.46%, respectively. In contrast, round sardinella adults only overlapped the isotopic niche with anchovy juveniles in a low proportion (5.77%; Fig. 5).

When assessing all seasons together and the SEAc as a measure of trophic niche width, we observed that round sardinella juveniles had a wider trophic niche width than adults (Fig. 6). Therefore, round sardinella juveniles overlapped in high proportion with anchovy juveniles (40.9%), which also present a large trophic niche width, and with sardine juveniles (25.83%; Fig. 6). In contrast, the SEAc of round sardinella juveniles overlapped in low proportion with that of anchovy adults (9.91%). The low overlap ob served with sardine adults was not considered as a potential trophic overlap between these 2 groups (round sardinella juveniles and sardine adults; 0.03%). Round sardinella at adult stages only overlapped with sardine juveniles in low proportion (8.21%) and segregated the trophic niche from both ontogenetic stages of anchovy (Fig. 6).

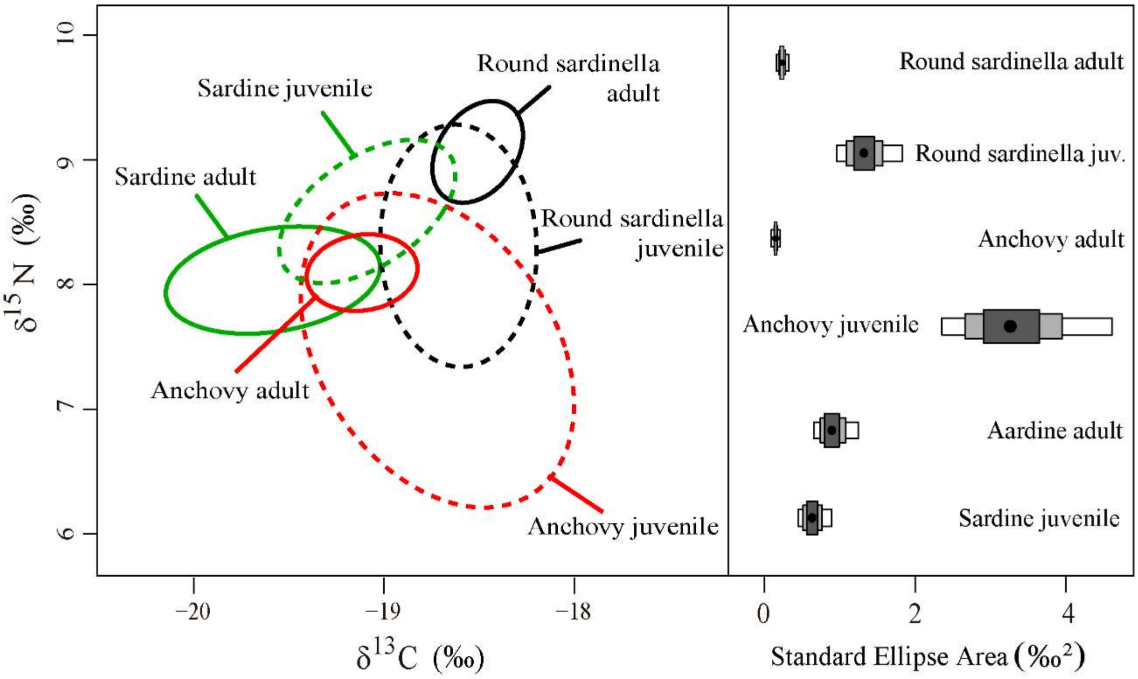

Fig. 6. Isotopic ellipse areas (δ^{15}N and δ^{13}C, in ‰^{2}) of adults and juveniles of round sardinella *Sardinella aurita*, anchovy *Engraulis encrasicolus* and sardine *Sardina pilchardus* combining isotopic data from all seasons (spring, summer, fall 2012 and winter 2013). The right side shows density plots of Bayesian ellipse area as a proxy of trophic niche width. Black points correspond to the mean standard ellipse area, while boxes show 50 (black), 75 (grey) and 95% (white) credible intervals.

Regarding the isotopic niche width, round sardinella and anchovy both showed larger isotopic niche width at the juvenile than at the adult stage. Conversely, sardine had a slightly larger isotopic niche at the adult stage (Fig. 6). These trends were also supported by the relationship between body length and $\delta^{15}N$ values (Fig. 7). Body length of anchovy and round sardinella were positively correlated to $\delta^{15}N$ values, suggesting a higher trophic position of larger sized individuals even when smaller-sized individuals (juveniles) showed high variability in $\delta^{15}N$ values (Fig. 7A,B). In contrast, body length and $\delta^{15}N$ values of sardine were negatively correlated (Fig. 7C), indicating lower trophic positions of larger-sized sardines. In anchovy and sardine, body length had a significantly negative relationship with $\delta^{13}C$ (Fig. 7E,F), whereas round sardinella body length and $\delta^{13}C$ did not show any relationship (Fig. 7D).

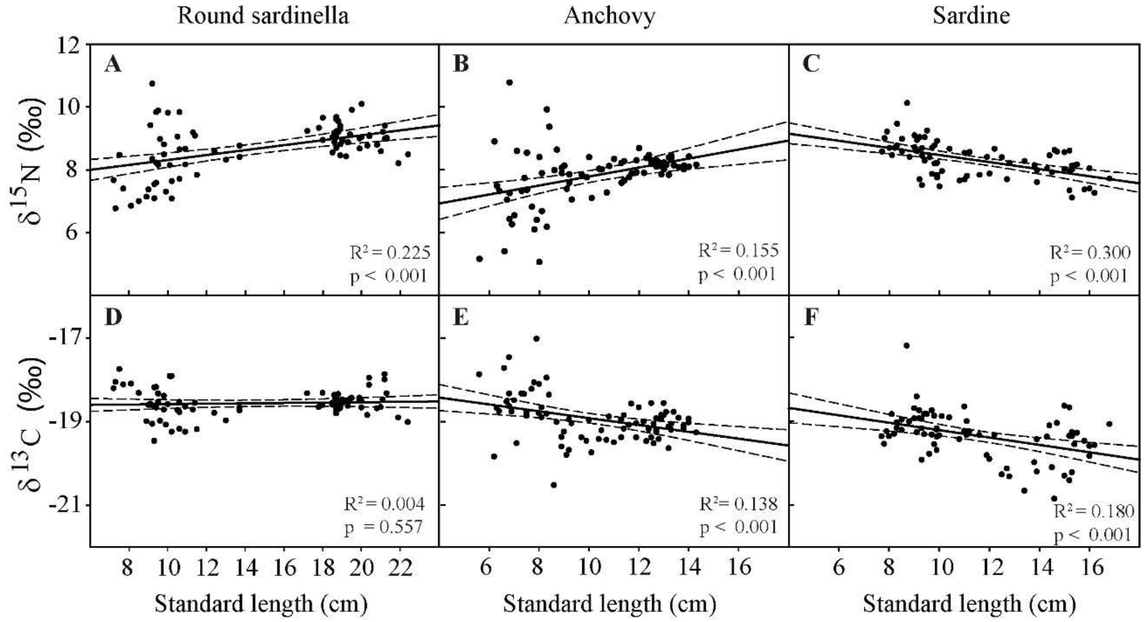

Fig. 7. Relationships between the body length (standard length, in cm), $\delta^{15}N$ and $\delta^{13}C$ values of (A,D) round sardinella *Sardinella aurita*, (B,E) anchovy *Engraulis encrasicolus* and (C,F) sardine *Sardina pilchardus*. Solid line: linear regression; dashed line: 95% confidence interval.

2.4 Discussion

In this study, we present new information on the trophic ecology of round sardinella, a range expanding pelagic fish in the NW Mediterranean Sea, by combining stomach content and isotopic analyses. Overall, stomach contents revealed that round sardi nella mainly preys on gelatinous zooplankton, copepods, cladocerans, tintinnids and diatoms, indicating a generalist diet. Likewise, isotopic results revealed that round sardinella partially overlaps in its trophic niche with anchovy and sardine, mostly at the juvenile stages, whereas it mostly segregates its trophic niche at the adult stage. The observed seasonal variation in the diet of round sardinella is in accordance with the opportunistic feeding behaviour described for round sardinella in other areas (Cury & Fontana 1988, Tsikliras et al. 2005). However, to our knowledge, this is the first time that gelatinous zooplankton, mainly salps, have been described as the main prey for round sardinella and other small pelagic fish in the Mediterranean Sea (Karachle & Stergiou 2017).

Feeding on salps by small pelagic fish has been reported in the southwestern Atlantic Ocean, where the analysis of stomach contents of Argentine anchoveta *Engraulis anchoita* revealed that this species consumed large quantities of salps when the availability of other zooplankton groups, such as copepods and cladocerans, was lower in the ecosystem (Mianzan et al. 2001). However, because we did not have data on the zooplankton abundance during the fish-sampling period, we cannot establish if round sardinella selected salps over other prey or fed on salps because they were more abundant at the time than other planktonic groups. Salps generally form large swarms (blooms), especially during spring (Pascual 2016), so it is likely that round sardinella prey on salps due to the high availability of this prey in certain periods. Although gelatinous zooplankton has generally been considered a prey of low nutritional quality without relevance for predators (Doyle et al. 2007), in recent years it has been found that salps are more nutritional than previously thought, with a high protein content (Henschke et al. 2016). The energy demand of marine predators may be achieved by a high consumption of salps (Dubischar et al. 2012, Henschke et al. 2016), as has been observed for top predators in the NW Mediterranean Sea (Cardona et al. 2012) and similar to what we observed for round sardinella in spring. Moreover, we also found amphipods of the family Hyperiidae in many stomachs of round sardinella, which are common parasites of salps (Laval 1980). The ingestion of these amphipods may increase the energy gain from the consumption of salps. Other tunicates (mainly appendicularians) were an important part of the diet of juvenile round sardinella during winter. This could be related to the large aggregations of this prey that are sometimes found in the western Mediterranean (Champalbert 1996). This result is in agreement with a previous study that observed dietary preference of round sardinella for tunicates during the cold season in the central Mediterranean Sea (Lomiri et al. 2008).

Recently, qualitative ecosystem food-web models representing the NW Mediterranean pelagic ecosystem highlighted the key role of gelatinous zooplankton when describing the temporal dynamics of small pelagic fish (Coll et al. 2019). The ability of round sardinella to eat gelatinous zooplankton compared to other small pelagic species (see Table 1) may represent an advantage in future potential scenarios of global warming (opportunism hypothesis), since gelatinous zooplankton is expected to positively benefit from future environmental changes (Mo li - nero et al. 2009, Brotz & Pauly 2012, Brotz et al. 2012, Grémillet et al. 2017). Moreover, if round sardinella have the capacity to consume salps and other clupeiforms do not (see Table 1), round sardinella could be in an advantageous fitness position compared to other small pelagic species in future scenarios where plankton biomass could decrease (Chust et al. 2014). Future ecological models should consider gelatinous zooplankton with a higher resolution to better describe the present and future dynamics of the pelagic ecosystems (Jaspers et al. 2014).

The diet of round sardinella was composed of other preferred prey in other seasons. The high proportion of tintinnids and diatoms observed in winter (higher for adults than for juveniles) was probably related to the high abundance of phytoplankton in this season (Arin et al. 2005). In our study area, the increase in phytoplankton biomass is a consequence of the water discharges of the Ebro River and strong northern winds, which may generate local upwelling processes triggering nutrient-enrichment events, which in turn fuel high primary production (Lloret et al. 2004, Arin et al. 2005, Costalago & Palomera 2014, Barroeta et al. 2017). The presence of phytoplankton in the stomach contents of round sardinella was previously described in areas with upwelling conditions or important inputs of nutrients, such as Senegalese or Egyptian waters (Nieland 1982, Madkour 2012). In contrast, in oligotrophic areas without upwelling events or river discharges, such as the Aegean Sea and the central Mediterranean, the presence of phyto plankton was not reported in the diet of this species (Tsikliras et al. 2005, Lomiri et al. 2008, Karachle & Stergiou 2014).

Copepods have also been described as the main prey of round sardinella in many studies from the Mediterranean Sea (Tsikliras et al. 2005, Lomiri et al. 2008, Madkour 2012, Karachle & Stergiou 2014, Bayhan & Sever 2015). In our study, they were the principal prey (both in number and weight) only during summer, whereas in winter they were the main prey for adults exclusively in terms of weight since phytoplankton was numerically the most abundant prey as mentioned above.

During summer, coinciding with the reproductive season of round sardinella (Palomera et al. 2007), cladocerans were found in high proportions in the stomach contents of both adults and juveniles. Cladocerans are an abundant prey in summer (Calbet et al. 2001) and are probably easily captured by round sardinella. Moreover, compared to winter, adults clearly fed on larger prey, which have higher energy content than smaller prey (Barroeta et al. 2017). This coincides with the higher energy demands for reproduction that round sardinella have in summer (Albo-Puigserver et al. 2017). In previous studies, while size-related variations in diet were correlated with morphological changes in gill structure, observed seasonal changes in diet were attributed to prey availability (Lomiri et al. 2008). Thus, the observed seasonal changes in the diet of round sardinella in this study are likely related to food availability. However, further studies combining stomach content and plankton composition are needed to confirm this hypothesis.

In winter, although round sardinella preyed on phytoplankton, similarly to that described for sardine, we found no overlap with sardine in terms of isotopic values. In addition, sardine showed a negative relationship of body length to $\delta^{15}N$, which highlights that adults of sardine are feeding at a lower trophic level than juveniles, suggesting an active filter-feeding activity as the individuals become larger (Costalago et al. 2012). In contrast, in round sardinella, this relationship was positive, showing that the importance of phytoplankton for round sardinella decreases as individuals grow in size. The observed feeding differences between juveniles and adults of round sardinella in this study were also found in round sardinella in the Aegean Sea, where Tsikliras et al. (2005) observed an increasing trophic level of consumed prey with the increase in fish body size, and explained it as a consequence of the different energy needs at different life stages. Juveniles of round sardinella preyed on smaller-sized prey categories than adults in the central Mediterranean Sea (Lomiri et al. 2008), similar to our results, and were related to the changes in the number and separation of gill rakers (Rincon et al. 1988). Although we found that larger individuals fed on larger prey, smaller individuals also had the capacity to prey on larger animals such as salps. The lower mean values of $\delta^{15}N$ of round sardinella adults could be a result of predation on lower trophic level species, such as salps (Cardona et al. 2012).

Our results show that adult individuals of round sardinella seem to segregate their niche from other small pelagic fish. The higher values of $\delta^{15}N$ observed in almost all seasons may be related to consumption of bigger prey at higher trophic levels than other small pelagic fish. Anchovy and sardine in the Gulf of Lions in summer consume prey mainly in the 0.2–0.6 mm size class (Le Bourg et al. 2015), while in our study, adult round sardinella consumed mainly 0.5–0.9 mm prey. However, during the juvenile stages, round sardinella partially overlapped with juvenile sardine and anchovy (supporting the ‘competition hypothesis’ at the juvenile stages), and the size class of prey consumed in summer was similar to that described for sardine and anchovy in the Gulf of Lions (Le Bourg et al. 2015). We can thus hypothesize that in situations of food limitation, juveniles of round sardinella, anchovy and sardine could be competitors. However, the wide diversity of prey and the dominant presence of salps in the diet of round sardinella could be a mechanism to reduce the interspecific trophic competition, thus favouring its coexistence with other sympatric small pelagic species. If round sardinella abundance and northward distribution continue to increase with time, its high trophic plasticity associated with its capacity to consume prey not exploited by similar pelagic species could represent an important advantage for this species in the face of changes in zooplankton composition.

We did not observe changes in $\delta^{13}C$ values with sardinella body length, indicating no changes in the source of carbon between juveniles and adults. This may be related to the habitat sharing between adults and juveniles and the preference for shallower waters of round sardinella (Schismenou et al. 2008). In contrast, a decline in $\delta^{13}C$ values with increasing body lengths, found for both sardine and anchovy, indicated an increase in carbon sources of pelagic origin with fish size, which is probably due to an expansion of adult distribution ranges from coastal productive areas to the continental shelf as they grow (Giannoulaki et al. 2011, 2013). However, the potential spatial overlap or segregation of juveniles and adults of round sardinella, anchovy and sardine remains to be quantitatively investigated.

In conclusion, this study confirms the capacity of round sardinella to feed on different prey groups and potentially adapt its diet to environmental prey availability, and highlights the importance of the trophic link between gelatinous plankton and this species. Future feeding ecology studies of clupeiforms in the Mediterranean should focus on detecting potential shifts in diet towards a more gelatinous zooplankton preference, which could lead to important consequences in energy fluxes, as it has been observed in small pelagic fish of the Pacific Ocean (Brodeur et al. 2019). Moreover, prey availability and nutritional quality of prey should be included in future trophic studies of small pelagic fish in order to better understand the advantages or disadvantages of preying on gelatinous zooplankton. Due to the differences found in trophic overlap between adult and juvenile round sardinella with sardine and anchovy, it is advisable to integrate different ontogenetic stages in future assessments in order to capture the different trophic interactions.

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The following supplement accompanies the article

Trophic ecology of range-expanding round sardinella and resident sympatric species in the NW Mediterranean

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Marine Ecology Progress Series 620: 139–154 (2019)

Table S1. Dry weight (DW; µg) of prey and morphometric relationships used to calculate DW. TL = Total length (µm).

Prey item	DW (µg)	Regression (length-DW)	Reference
Euterpina acutiforms		$DW = (1.389*10^{-8})TL^{2.857}$	Ara (2001)
Acartia spp.		LogDW = 2.71*LogPL-7.28	Cataletto & Fonda Umani (1994)
Temora longicornis		LogDW = 2.815*LogPL-7.181	Hay et al. (1991)
Temora spp.		LogDW = (2.71*LogPL-3.685)/1000	Razouls (1981)
Calanoida		LogDW = 2.738*LogPL-6.934	Hay et al. (1991)
Corycaidae		LnDW = 1.96*LnPL-11.64	Van der Lingen (2002)
Oncaea spp.	1.34	Mean	Borme et al. (2009)
Microsetella spp.		LnDW= 1.15*LnTL-7.79	Satapoomin (1999)
Oithona spp.	2.2	Mean	Pitois & Fox (2006)
Clytemnestra scutellata		LnDW = 1.96*LnPL-11.64	Van der Lingen (2002)
Harpacticoida		LnDW = 1.96*LnPL-11.64	Van der Lingen (2002)
Sapphirina spp.		$DW = 6.333*TL^{1.142}$	Lopes et al. (2007)
Candacia spp.	106.20	Mean	Pitois & Fox (2006)
Copepoda		LogDW = 3.13*LogPL-8.18	Uye (1982)
Centropages spp.		LogDW = 2.451*LogPL-6.103	Hay et al. (1991)
Decapoda	27.798	Mean	La Mesa et al. (2008)
Copepod nauplii		LogDW = 2.848*LogL-7.265	Durbin & Durbin (1978)
Copepod copepodites		LogDW = 3.095*LogPL-8.195	Durbin & Durbin (1978)
Cirripedia cyprid		LogDW = -5.375+2.191*LogTL	Muxagata & Williams (2011)
Cirripeda nauplii		LogDW = -9.088+3.377*LogTL	Muxagata & Williams (2011)
Bivalvia	3.758	Mean	La Mesa et al. (2008)
Evadne spp.		$DW = 3.946*L^{2.436}$	James (1987)
Penilia avirostris		LogDW= 4.99*Log L-13.77	Uye et al. (1982)
Podonidae (no *Evadne* spp.)	1.6	Mean	Fonda Umani et al. (1979)
Cladocera	1.4	Mean (Podon and Penilia)	Fonda Umani et al. (1979)
Gastropoda	0.6	Mean	Sautor & Castel (1995)
Ostracoda juveniles	6.035	Mean	Borme et al. (2009)
Tunicata Appendicularian		LogDW = 2.51*LogL-6.54	Gorsky et al. (1987)
Salpida		$DW=11.33*L^{1.77}$	Heron et al. (1998)
Polychaeta	5.67	Mean	La Mesa et al. (2008)
Chaetognatha	1430	Mean	Omori (1969)
Amphipoda		LogDW=-2.348+2.793*Log L*1000	Percy (1993)
Fish eggs	30	Mean	Hunter & Dorr (1982)

Table S2. Numerical percentage (%N) and weight percentage (%W) obtained in stomach contents of round sardinella.

Category	Taxon	Spring 2012				Winter 2013				Summer 2013			
		Juvenile		Adult		Juveniles		Adults		Juvenile		Adult	
		%N	%W	%N	%W	%N	%W	%N	%W	%N	%W	%N	%W
Copepoda	Copepod naupli	6.68	0.06	0.57	<0.01	3.85	1.13	1.89	0.32	0.88	0.02	1.56	0.17
	Copepodita					0.16	0.01	0.81	0.18				
	Copepoda eggs			0.07		3.47		7.57				1.18	
	Calanoida												
	Acartia spp.	0.49	0.05	0.21	<0.01	0.41	0.33	4.59	23.14	2.65	4.41	27.99	21.36
	Centropages spp.	8.57	5.87	0.07	<0.01	0.35	3.46	0.27	7.55	9.12	21.71	0.31	0.69
	Diaixis pygmaea											0.01	<0.01
	Temora spp.	1.02	0.09					0.27	5.89				
	Candacia spp.									0.29	7.60		
	Calanoida n.d.	4.59	1.85	0.82	0.01	2.81	11.63	2.43	16.78	4.12	6.76	22.53	31.33
	Cyclopoida												
	Oncaea spp.	1.35	0.09	0.05	<0.01	0.16	0.09	0.27	0.41	0.59	0.19	0.79	0.19
	Oithona spp.	0.20	0.02	0.11	<0.01	0.00	0.00	0.27	0.67	0.59	0.31	0.19	0.08
	Corycaeus spp.					0.30	0.31					0.13	0.02
	Sapphirina spp.			0.21	0.01								
	Harpacticoida												
	Euterpina acutiforms	3.36	0.05	0.05	<0.01	1.50	0.68	5.14	8.68	4.71	1.01	3.95	0.64
	Microsetella spp.	0.04	<0.01	0.05	<0.01	2.51	0.53	0.54	0.19	2.06	0.19	0.61	0.04
	Clytemnestra spp.	0.49	0.07	0.00		0.35	0.66						
	Harpacticoida n.d.									1.18	0.17	0.05	0.01
	Copepoda nd.	26.97	9.13	1.79	0.03	5.92	10.50	7.03	33.34	5.00	2.71	16.65	6.91
Cladocera	*Evadne nordmanni*											0.19	0.03
	Evadne spinifera											0.31	0.03
	Podonidae									1.76	0.69	2.68	0.78
	Penilia avirostris									55.59	33.50	11.69	4.90
	Cladocera n.d..			0.02	<0.01							0.03	0.01
	Cladocera eggs									0.29	0.02	0.05	<0.01
Ostracoda	Ostracoda			0.07	<0.01	0.23	0.57				0.00	0.12	0.13
Cirripedia	Cirripedia cypris	3.28	1.50	0.07	<0.01	0.43	0.58					0.02	0.01
	Cirripedia nauplii			0.02	<0.01	0.56	0.08						
Mollusca	Bivalves larvae	5.45	0.97			0.73	1.13	0.27	1.14	3.24	2.96	1.13	0.77
	Gastropod larvae	0.66	0.06			0.16	0.54			0.88	0.41	0.76	0.26
	Mollusca n.d.					0.68							
Decapoda	Zoea Carcinus					0.07	0.85			0.29	1.99		
	Anomura larvae	0.16	0.22										
	Caridea larvae									0.59	3.98		
	Decapoda larvae	0.08	0.11	0.02	<0.01					1.47	9.95	0.05	0.12
Chaetognatha	Chaetognatha n.d.	0.12	8.32	0.02	0.09	0.07	11.79				0.00	0.11	28.13
Polychaeta	Polychaeta n.d..	0.08		0.07	<0.01	1.18	2.76	0.27	1.73	0.29	0.41	0.13	0.13
Tunicata	Appendicularia	0.70	0.35	0.21	<0.01	10.46	37.13			0.29	0.76		
	Pirosoma	0.08	0.01	0.00						0.29	0.26		
	Salpida	25.16	70.96	85.11	99.20	0.69	1.27					0.18	0.01
	Tunicata n.d.	0.00		0.14	<0.01	18.65	11.96						
Amphipoda	Amphipoda hyperiidea	0.45	0.23	0.92	0.59	0.14	0.31						
	Phoronimia sedentaria			0.02	0.05								
	P. sedentaria eggs			0.92									
Invertebrates	Eggs					1.39		0.54				0.10	
Eggs Teleost	*E. encrasicolus*			0.07	0.01								
	Teleostea n.d.			0.07	0.01	0.14	1.71					0.60	3.27
Ctennophora	Ctenophora					0.21						0.02	
Cnidaria	Cnidaria ephyra					0.07		0.27					
Foraminifera	Foraminifera					0.00		0.27				0.01	
Tintinnina	Rhabdonellidae	0.49		0.00				10.54		1.18		1.44	
	Stenosemella ventricosa	5.61		2.86		5.84		9.19				0.99	
	Codonella spp.					0.40		4.05					
	Propectella spp.					0.88		0.27					
	Tintinnida n.d.					7.16				0.29		0.20	
Dinoflagellata	*Noctiluca* spp.	0.49										0.01	
	Protoperidium spp.	1.02		0.02		6.46		3.51		1.76		0.41	
	Ceratium spp.					3.18						0.09	
	Prorocentrum spp.					0.40						0.05	
	Dynophysis spp.					0.07							
	Dinoflagellata n.d.					0.33							
Diatoms	*Pleurosigma* spp.	0.49		2.63		6.10		5.41		0.29		0.48	
	Coscinodiscus spp.			0.02									
	Bacillariphyceae n.d.	0.04		0.02		1.06		30.00				0.06	
Radiolaria	Radiolaria					0.23		0.54					
Pollen grain	Pollen grain	1.68		2.68		8.79		3.51		0.29		1.16	
Vascular plant	Hair vascular plant	0.16		0.02		0.34		0.27				0.12	
Microalgae	Microalgae					0.56							

***n.d.** : not determined

Table S3. SIMPER analysis results between seasons. Only prey groups that contributed with more than 5% of the dissimilarity are reported.

Prey	Abund. 1	Abund. 2	Average Dissimilarity	Contribution dissimilarity (%)	Cumulative contribution (%)
(1) Spring 2012 – (2) Winter 2013					
Diatoms	0.47	3.65	7.89	10.25	10.25
Tintinnina	0.51	3.95	7.31	9.49	19.75
Dinoflagellata	0.11	3.35	6.72	8.73	28.48
Copepoda	1.45	4.01	5.70	7.41	35.88
Tunicata n.d.	0.09	3.03	5.33	6.93	42.81
Pollen grain	0.71	3.20	5.29	6.87	49.68
Copepod nauplius	0.43	2.78	5.18	6.72	56.40
Appendicularia	0.28	8.82	4.27	5.55	61.96
Salpida	2.78	0.63	4.22	5.48	67.43
Mollusca	0.11	2.03	4.02	5.23	72.66
(1) Spring 2012 – (2) Summer 2013					
Copepoda	1.45	5.62	12.30	15.74	15.74
Cladocera	0.03	3.59	8.20	10.49	26.23
Mollusca	0.11	2.82	7.76	9.92	36.15
Tintinnina	0.51	2.89	7.42	9.50	45.65
Copepod nauplius	0.43	2.27	5.65	7.23	52.88
Salpida	2.78	0.79	5.56	7.12	60.00
Dinoflagellata	0.11	2.09	5.36	6.86	66.86
Diatoms	0.47	1.93	4.20	5.37	72.23
Pollen grain	0.71	1.93	3.99	5.10	77.33
(1) Winter 2013 – (2) Summer 2013					
Cladocera	0	3.59	5.96	13.10	13.10
Diatoms	3.65	1.93	4.26	9.37	22.47
Tintinnina	3.95	2.89	3.43	7.55	30.02
Pollen grain	3.20	1.93	3.02	6.63	36.65
Tunicata n.d.	3.03	0.30	2.75	6.05	42.70
Dinoflagellata	3.35	2.09	2.48	5.45	48.15
Appendicularia	2.82	0.30	2.47	5.43	53.58

***n.d. :** not determined

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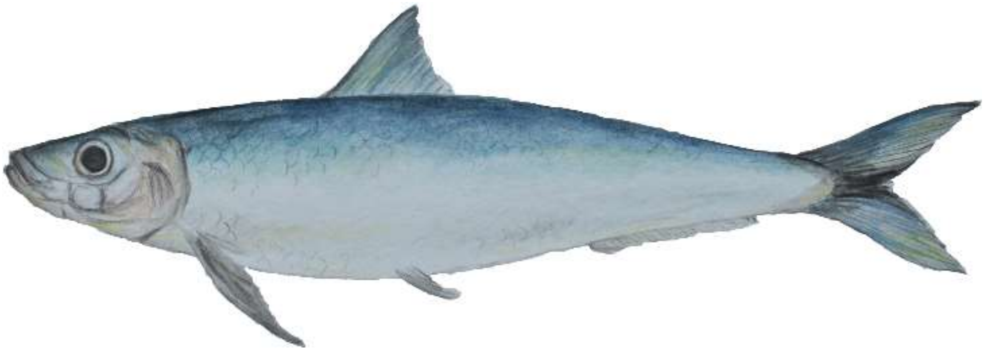
PART II

Ecological Energetics



Chapter 3

Ecological energetics of forage fish from the Mediterranean Sea: Seasonal dynamics and interspecific differences

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Abstract

Small and medium pelagic fishes play a central role in marine food webs by transferring energy from plankton to top predators. In this study, direct calorimetry was used to analyze the energy density of seven pelagic species collected over four seasons from the western Mediterranean Sea: anchovy *Engraulis encrasicolus*, sardine *Sardina pilchardus*, round sardinella *Sardinella aurita*, horse mackerels *Trachurus trachurus* and *T. mediterraneus*, and mackerels *Scomber scombrus* and *S. colias*. Inter-specific differences in energy density were linked to spawning period, energy allocation strategies for reproduction and growth, and feeding ecologies. Energy density of each species varied over time, with the exception of *S. colias*, likely due to its high energetic requirements related to migration throughout the year. In general, higher energy density was observed in spring for all species, regardless of their breeding strategy, probably as a consequence of the late-winter phytoplankton bloom. These results provide new insights into the temporal availability of energy in the pelagic ecosystem of the Mediterranean Sea, which are pivotal for understanding how the population dynamics of small and medium pelagic fishes and their predators may respond to environmental changes and fishing impacts. In addition, the differences found in energy density between species highlighted the importance of using species specific energy values in ecosystem assessment tools such as bioenergetic and food web models.

Keywords: Energy density; Bioenergetics; Food webs; Mediterranean Sea; Environmental variability.

3.1 Introduction

The amount of energy available to an individual during its lifecycle affects its ability to grow and reproduce and this in turn, affects ecological energetics, that is, how energy is acquired, retained and transferred from one trophic level to another in the food web (Rossoll et al., 2012). Ecosystem energetics underpins many food web models that are increasingly being used to assist ecosystem-based fisheries and conservation management (Christensen and Maclean, 2011). Knowing the energetic value of the main ecological groups is needed in these models to understand energy transfer from lower to higher trophic levels and also to validate model outputs. One of the most cost-effective and quantitative ways to assess ecological energetics is through energy density analysis which provides a direct measure of the nutritional condition and quality of an individual or population.

Energy density is positively correlated with lipid content (Rand et al., 1994; Van Pelt et al., 1997), which is important for energy storage and often the first macro-molecule to be catabolized (Lloret et al., 2014). Instead, proteins and carbohydrates remain rather constant in most species and have a much lower energy density than lipids (Anthony et al., 2000; Lloret et al., 2014). Energy storage and nutritional quality of fish may fluctuate during the year due to different biological processes (i.e. growth, maintenance or reproduction) and external factors (i.e. temperature). In temperate regions, pelagic marine environments show important seasonal fluctuations with cyclical changes in temperature and food availability (Coma et al., 2000; Mazzocchi and Ribera d'Alcalà, 1995). In response to this, marine organisms have developed different life-history and energy allocation strategies in order to optimise their reproduction and population growth (Houston et al., 2006). Some marine species acquire and store energy in periods of high food abundance, which is then used for reproduction (they are named 'capital breeders'). Others use their current energy income for reproduction (named 'income breeders'), and there are also intermediate strategies (Alonso-Fernández and Saborido-Rey, 2012; McBride et al., 2015).

Species of fish with faster life cycles and smaller body size, such as small and medium sized pelagic fish, also known as forage fish, are thought to respond rapidly to environmental fluctuations that have been shown to have important implications for fish recruitment and ecosystem structure (Perry et al., 2005; Peck et al., 2013).

Changes in the energy density of forage fish have been shown to be responsible for declines in top-predator populations through negatively impacting their breeding success and fitness (Doney et al., 2012; Österblom et al., 2008). For example, the decline of the common guillemots (*Uria aalge*) in the North Sea was linked to a reduction in the energetic value of their main prey (sprat *Sprattus sprattus* and lesser sandeels *Ammodytes marinus*) (Wanless et al., 2005). Furthermore, the decline of Steller sea lions (*Eumetopias jubatus*) in the Gulf of Alaska was partially related to a reduction of fattier fishes such as herring (*Clupea harengus*) within their feeding grounds (Rosen and Trites, 2000). Moreover, small and medium pelagic fishes are a good source of food nutrition for humans and in some areas, such as sub-Saharan countries, their consumption represent the main protein supply (Kawarazuka and Béné, 2011; Tacon and Metian, 2013).

In the Mediterranean Sea, small pelagic fishes such as *Sardina pilchardus* (sardine), *Engraulis encrasicolus* (anchovy) and *Sardinella aurita* (round sardinella), and medium pelagic fishes such as *Trachurus* spp. (horse mackerels) and *Scomber* spp. (mackerels) play key roles in the food-web, due to their high abundances and fast population turnover rates (Coll et al., 2008; Palomera et al., 2007). Despite this, while the energy density of multiple forage fishes has been reported in the Atlantic and the Pacific Oceans (Anthony et al., 2000; Dubreuil and Petitgas, 2009; Pedersen, 2001; Spitz and Jouma'a, 2013), such data are very scarce in the Mediterranean Sea (Tirelli et al., 2006; Harmelin-Vivien et al., 2012).

In the present study, we investigated the ecological energetics of seven forage fishes, *E. encrasicolus*, *S. pilchardus*, *S. aurita*, *T. trachurus*, *T. mediterraneus*, *S. scombrus* and *S. colias*, that are ecologically and economically important in the western Mediterranean Sea (see Table 1). The study aims were to (1) provide reference values of the energy densities of their whole-body, (2) examine potential seasonal changes, and (3) make inter-specific comparisons. These results are needed to better understand the different life-history strategies of these fishes and regional ecological energetics. Energy density analyses are relevant as input data for bioenergetics and food web models, increasingly used to predict the cumulative effects of climate change and fishing impacts on population and ecosystem dynamics.

Table 1. Species sampled in this study and information on their typical spawning period, breeding strategy (Capital – Income), main diet and known predators in the Mediterranean Sea.

Species	**Spawning period**	**Capital/ Income**	**Diet**	**Predator species**	**References**
Engraulis encrasicolus	Apr-Oct	Income	Copepods, cladocerans	*Etmopterus spina, Hexanchus griseu, Larus audouinii, Merluccius merluccius, Prionace glauca, Puffinus mauretanicus, P. yelkouan, Sarda sarda, Seriola dumerili, Thunnus thynnus, Tursiops truncates, Xiphias glaudius*	Barría et al., 2015; Blanco et al., 2001; Bourgeois et al., 2011; Costalago et al., 2012; Kabasakal, 2004; Matallanas et al., 1995; McBride et al., 2015; Navarro et al., 2009; Navarro et al., 2016; Oro et al., 1997; Palomera, 1992; Stergiou and Karpouzi, 2002
Sardina pilchardus	Oct-Apr	Capital	Copepods, cladocerans, diatoms	*Larus audouinii, Merluccius merluccius, Mustelus mustelus, Prionace glauca, P.yelkouan, Puffinus mauretanicus, Puffinus yelkouan, Sarda sarda, Scomber scombrus, Seriola dumerili, Thunnus thynnus, Xiphias glaudius*	Barría et al., 2015; Bourgeois et al., 2011; Costalago and Palomera, 2014; Kabasakal, 2004; McBride et al., 2015; Navarro et al., 2009; Nikolioudakis et al., 2012; Stergiou and Karpouzi, 2002
Sardinella aurita	Jul-Sep	Capital*	Copepods, decapods larvae, fish larvae	*Coryphaena hippurus, Larus audouinii, Sarda sarda, Tetrapturus belone, Xiphias glaudius*	Campo et al., 2006; Castriota et al., 2008; Freón et al., 1997; Karachle and Stergiou, 2014; Lomiri et al., 2008; Stergiou and Karpouzi, 2002
Trachurus trachurus	Nov-May	Income	Copepods, euphasiids, fish	*Coryphaena hippurus, Euthynnus alleteratus, Hexanchus griseu, Seriola dumerili*	Andaloro and Pipitone, 1997; Ivan Jardas et al., 2004; Massutí et al., 1998; Šantić et al., 2005; van Damme et al., 2014
Trachurus mediterraneus	May-Aug	Capital*	Copepods, euphasiids, fish	*Coryphaena hippurus, Euthynnus alleteratus, Hexanchus griseus, Seriola dumerili*	Andaloro and Pipitone, 1997; Bayhan et al., 2013; Šantić et al., 2004; Tsikliras et al., 2010; Viette et al., 1997; Yankova et al., 2008
Scomber scombrus	Jan-Mar	-	Euphasiids, decapod larvae, fish	*Coryphaena hippurus, Euthynnus alleteratus, Puffinus yelkouan, Thunnus thynnus*	Bourgeois et al., 2011; Massutí et al., 1998; Olaso et al., 2005; Stergiou and Karpouzi, 2002
Scomber colias	May-Jul	-	Copepods, mysids, decapod larvae, fish	*Coryphaena hippurus, Euthynnus alleteratus, Puffinus yelkouan,*	Bourgeois et al., 2011; Castro, 1993; Keč et al., 2012; Massutí et al., 1998; Stergiou and Karpouzi, 2002

* The breeding strategy has not been described using capital- income terminology. However, from published data on conditional and lipid indices, these species have been classified in this study.

3.2 Material and methods

Study area

The present study was conducted in the continental shelf and upper slope areas associated with the Ebro River Delta, northwestern Mediterranean Sea (Fig. 1). As a consequence of particular oceanographic conditions, such as wind conditions, vertical mixing and river discharges (Salat et al., 2002), this area is an important fishing ground of the Mediterranean Sea (Lleonart and Maynou 2003) and has been identify as a priority area for conservation (Coll et al., 2015; Piante and Ody, 2015). Sea surface temperature and primary production follow annual cycles characterized by strong seasonality. From May to October the ecosystem is characterized by stratification of the water column, resulting in a reduction of nutrients in the photic zone. In contrast, from November to April, the water temperature is at its lowest and the water column is mixed, leading to higher nutrient availability at the surface, with a peak of phytoplankton in late-winter and spring (Salat et al., 2002).

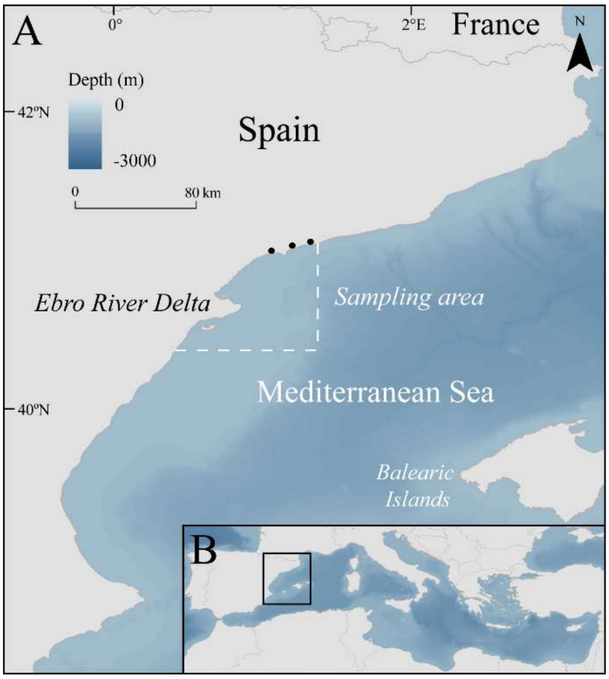

Fig. 1. (A) Map of the study area where small and medium sized pelagic fishes were collected. The sampling area is indicated with a dashed line and black dots are the harbours where samples were landed (Torredembarra, Tarragona and Cambrils). (B) Position of the study area in the Mediterranean Basin.

Sampling procedures

Individuals of sardine, anchovy, round sardinella, Atlantic horse mackerel (*Trachurus trachurus*), Mediterranean horse-mackerel (*Trachurus mediterraneus*), Atlantic mackerel (*Scomber scombrus*) and Atlantic chub mackerel (*Scomber colias*) were collected during spring-2012, summer-2012, fall-2012 and winter-2013 from commercial vessels of the harbours of Tarragona, Torredembarra and Cambrils working in the study area (Table 1; Fig. 1). Due to the lack of catches of *T. trachurus* in summer 2012 in the area of study, this species was not sampled that summer.

All individuals were immediately frozen after capture and stored at –20 °C. Total body length and weight was recorded for all individuals. From the samples collected, for each species, from 10 to 20 individuals per season with similar body size and both sexes were selected to measure the energy density (kJ g^{-1}). To reduce the potential variation in energy density due to ontogenetic changes, only individuals larger than the size at first maturity of each species (length at which 50% of the fish at that size are mature) were selected.

Energy density analyses

In total, the energy density of 354 individuals of the seven forage fish species was determined. This metric is widely used as a proxy of food quality and is thus perfectly suitable for comparative studies (Spitz et al., 2012). For determination of energy density, we used direct calorimetry measuring the heat of combustion of small samples (Parr 6725 Semimicro Oxygen Bomb Calorimeter). The calorimeter system was calibrated with the combustion of benzoic acid standard. Prior to the determination of energy density, each individual was homogenized with a blender and the entire individual in the case of *E. encrasicolus*, *S. pilchardus* and *S. aurita* and two subsamples in the case of *T. trachurus*, *T. mediterraneus*, *S. scombrus* and *S. colias* were oven-dried at 70 °C to constant mass for 48–72 hours. After determining the dry weight, each sample was homogenized in a mixer (Retsch Mixer Mill MM-200) and two pellets of 100–200 mg for each individual were obtained with a press for the determination of the energy density. If energy densities of the two pellets from an individual differed by more than 3%, a third pellet was analysed. The average of the two or three subsamples was used to calculate the energy density of each individual.

The energy density was originally measured on dry samples (kJ g^{-1} of dry weight) and was converted to wet mass basis (kJ g^{-1} of wet weight) by taking into account the water content of each fish (% dry weight=100· dry weight· wet weight–1; Lloret et al., 2014). The prey quality of each species was determined following the classification proposed by Spitz et al. (2010): Low Quality (ED < 4 kJ $^{g-1}$), Moderate Quality (4 < ED < 6 kJ $^{g-1}$) and High Quality (ED > 6 kJ $^{g-1}$).

Statistical analyses

Inter-specific and seasonal differences in energy density were tested using one-way semi-parametric permutation multivariate analyses of variance tests (PERMANOVA test) on the Euclidean distance matrix (Anderson et al., 2008). In the case of a significant difference in the energy density of each species between seasons or between species, pairwise tests were performed. Potential differences between sexes were not tested since the number of females or males was not always sufficient for all species and seasons. Nonetheless, previous studies on small pelagic fishes have revealed no relationship of gender with lipid dynamics of the entire fish (Garrido et al., 2008). PERMANOVA allows for the analysis of complex designs without the constraints of multivariate normality and homoscedasticity (Anderson et al., 2008). The method calculates a pseudo-F statistic directly analogous to the traditional F-statistic for multifactorial univariate ANOVA models, using permutation procedures to obtain p-values for each term in the model (Anderson et al. 2008). PERMANOVA tests were carried out with PRIMER-E 6 software.

Table 2. Annual mean and standard deviation of Energy Density (ED; kJ · g^{-1} of wet weight), weight (g) body length (cm), % of dry weight of individuals used for analyze the ED of the seven small and medium sized pelagic fishes sampled in the western Mediterranean Sea. Species are classified according to their mean ED values following Spitz et al. (2010) as Moderate Quality (4 < ED < 6 kJ g^{-1}) and High Quality species (ED > 6 kJ g^{-1}).

Species	*Engraulis encrasicolus*	*Sardina pilchardus*	*Sardinella aurita*	*Trachurus trachurus*	*Trachurus mediterraneus*	*Scomber scombrus*	*Scomber colias*
ED (kJ·g^{-1})	5.35±0.61	6.03±1.17	5.64±1.03	6.03±0.61	5.82±1.02	7.17±1.30	6.93±0.87
Length (cm)	13.03±1.55	14.33±1.38	20.63±3.48	23.89±3.81	29.72±4.37	28.72±2.82	31.96±5.80
Weight (g)	14.65±5.75	22.90±7.78	73.14±33.70	125.41±61.31	217.41±94.62	200.70±67.44	344.41±157.24
%Dry weight	26.01±1.74	27.25±3.14	27.33±2.93	28.25±1.77	27.26±2.69	30.10±3.14	30.29±2.11
Quality type	Moderate	High	Moderate	High	Moderate	High	High

3.3 Results

Inter-specific differences in energy density

The annual energy density was significantly different between species (Pseudo-F6,348= 22.45; p-value < 0.01) with the lower values for *E. encrasicolus* and the higher values for *S. scombrus* (Table 2; Fig. 2). Within small pelagic fish, *S. pilchardus* showed

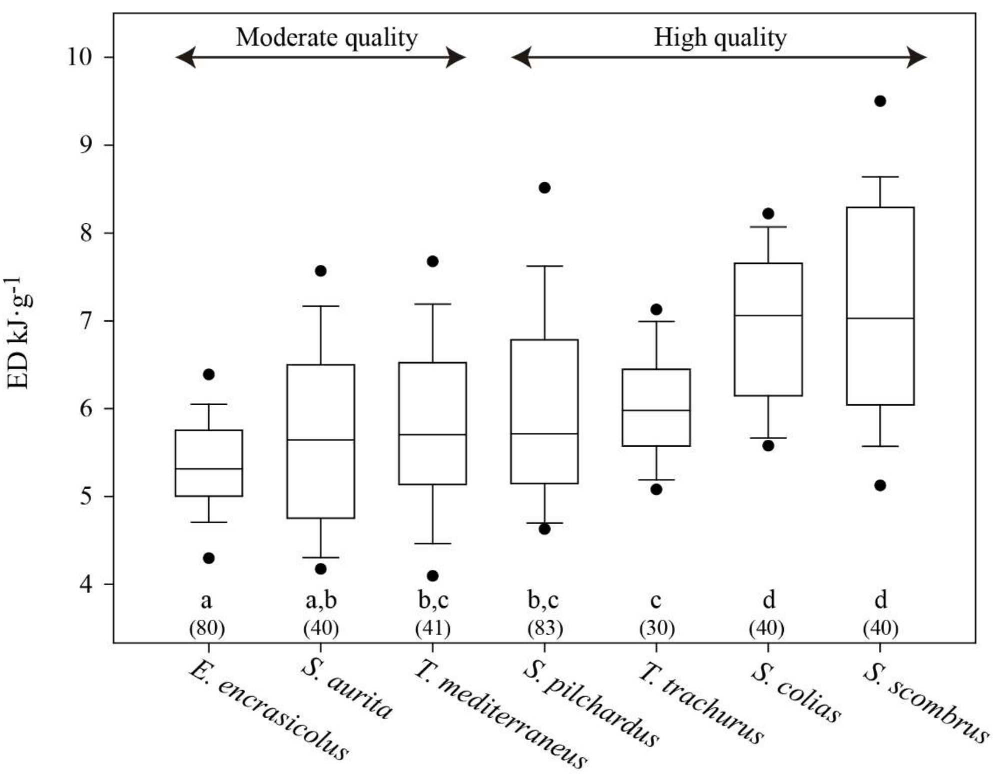

Fig. 2. Annual energy density (kJ g–1 of wet weight) of seven species of small and medium sized pelagic fishes from the western Mediterranean Sea. Box length represents interquartile range, bar length represents range and horizontal lines represent median values. Black dots are outliers. Pairs of means differing significantly ($P < 0.05$) by pairwise tests between species are indicated by the letters – species with the same letter were not significantly different.

significantly higher energy density than *E. encrasicolus* and similar to *Trachurus* spp. *S. aurita* had similar energy density to both, *E. encrasicolus* and *S. pilchardus*. Within the medium pelagic fishes, *T. trachurus* and *T. mediterraneus* had similar energy density, which was lower than the *Scomber* spp. Both *Scomber* species had significantly higher energy density than the rest of the species analysed (Fig. 2). The body length of the individuals analysed was also significantly different between all species (Pseudo-F6,348= 309.47; p-value < 0.01). Body lengths of *S. pilchardus* and *E. encrasicolus* were smaller than 20 cm, *S. aurita* and *T. trachurus* had mean body lengths between 20 and 25 cm while *T. mediterraneus* and *Scomber* spp. had body lengths larger than 25 cm (Table 2).

None of the seven species studied here were classified as species with low nutritional quality. *E. encrasicolus*, *S. aurita* and *T. mediterraneus* were classified as forage species of moderate nutritional quality, while *S. pilchardus*, *T. trachurus*, *S. scombrus* and *S. colias* were classified of high prey quality (Table 2). However, within the moderate prey quality category, only *E. encrasicolus* could be classified as moderate during all seasons, while within the high quality group only the two *Scomber* spp. had values greater than 6 kJ g–1 across seasons.

Seasonal differences in energy density

Seasonal differences in energy density were found in most of the species; only *S. colias* showed similar energy density through the year (Table 3). Among the small pelagic fishes, energy density of *E. encrasicolus* was significantly highest during spring and lowest during fall. The energy density of *S. pilchardus* increased in spring and were not significantly different in summer, and declined to a minimum during fall and winter. For *S. aurita* energy density in spring and fall were not significantly different and higher than in summer and winter (Figure 3).

Table 3. Mean and standard deviation of energy density (kJ · g^{-1} of wet weight) and number of individuals analyzed (n) of seven small and medium sized pelagic fishes species sampled in the western Mediterranean Sea during spring-2012, summer-2012, fall-2012 and winter 2013. Results of the PERMANOVA tests (Pseudo-F values) between seasons for each species separately are shown. Pairs of means differing significantly (P <0.05) by pairwise tests are indicated by the letters - seasons with the same letter were not significantly different.

Species	**Pseudo-F (p-value)**	**n**	**Spring**	**n**	**Summer**	**n**	**Fall**	**n**	**Winter**
E. encrasicolus	8.96 (<0.01)	20	5.80±0.57 ^{a}	20	5.39±0.57 ^{b}	20	4.93±0.28 ^{c}	20	5.30±0.66 ^{b}
S. pilchardus	22.69 (<0.01)	20	7.11±1.03 ^{a}	20	6.56±1.23 ^{a}	20	5.40±0.55 ^{b}	23	5.21±0.45 ^{b}
S. aurita	10.55 (<0.01)	10	6.56±0.75 ^{a}	10	5.16±0.59 ^{b}	10	6.04±1.10 ^{a}	10	4.80±0.54 ^{b}
T. trachurus	5.33 (0.01)	10	5.83±0.58 ^{a}	-	-	10	5.79±0.47 ^{a}	10	6.48±0.54 ^{b}
T. mediterraneus	6.54 (<0.01)	10	6.58±0.55 ^{a}	10	5.08±0.51 ^{b}	11	5.44±0.72 ^{b,c}	10	6.23±1.36 ^{a,c}
S. scombrus	5.31 (<0.01)	9	8.05±0.92 ^{a}	11	7.02±1.53 ^{a,b}	10	7.56±0.81 ^{a}	10	6.14±1.09 ^{b}
S. colias	2.82 (0.05)	10	6.88±0.66	10	7.57±0.76	10	6.64±0.98	10	6.66±0.82

Among the medium pelagic fishes, the energy density of *T. trachurus* slightly increased in winter and had similar low values in spring and fall. For the congeneric species, *T. mediterraneus*, energy density was lowest in summer and fall, and increased during winter with a peak in spring. *S. scombrus* had significantly lower energy density in winter than in spring and fall. On the contrary, the energy density of *S. colias* was significantly similar between seasons (Table 3, Figure 3).

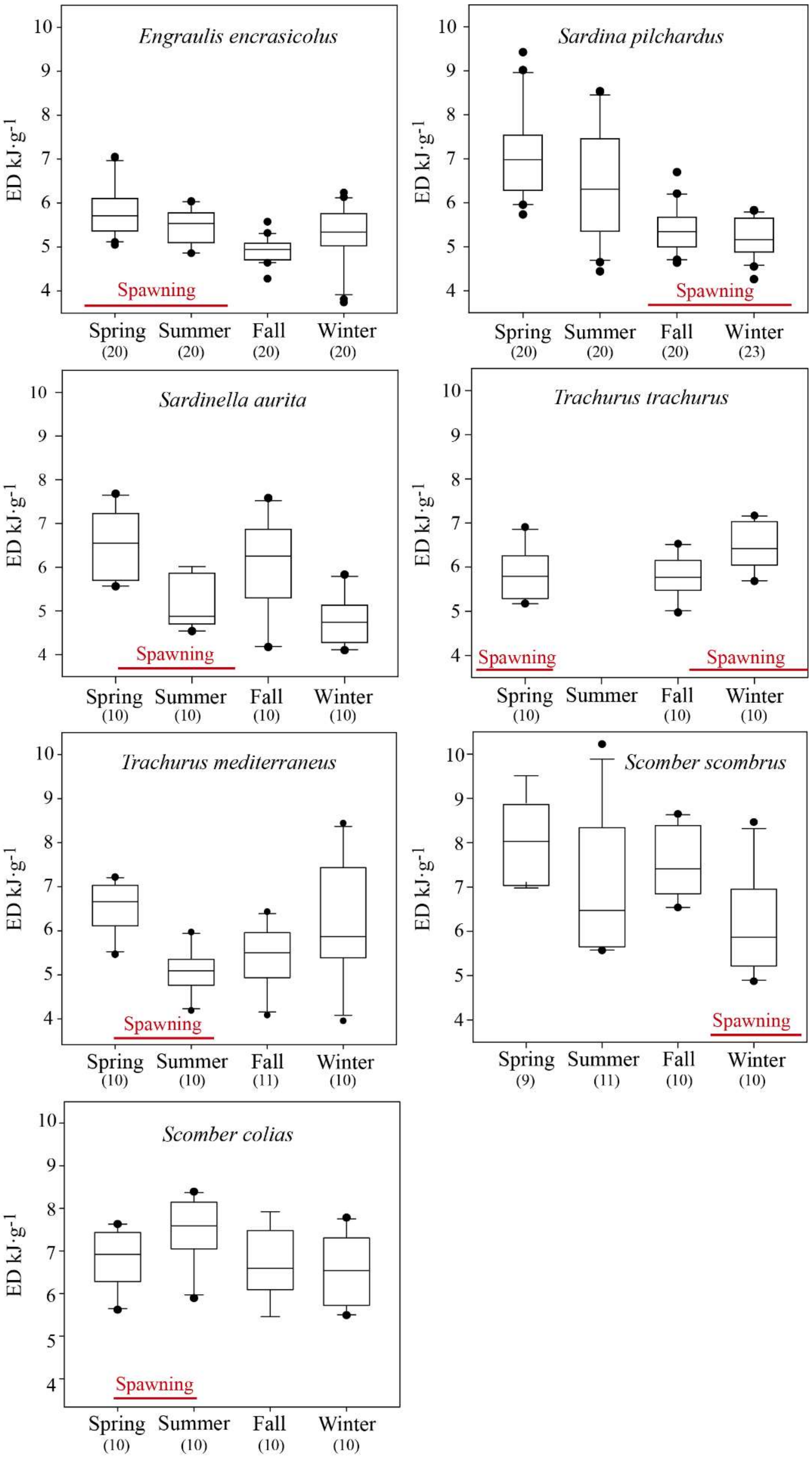

Fig. 3. Seasonal variation in the energy density (kJ g^{-1} of wet weight) of seven species of small and medium sized pelagic fishes from the western Mediterranean Sea. Box length represents interquartile range, bar length represents range and horizontal lines represent median values. Black dots are outliers.

3.4 Discussion

This study reports new energetic data on 354 individuals of seven ecologically and economically important pelagic fish species in the Mediterranean Sea. Results revealed clear differences in energy density between species and between seasons. These differences are related to species life-history and energy allocation strategy in addition to their feeding ecology. In consequence, predators of these small and medium sized pelagic fish could gain more or less energy per unit of food intake depending on the preyed species and period of the year.

Inter-specific differences in energy density

The two fishes that had the lowest energy density, *E. encrasicolus* and *S. aurita*, are species with fast growth, small body size and a lifespan of 4–5 years (Morales-Nin and Pertierra, 1990; Tsikliras and Antonopoulou, 2006). Both species have a greater reliance on a planktivorous diet (Palomera et al., 2007). In contrast, the two species with higher energy density, *Scomber scombrus* and *S. colias* are both highly migratory species that feed on wide range of prey sizes, have bigger body sizes than clupeiformes and a longer lifespan (10–15 years; Bachiller and Irigoien, 2013, 2015; Velasco et al., 2011; Table 1).

Previous studies in the same study area found that *Scomber* species occupy a higher trophic level in the food web than the small pelagic fish and segregate completely their trophic niche (Albo-Puigserver et al., 2016). Trophic level and body size are often collinear and represent important features of species since larger size fishes may have larger mouth size and may be able to feed on larger prey with higher energy content (Cohen et al., 1993; Jennings et al., 2008; Bachiller and Irigoien, 2013). Moreover, prey species described in the diet of *Trachurus* spp. and *Scomber* spp., such as mysids, decapods and fish, have higher energy density than copepods that are the main prey of clupeoid species (Mintenbeck et al., 2012; Table 1).

Apart from differences in the feeding habits, older (and often larger) fishes tend to have higher energy reserves since they do not invest energy in rapid growth as juveniles (Anthony et al., 2000; Røjbek et al., 2014). Moreover, species such as mackerels have large fat reserves in their muscle to cope with the energetic requirements of the migration (Ackman and Eaton, 1971; Ben Rebah et al., 2009; Graham et al., 1983). Larger fat reserves, in combination with the diet preferences, could explain the higher energy density showed in *S. colias* and *S. scombrus* in comparison with the other species analysed in this study.

In the case of *S. pilchardus*, the mean annual energy density was higher than the other clupeoid species and similar to those of *T. mediterraneus* and *T. trachurus*. However, in terms of dietary habits *S. pilchardus* is more closely related to *E. encrasicolus* than to *Trachurus* spp. that has a migratory behaviour, larger body size and might prey on larger species (Bachiller and Irigoien 2013, 2015). Opposite to *E. encrasicolus*, *S. pilchardus* has been described as a capital breeder that accumulates large amounts of energy before spawning in winter (McBride et al., 2015). Then, if the accumulation of energy before spawning is high enough, the capital breeding behaviour could explain the high mean annual energy density of *S. pilchardus*.

Seasonal differences in relation to spawning

Temporal variations in energy density, proximate composition and feeding preferences have been reported for several fish species worldwide. Over time, different species have adapted to environmental changes and ecological processes (i.e. competition and density dependency) by developing individual life-history strategies and spawning periods, which are reflected in their whole-body energy density measure over time (Gonçalves et al., 2012; Hondolero et al., 2012).

Similar to previous studies, in our study *S. pilchardus* showed more seasonal variability in energy density than *E. encrasicolus* due to their contrasting spawning periods and breeding strategies (winter and summer spawner and capital and income breeder, respectively; Ganias et al., 2007; Pethybridge et al., 2013; Sánchez et al., 2013). As a capital breeder and winter spawner (November-March; Palomera and Olivar, 1996), *S. pilchardus* accumulates mesenteric fat before reproduction during spring and summer when zooplankton biomass is high (Sabatés et al., 2006). As a consequence, we found higher energy density in spring and summer for this species. These results are in accordance with the dietary habits described in the Gulf of Lions for *S. pilchardus*, which in winter mainly preys on diatoms using its filter feeding capacity and in summer feeds on larger prey that are more energetic (Costalago and Palomera, 2014). In contrast, *E. encrasicolus* spawns mainly in warm waters, between 17° and 23 °C from April to August. The peak of reproduction is in spring and matches the period of high food availability (Palomera, 1992), allowing this species to use the food intake for reproduction directly. In the southwestern Mediterranean, Bacha and Amara (2009) observed that during spring and summer *E. encrasicolus* preyed on larger copepods with higher energy, probably to satisfy its reproductive needs.

In the case of *S. aurita*, the spawning period begins in late spring and has a peak during July-August because this species prefers warmer waters for spawning (Palomera et al., 2007). The high energy density in *S. aurita* in spring is probably due to the accumulation of energy just before the spawning period, following a more capital breeding strategy, while the low energy density in summer might be due to the loss of energy for reproduction. This pattern is in accordance with previous studies on lipid content of *S. aurita* in the Gulf of Gabes (South-Central Mediterranean Sea; Ben Rebah et al., 2009) and the Adriatic Sea (Mustać and Sinovčić, 2012).

The high energy density observed during the spawning period (winter) for *T. trachurus* indicates that the energy used for reproduction is mainly derived from the current feeding intake (van Damme et al., 2014). This result differs from previous studies on feeding intensity in the Adriatic Sea (Jardas et al., 2004; Šantić et al., 2005), where it was suggested that this species accumulates energy to use it later for reproduction in winter. On the contrary, in the NE Atlantic *T. trachurus* was defined as an income breeder, similar to our results (Bonnet et al., 1998; Ndjaula et al., 2009). In the case of *T. mediterraneus*, our results show that it accumulates energy prior to spawning. Previous studies in the Adriatic and Aegean Sea reported low feeding intensity and low lipid content during the spawning period (Šantić et al., 2004; Tzikas et al., 2007). Therefore, *T. mediterraneus* follows a breeding strategy more similar to a capital breeder. It is interesting to note that the two congeneric species of *Trachurus* spp. studied here utilize different breeding strategies that could play an important role in the resource partitioning that has been observed between these species in the northwestern Mediterranean Sea (Albo-Puigserver et al., 2016). However, given the single annual period sampled, further studies on the energy and feeding dynamics are needed to confirm these results.

Regarding the two *Scomber* species, both had low seasonal variability in energy density. The energy density of *S. scombrus* slightly decreased in winter, probably due to the energy investment in reproduction, while *S. colias* maintained similar energy density levels across all seasons, a characteristic of an income breeding species. Both species, besides preying on copepods, also have an active predation on larger prey such as euphasiids, decapods and fish larvae that are more energetic (Bachiller et al., 2015; Table 1). Moreover, opposite to small pelagic fishes, the migratory behavior of the larger *Scomber* spp. influences their energy requirements, resulting in higher demands.

Our results contrast with previous studies that proposed larger and more long-lived fish (i.e. mackerels) are more likely to use capital sources, and shorter-lived fish (i.e. sardine and anchovy) are more likely to use income sources for breeding (McBride et al., 2015; Somarakis et al., 2000). Instead, we suggest that the breeding strategy used in short and long-lived fish depends mainly if the spawning period is closely coupled with seasonal plankton blooms or not, a hypothesis that has been also suggested by others (Pethybridge et al., 2014; Røjbek et al. 2014). Furthermore, in long-lived fish other energetic requirements such as the large migratory movements might influence their life-history breeding strategy.

In general, we observed higher energy density in spring for all species, regardless of their breeding strategy. This result is in agreement with the higher energy input in the pelagic photic-zone during this period due to the late-winter phytoplankton bloom, when in the surface-water layers there is higher plankton food availability (Álvarez et al., 2012; Estrada, 1996). In other areas, it has been described that the composition of the phytoplankton drives the seasonal changes in energy reserves (i.e. lipids) of zooplankton (Peters et al., 2007; Røjbek et al., 2014). The greater food availability and its higher energy content explain the higher energy density of the small and medium pelagic fishes in spring.

We would like to highlight that this study only covers one year of sampling. Therefore, the capital -income breeding strategies identified for the studied species need to be confirmed with long-term (interannual) studies since food availability or zooplankton composition may vary from one year to another depending on environmental variability. Moreover, other aspects such as sex ratio might affect energy density during the reproduction period, since it has been observed that in some species the hydrated gonads of females are more energetic than the male gonads. In future studies, an interannual analysis of energy density on the different sexes is recommended.

Quality as prey

The pelagic fish species analysed in the present study are important components of the diet of different marine predators, including seabirds, marine mammals, elasmobranchs and large commercially fish such as tuna (see Table 1). These predators vary their own fitness not only by maximising quantity of prey, but also by prey selection (Österblom et al., 2008). In the case of the three small pelagic fish, only *S. pilchardus* was classified as a high quality prey. Previous studies in the Gulf of Lions and Bay of Biscay also measured high energy density in *S. pilchardus* (Vivien et al., 2012; Spitz and Jouma'a, 2013; Table 4).

However, as pointed out by Spitz and Jouma'a (2013), depending on the season, predators feeding on *S. pilchardus* will obtain more or less energy per unit of food. The energy densities observed in our study area for *E. encrasicolus* were lower than those reported in the Gulf of Lions, northwestern Mediterranean Sea (Table 4; Vivien et al., 2012) but similar to those reported in the Bay of Biscay (Atlantic Sea) and Adriatic Sea (Table 4).

Regarding medium pelagic fish species, *T. trachurus*, *S. scombrus* and *S. colias* were classified as high quality prey according to their energy density ranges. Similarly, in the Bay of Biscay these species were also classified as high quality prey mainly (Table 4). From a predator viewpoint, feeding on a species classified as high quality, such as, *S. pilchardus*, *T. trachurus*, *S. scombrus* and *S. colias*, results in more energy per unite of food intake than preying on *E. encrasicolus* or *S. aurita*. Therefore, for pelagic predators that prey mainly on small pelagic fish, such as Atlantic bonito, *Sarda sarda* and Bluefin tuna, *Thunnus thynnus*, it could be advantageous to prey on *S. pilchardus* during spring and summer when they are energetically better prey than *E. encrasicolus* (Navarro et al., 2017; Røjbek et al., 2014). However, other aspects such as the catchability of prey, time of searching, size of prey will also influence prey profitability (Bowen et al., 2002).

Table 4. Publish data on Energy Density (ED; kJ · g-1 of wet weight) for species of forage fish included in this study. Species are classified according to their ED content following Spitz et al. (2013) as Moderate Quality (4 < ED < 6 kJ g–1) and High Quality species (ED > 6 kJ g–1).

Species / Area	Year	Season	ED	References	Quality
E. encrasicolus					
Gulf of Lions	2002-2003	Spring	12.81±0.66	Vivien et al., 2012	High
Bay of Biscay	2002-2010	Spring	5.5±0.5	Spitz and Jouma'a, 2013	Moderate
Bay of Biscay	2002-2010	Fall	6.4±1.2	Spitz and Jouma'a, 2013	High
Bay of Biscay	2002-2010	Year mean	5.8±0.9	Spitz and Jouma'a, 2013	Moderate
Bay of Biscay	2001-2007	Winter	6.11±1.35	Dubreuil and Petitgas, 2009	High
Bay of Biscay	2001-2007	Spring	6.48±1.36	Dubreuil and Petitgas, 2009	High
Bay of Biscay	2001-2007	Fall	8.17±0.93	Dubreuil and Petitgas, 2009	High
Adriatic Sea	2002	Fall	5.56*	Tirelli et al., 2006	Moderate
Adriatic Sea	2003	Spring	4.51*	Tirelli et al., 2006	Moderate
S. pilchardus					
Gulf of Lions	2002-2003	Spring	14.14±1.49	Vivien et al., 2012	High
Bay of Biscay	2002-2010	Spring	5.8±0.8	Spitz and Jouma'a, 2013	Moderate
Bay of Biscay	2002-2010	Fall	8.8±1.6	Spitz and Jouma'a, 2013	High
Bay of Biscay	2002-2010	Year mean	7.5±2.0	Spitz and Jouma'a, 2013	High
T. trachurus					
Bay of Biscay	2002-2010	Spring	7.9±1.5	Spitz and Jouma'a, 2013	High
Bay of Biscay	2002-2010	Fall	6.5±1.0	Spitz and Jouma'a, 2013	High
Bay of Biscay	2002-2010	Year mean	7.0±1.3	Spitz and Jouma'a, 2013	High
S. scombrus					
Bay of Biscay	2002-2010	Spring	5.9±0.8	Spitz and Jouma'a, 2013	Moderate
Bay of Biscay	2002-2010	Fall	8.3±1.0	Spitz and Jouma'a, 2013	High
Bay of Biscay	2002-2010	Year mean	7.5±1.5	Spitz and Jouma'a, 2013	High

* Mean of two size class categories (110-119 mm and 120-129 mm).

Concluding remarks

This study reports reference values of the energy density of seven key pelagic fish species in an important fishing area of the western Mediterranean Sea for the first time. Results revealed clear differences in energy density between species and between seasons that are related to species life-history and energy allocation strategies in addition to their feeding ecology. These results highlight that when evaluating the energy intake by forage fish and the consequences of changes in population levels, attention should be paid to species and seasonal variability. The information derived from this study will enhance the accuracy of input data for bioenergetics-based population and food web models that are increasingly used as resource management assessment tools.

While this study shows that energy storage of small and medium pelagic fishes differs over time, the implications of long-term environmental changes (global warming and decreased primary productivity) on ecological energetics remains unknown. In the Mediterranean Sea an increase of the sea temperature and a decrease in plankton productivity have been already observed (Calvo et al., 2011; Mozetic et al., 2010; Vargas-Yáñez et al., 2008). These changes could impact the energy fluxes of marine food webs. In fact, recent studies have proposed that the main reason of the drastically decline in the biomass and in the mean size of *S. pilchardus* and *E. encrasicolus* in the western Mediterranean is a decrease in zooplankton quality and quantity due to environmental factors (Brosset et al., 2015; Saraux et al., 2014; Van Beveren et al., 2014, 2016). Moreover, an increase in the sea surface temperature during winter might cause a reduction in the reproduction window of *S. pilchardus* (winter spawner) (Palomera et al., 2007). Therefore, we could expect a change in the diet of marine predators from a diet dominated by *S. pilchardus* to one dominated by other species with lower nutritional quality, such as *S. aurita* that has been shown to expand its range northwards with the increase of temperature (Sabatés et al., 2006). Food web models that incorporate the energy density data provided in this study will assist in testing this, and other environmental hypotheses.

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Chapter 4

Year-round energetic dynamics of anchovy and sardine in the northwestern Mediterranean Sea

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Abstract

Body condition and energy storge variability has important implications for fish recruitment and population structure. It is essential to have a good understanding on the energy allocation strategy of fish in order to assess the differnet biological processes, reproduction, growth and maintenance, and evaluate the state of the fish stocks. In this study, we address the energetics dynamics of the annual cycle of anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) in the northwestern Mediterranean Sea using indirect and direct condition indices. We assessed and validated the use of morphometric, chemical and energetic indices for both species. Annual patterns of the relative condition index (Kn), gonadosomatic index (GSI), percentage of dry weight (%DW), lipid content (% lipids) and energy density (ED) were linked to the energy allocation strategy. Our results highlight that anchovy mainly rely on income energy to reproduce, while sardine accumulate the energy during the resting period to be used in the reproduction period. Variability in the % lipids and ED between seasons was lower in anchovy than in sardine. In both species, we observed an early decline in energy reserves in late summer-early fall, which may be related with unfavourable environmental conditions during spring and summer. Regarding the use of different condition indices, both direct indices, % lipids and ED, were highly correlated with Kn for sardine. Instead, ED was better correlated with Kn than lipids for anchovy. ED in gonads was highly correlated with GSI highlighting the importance of energy invested in reproduction. We produced the empirical relationship between ED and % DW and ED of gonads and GSI, which can be used to indirectly estimate ED of individuals and gonads in the study area. This work provides new information about energy dynamic of sardine and anchovy in the northwestern Mediterranean Sea and highlights the importance of understanding their seasonal energetic variation to understand population dynamics.

Keywords: anchovy, sardine, capital breeder, income breeder, condition, energy allocation, energy density, lipids.

4.1 Introduction

Small pelagic fish are a key component of pelagic ecosystems and support important fisheries in the world (Cury et al., 2000; FAO, 2018). Their significant biomass at mid-trophic levels makes these forage fish a main prey for numerous marine predators, playing a major role in energy transfer from lower to higher trophic levels (Bakun et al., 1996; Cury et al., 2011). As a consequence of their fast growth, short lifespan and their strong coupling to planktonic production, small pelagic fish populations are highly subjected to environmental variability and face strong variations in abundance and biomass all over the world (Checkley et al., 2009). Such fluctuations have a considerable impact on the whole ecosystem (Coll et al., 2008; Peck et al., 2013).

The two most important small pelagic fish in the Mediterranean Sea, in terms of biomass and commercial interest, are European anchovy (*Engraulis encrasicolus*) and European sardine (*Sardina pilchardus*) (Palomera et al., 2007). However, important changes in abundance, landings and biological features (such as growth and body condition) have been reported for both species in the northwestern Mediterranean (Brosset et al., 2017; Quattrocchi & Maynou, 2017). These changes have been partially attributed to particular oceanographic parameters and increase in fishing pressure (Van Beveren et al., 2014; Brosset et al., 2017; Coll et al., 2019; Saraux et al., 2019).

As a consequence of the annual and seasonal fluctuation that the pelagic marine environment presents, marine organisms have developed several strategies for energy acquisition and allocation to reproduction. The classical division of these strategies is made between capital and income breeders (Drent & Daan, 1980; Stearns, 1989). In capital breeders, the primary energy source for reproduction comes from reserves stored prior to the spawning season, while in income breeders, reproduction is fully supplied by concurrent energy intake, i.e. current feeding. In practice, life-history strategies are represented along the whole continuum of these two extremes (McBride et al., 2015).

According to previous studies in the Mediterranean Sea, anchovy, that spawns in spring and summer, seems to be mainly an income breeder (Somarakis et al., 2004; Somarakis, 2005; Pethybridge et al., 2014; Brosset et al., 2015a), while sardine, that spawns in fall and winter, seems to be mainly a capital breeder (Ganias et al., 2007; Ganias, 2009; Mustać & Sinovčić, 2009; Pethybridge et al., 2014). Due to their different energy use strategies towards growth, reproduction and maintenance, and their opposite reproduction periods, it is plausible to to expect that they could have different ecological responses to environmental change that are currently undergoing in the Mediterranean Sea, such as an increase in sea surface temperature and changes in primary productivity (Giorgi, 2006; Piroddi et al., 2017; Hoegh-Guldberg et al., 2018; Oliver et al., 2018). Yet, it is not well known how those changes will affect the energy acquisition and allocation of anchovy and sardine populations and will ultimately affect their reproduction and growth (Nunes et al., 2011).

The evaluation of the nutritional and physiological status of a population is increasingly used as an indicator of fish stock state and is directly linked to fitness (Rosa et al., 2010; Lloret et al., 2013; Brosset et al., 2017). Individuals with better physiological condition, which means higher nutritional reserves, may have higher growth and survival rates, and greater reproductive success (Brosset et al., 2015a). Several condition indexes to evaluate the physiological status of fishes are available (Lloret et al., 2013). Condition of fish is mainly a measure of stored energy that can be evaluated with direct measures (e.g., lipid content and energy density) or indirect measures (e.g., morphometric index) (Lloret et al., 2013; Schloesser & Fabrizio, 2017; Gatti et al., 2018). However, not all indices reflect exactly the same type of energy stored.

The main energy store in fish is in the form of lipids and proteins (Lloret et al., 2013). Lipids are the preferred source of metabolic energy for growth, reproduction, and swimming in fish and the first macro-molecule to be catabolized (Shulman & Love, 1999; Tocher, 2003). Instead, proteins and carbohydrates, that are the main compounds of body structure, usually remain rather constant and are less energetic than lipids (Anthony et al., 2000). However, in cases of high lipid depletion proteins can be mobilized and used as energy source (Black & Love, 1986). Therefore, when evaluating the condition of fishes, it is important to understand what index to use and what it is measuring. In addition, it is important to account for the period when measurements are taken (reproductive or non-reproductive period), since during the non-reproductive period lipids are mainly allocated in the muscle, while during the reproduction period lipids are also allocated in the gonads, as it has been described for small pelagic fish in the Mediterranean Sea (Brosset et al., 2015a).

Although indirect condition indices and lipid content of muscle tissue have been preferably used in the study of small pelagic fish condition (Ganias et al., 2007; Rosa et al., 2010; Pethybridge et al., 2014; Brosset et al., 2015a, 2017), the amount of energy per unit of mass (Energy Density; ED) is the only measure that directly gives information on the average proximate composition of fish (weighted average of protein, lipid and carbohydrates energy densities; Gatti et al., 2018). Two previous studies in the Mediterranean have used ED to study the condition of sardine and anchovy (Tirelli et al., 2006; Albo-Puigserver et al., 2017). Although it is recommended to validate the body condition indeces before using them to define their suitability (McPherson et al., 2010; Brosset et al., 2015b; Gatti et al., 2018), the use of ED in sardine and anchovy has not been previously compared with other indirect and direct body condition indeces in the Mediterranean Sea.

Considering all the above, the aims of this study were (1) to assess seasonal dynamics on the condition and energy allocation of sardine and anchovy in the northwestern Mediterranean Sea, and (2) to determine the relationship between different condition indices in anchovy (income breeder) and sardine (capital breeder). These comparisons allowed us to understand which of the condition measurements better capture the variability in the physiological state of small pelagic fish populations. Specifically, indirect morphological condition indices, total water content, and direct chemical (lipid content of muscle tissue) and energetic (direct calorimetric analyses of whole specimens and gonads) analyses of condition were performed. Moreover, the used of direct calorimetry as a measure of physiological condition was assessed and a relationship with total water content was developed. Understanding how these species allocate their resources over a year is fundamental to predict the responses of small pelagic fish to environmental variability and changes, and the ultimate effects on marine food webs, which has a direct informative value for the management of marine resources and ecosystems.

4.2 Material and Methods

Sampling and study area

European anchovy (*Engraulis encrasicolus*) and European sardine (*Sardina pilchardus*) samples were collected monthly from purse-seine landings off the Tarragona harbour (Spain; northwestern Mediterranean) that operate in the Ebro Delta continental shelf area from April 2012 to March 2014. Due to fishing closures, no samples were collected for January 2013 and January 2014.

The Ebro Delta continental shelf is a major spawning ground for anchovy and sardine (Palomera, 1992; Tugores et al., 2011; Giannoulaki et al., 2014). Their primary productivity is largely subjected to the environmental features of the region. In the northwestern Mediterranean Sea there is typically a late winter – early spring phytoplankton bloom, enhanced by strong riverine nutrient input (Salat, 1996; Lloret et al., 2001, 2004), followed by a spring increase in zooplankton (Sabatés et al., 2007; Fig. 2A). Anchovy spawns in warm waters, between 17 and 23 ºC; which in the northwestern Mediterranean begins at the end of spring and extends throughout the summer (April – September) (Palomera, 1992; Palomera et al., 2007). Sardine prefers colder waters to spawn, between 12 and 14ºC; therefore, the spawning period of sardine in the northwestern Mediterranean is from middle fall until the end of winter (November – March) (Palomera & Olivar, 1996; Palomera et al., 2007).

Body condition indices

In total, 2078 anchovy and 1957 sardine were sampled. Total length (TL ± 0.1 cm), total weight (TW ± 0.01 g), gutted weight (GW ± 0.01 g), sex (M = male, F = female) and gonad weight (W_G ± 0.1 mg) were recorded for all fish. The macroscopic maturity phase was determined for all individuals using the anchovy and sardine maturity stage keys of (ICES, 2008): 1 = immature; 2 = developing; 3 = spawning capable; 4 = spawning; 5 = post-spawning/spent, 6 = resting.

Only individuals larger than the minimum landing size (TL ≥ 9 cm for anchovy and TL ≥ 11 cm for sardine; Ganias et al. 2007) were used in the analysis in order to avoid possible size-related bias due to variation in monthly length frequency distributions of smaller individuals. After dissection, individuals were conserved at -20ºC.

a) Indirect body condition indices

The somatic condition of both species was evaluated by calculating the relative condition factor (Kn, Le Cren 1951). The Kn was obtained as the ratio of the gutted weight (GW) to the corresponding predicted gutted weight (Wp) for a fish of the same length (Le Cren, 1951):

$$(1) \qquad \mathrm{Kn} = \frac{\mathrm{GW}}{\mathrm{W_p}}$$

The Wp was obtained by performing a nonlinear regression of GW as a function of $a \cdot TL^b$, where a and b are coefficients estimated from all fish sampled during the years of 2012–2014 (with values for anchovies: a = 0.0029, b = 3.2538; and for sardines; a = 0.0037, b = 3.2309). We used the Kn index as a proxy of somatic condition for fish. Gutted weight is preferred to the total weight to avoid the influence of gonad development on the true somatic condition of individuals (Millán, 1999; Nunes et al., 2011).

The measure of water content of fish has been used as a proxy of lipids, as it is often inversely related to total lipid content (Lloret et al., 2013). The relationship between energy density and dry weight has also been well documented for different fish species. The percentage of dry weight was calculated for 80 specimens of each species (20 per season). Individuals were oven-dried at 60ºC to constant mass for 48 – 72 hours. After determining their dry weight (W_{Dry} ± 0,001 g), they were stored and used for direct calorimetric analysis.

To relate the reproductive cycle with the relative condition factors, the gonadosomatic index (GSI) and the percentage of reproductively active individuals were calculated as a measure of reproductive activity (Somarakis et al., 2004; Basilone et al., 2006; Ferrer-maza et al., 2016). GSI was obtained as the ratio of gonad weight (W_G) to gutted weight (GW):

$$(2) \qquad \mathrm{GSI} = \frac{\mathrm{W}_G}{\mathrm{GW}} \cdot 100$$

The proportion of reproductive individuals during the year was obtained considering as actively spawning individuals those with maturity stage 3, 4 and 5 and as not actively spawning individuals those with maturity stage 1, 2 and 6 (ICES, 2008).

In order to qualitatively relate the variability in Kn and GSI with the seasonal environmental variability, monthly satellite-derived sea surface temperature (SST; ºC) and chlorophyll-a concentration (Chl-a; mg·m^{-3}, at 2km resolution) were obtained for the study area during the sampling period (April 2012 to March 2014) from Environmental Marine Information System(EMIS, JRC, https://data.jrc.ec.europa.eu; Melin, 2013).

b) Direct body condition indices

We used biochemical and direct calorimetry analysis to measure two direct condition indices: lipid content (% lipids) and energy density (ED).

Lipid content was analysed in 80 individuals of each species (20 per season) from the first year of sampling (spring 2012 to winter 2013). The lipid content of each individual was extracted from a sample of dorsal muscle (200 to 500 mg) using the Folch method (Folch et al., 1957). The total lipids extracted from each sample were weighted (± 0.0001 g) and were expressed as the percentage of wet weight (W_{Wet}, ± 0.0001 g), which was calculated as follows:

$$(3) \qquad \%\,\text{lipids} = \frac{lipids\ weight\ (g)}{sample\ Ww\ (g)} \cdot 100$$

Analyses of the energy density (ED, kJ·g$^{-1}W_{Wet}$) were performed on whole individuals and gonads of both anchovy and sardine from the first year of sampling by direct calorimetry using a Parr 6725 Semimicro Oxygen Bomb Calorimeter (Moline, Illinois, USA). In the case of the ED of the whole individual, we used the 80 specimens of each species previously oven-dried (20 per season). ED was determined individually according to the protocol used in previous studies (Tirelli et al., 2006; Dubreuil & Petitgas, 2009; Albo-Puigserver et al., 2017). The oven-dried individuals were mixed to obtain a homogenised powder of each individual, from which pellets of 150 to 200 mg were obtained with a press. Two of these pellets were used for the determination of the ED, and if the values differed by more than 3%, a third pellet was combusted. The average of the two or three samples was used to estimate the ED of each individual. The ED was converted to a wet-weight basis (kJ g$^{-1}W_{Wet}$) using the percentage of dry weight ($\%W_{Dry}=100 \cdot W_{Dry}/W_{Wet}$) of each fish. In the case of ED analysis of gonads, if the gonads of an individual fish were not large enough to perform the analysis (the calorimeter can only process samples that range from 25 to 200 mg), they were pooled by sex, body length and maturity stage to obtain an adequate weight for the analysis (gonad weight: W_G). The analysis was determined for 129 anchovy (29 from spring, 33 from summer, 37 from autumn, and 30 from winter) and 131 sardines (45 from spring, 27 from summer, 29 from autumn and 30 from winter). The same protocol mentioned above for the entire individuals was followed for the ED determination of gonads (from an individual or group).

Statistical analyses

Differences in Kn and GSI of anchovy and sardine between months and sexes were statistically compared using a two-way semi-parametric permutation multivariate analyses of variance tests (*PERMANOVA* test) based on Euclidean distance matrices with a previous square-root transformation (Anderson et al., 2008). Spearman's rank non-parametric correlation test was used to examine the relationships between Kn and GSI.

Differences in lipid content and energy density between seasons, sexes or between maturity stages, in the case of gonad analysis, for sardine and anchovy and between species were also tested using a two-way semi-parametric permutation multivariate analyses of variance tests (*PERMANOVA* test) based on Euclidean distance matrices with a previous square-root transformation (Anderson et al., 2008). In the case of significant differences pairwise tests were performed. Analyses were run using PRIMER-E v6 software (Clarke & Gorley, 2006).

The relative condition index (Kn) was plotted against the other direct and indirect condition indexes (% lipids, ED and % dry weight), and their correlation was calculated by means of the Spearman's rank non-parametric correlation coefficient. Relationship between energy density of individuals with the percentage of dry weight and the relationship between energy density of gonads and the percentage of gonadosomatic index were explored using linear and logarithmic regressions, respectively, and adopting a significance level of $p< 0.05$. Spearman's rank non-parametric correlation tests and linear regression analyses were performed with R v3.6.0. (R Core Team, 2019).

4.3 Results

Seasonal variation in the indirect body condition indices

Monthly variation in the GSI during the 2012-2014 annual cycle exhibited opposite annual patterns for anchovy and sardine of both sexes (Fig. 1). GSI of anchovy reached the maximum values between April and August while sardine reached maximum values between November and March (Fig. 1B and 1D). Differences between sexes in GSI were observed in both species (anchovy: Pseudo-$F_{1,1985}$=30023, p=0.001; sardine: Pseudo-$F_{1,1866}$=105.61, p=0.001).

The percentage of active spawning individuals showed similar patterns to GSI (Fig. 2). Active spawning individuals of anchovy were observed from April, one month after the peak of Chl-a and when SST started to increase, to October, when SST started to decrease (Fig. 1A and 2). More than 90% of females were actively spawning in June and July of 2012 and in July, August and September of 2013, coinciding with the period of higher SST and lower Chl-a concentrations (Fig. 2A and 3). Whereas, sardine actively spawned from October to March, coinciding with the decrease of the SST. However, in April and May of 2012 the proportion of actively spawning individuals reached almost 50% and 40%, respectively. The peak of active spawning was in December and February of 2012 and December 2013 when SST was at the lowest and the Chl-a concentration started to increase (Fig. 1A and 2).

The Kn of anchovy exhibited high intra-annual and even intra-seasonal variability and was synchronous between sexes (Fig. 2C). There were significant differences in Kn between months (Pseudo-$F_{21,1999}$=27.566, p=0.001), but not between sexes (Pseudo-$F_{1,1999}$=0.18577, p=0.671). High values of Kn were observed in spring and low values in the fall. Kn and GSI values exhibited a weak but significant positive correlation (r_s= 0.26, p < 0.001).

For sardine, significant differences in Kn between months and sexes were observed (Pseudo-$F_{21,1877}$=67.459, p=0.001; Pseudo-$F_{1,1877}$=5.7722, p=0.023, respectively) (Fig. 1E). However, the differences between sexes were only observed in August 2013 (pairwise comparison t=2.3608, p=0.027). Individuals had higher Kn values during spring and summer and lower values of during fall and winter (Fig. 1D, E). Kn and GSI exhibited a significant negative correlation (r_s= 0.44, p < 0.001).

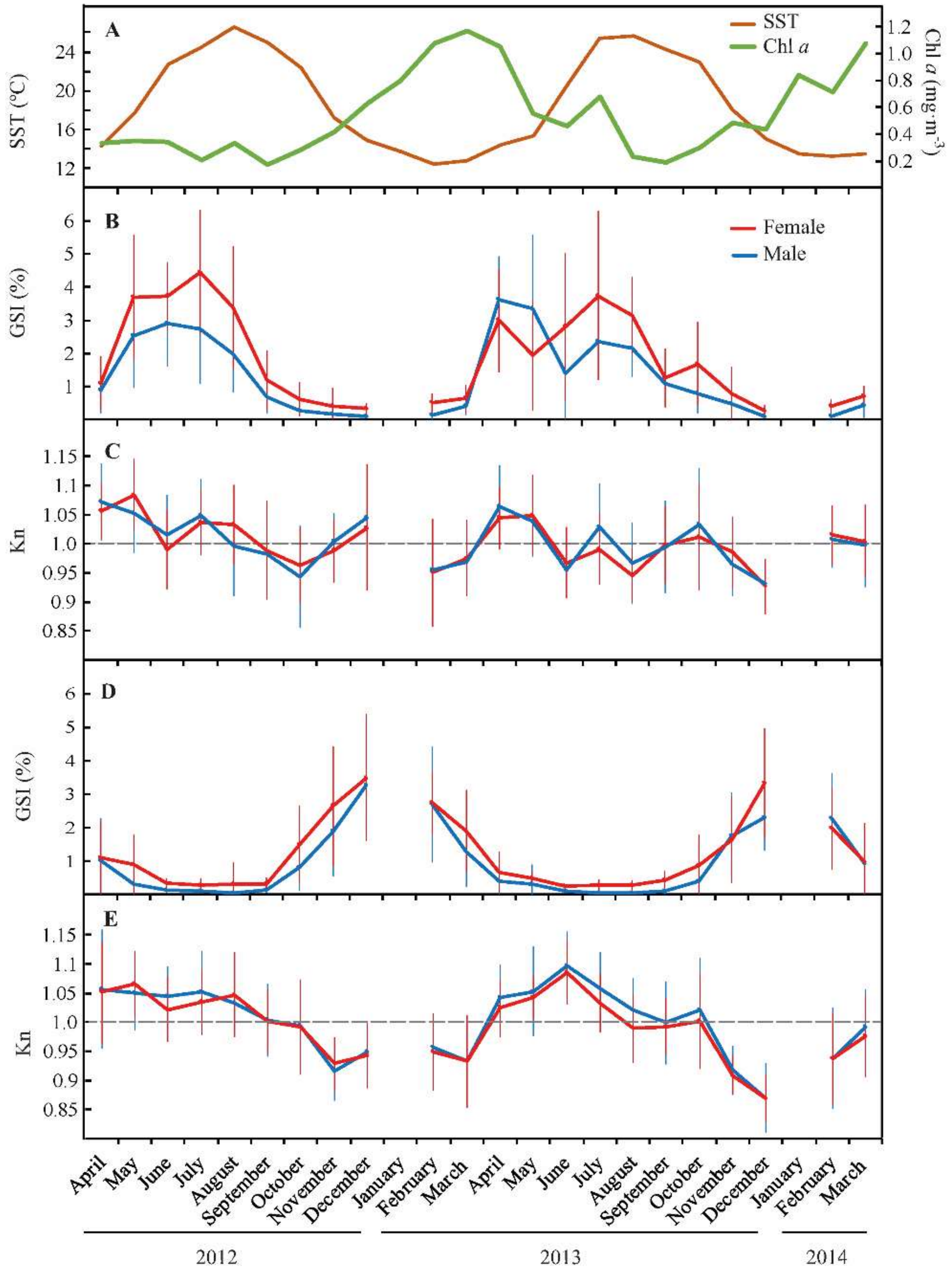

Fig. 1. (A) Monthly mean sea surface temperature (SST; orange line) and chlorophyll *a* concentration (Chla; green line) of the area of study (source: EMIS JRC, https://data.jrc.ec.europa.eu/). Mean and standard deviation of monthly variation of gonadosomatic index (GSI) and relative condition index (Kn) for females (red) and males (blue) of anchovy (B and C) and sardine (D and E), respectively.

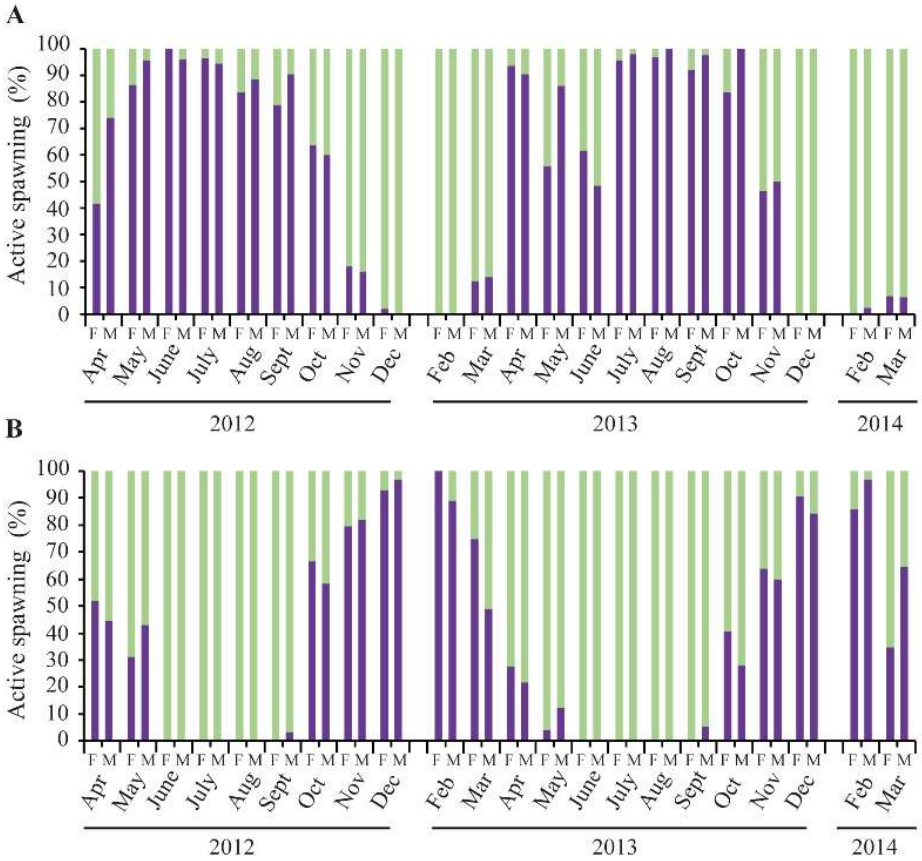

Fig. 2. Monthly variation of the percentage of mature active individuals in blue (maturity stage 3, 4 and 5; ICES 2008) and immature and resting individuals in green (maturity stage 1, 2 and 6) for anchovy (A) and sardine (B). Females (F) and males (M) proportions are represented separately.

Seasonal variation in the direct body condition indices

In both species significant seasonal variations in lipid content were observed (Pseudo-$F_{3,71}$=20.33, $p<0.001$; Pseudo-$F_{3,70}$=19.15, $p<0.001$, for anchovy and sardine, respectively). In the case of anchovy, only spring had significantly higher lipid content (Fig. 3A). Regarding sardine, lipid content in spring and summer was similar and significantly higher than values in fall and winter (Fig. 3B). Lipid fraction in the muscle of anchovy and sardine was similar between sexes (Pseudo-$F_{1,73}$=3.69, $p=0.05$; Pseudo-$F_{1,72}$=1.44, $p=0.24$, respectively).

Similar to lipid content, in both species differences in ED were only found between seasons (Pseudo-$F_{3,71}$=8.55, $p<0.001$ for anchovy and Pseudo-$F_{1,73}$=21.21, $p<0.001$ for sardine) and not between sexes (Pseudo-$F_{2,71}$=0.35, $p=0.68$ for anchovy and Pseudo-$F_{1,73}$=1.95, $p=0.15$ for sardine). In the case of anchovy, the pairwise comparison of ED between seasons showed that ED was maximum in spring and declined in summer and fall with significantly different ED values, while in winter the ED of anchovy was similar to the ED levels of summer (Fig. 3C). For sardine, in spring and summer on one hand and in fall and winter on the other hand the individuals had similar ED values. Between the two periods (spring-summer and fall-winter) significant differences were found in ED, similar to what we observed for the lipid content (Fig. 3D).

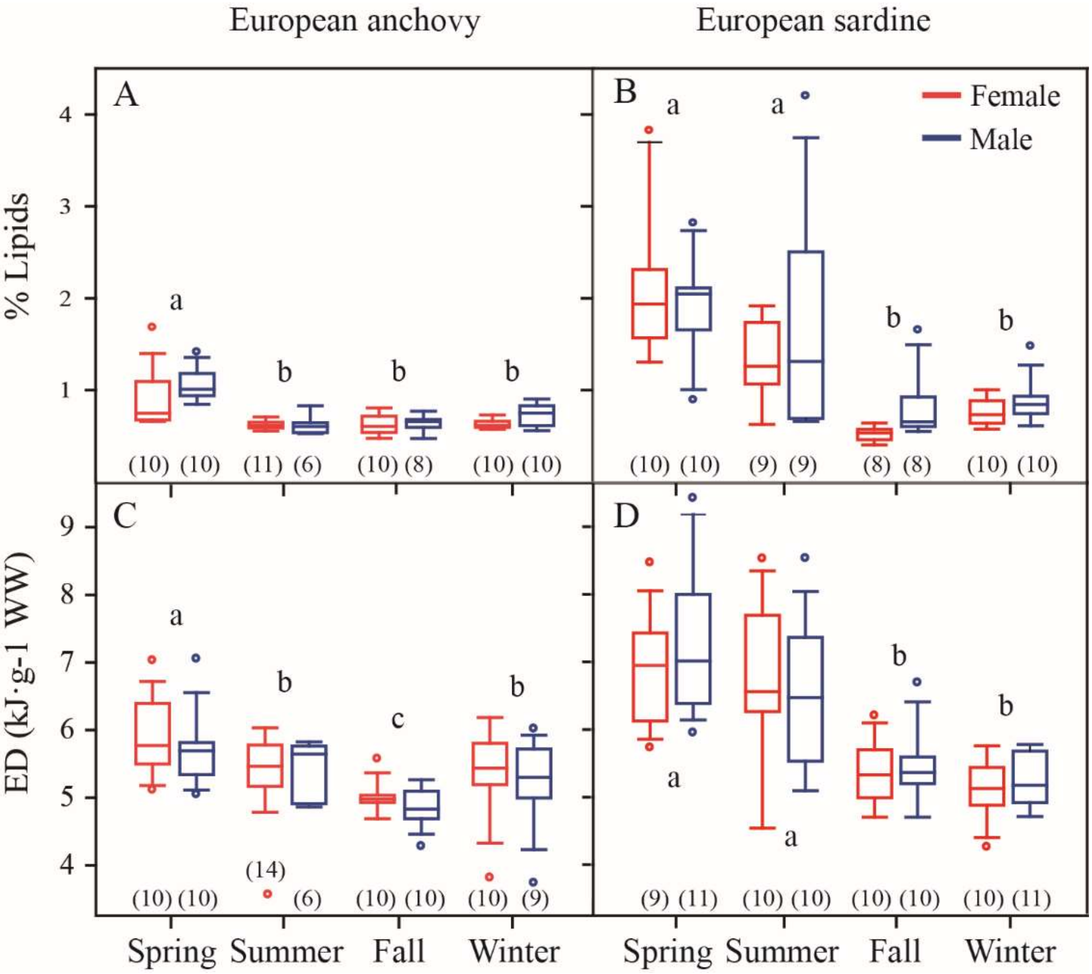

Fig. 3. Boxplots of seasonal lipid content (A-B) (% lipids·g^{-1} wet weight) and energy density (C-D) (kJ·g^{-1} wet weight) of European anchovy (*Engraulis encrasicolus*) at left and European sardine (*Sardina pilchardus*) at right. Females in red and males in blue. Box length represent interquartile range, bar length represent range and horizontal lines represent median values, dots are outliers. Number in brackets are the sample size of each boxplot. Pairs of means differing significantly ($P < 0.05$) by pairwise test between seasons within each graph and both sexes together are indicated by letters- seasons with the same letter were not significantly different.

Comparing both species, the lipid content of sardine in spring, summer and winter was significantly higher than in anchovy ($\text{Pseudo-F}_{1,141}=64.99$, $p<0.001$), and no differences in the lipid content were observed in fall between species (Figs. 3A and 3B).

Similarly, in the case of the ED results, sardine values where significantly higher in spring, summer and fall than in anchovy (Pseudo-$F_{1,154}$=35.19, p<0.001), and no differences in ED were observed in winter between species (Figs. 3C and 3D).

Comparison of body condition indicators

The ED on a wet weight basis was linearly correlated to the DW for both species. The linear models fitted for each species were statistically significant and explained 91% of the total variation in ED in anchovy and 94% of the total variation in ED in sardine (Fig.4).

The relationship between relative condition factor (Kn) and energy density, lipid content and dry weight were positively correlated for anchovy and sardine (Fig. 5). For anchovy, the correlation was stronger between Kn and ED (Fig. 5A) or %DW (Fig. 5E) than in the correlation between Kn and % Lipids (Fig 5C). Instead, for sardine the higher correlation was between Kn and %Lipids (Fig. 5D) followed by ED (Fig. 5B) and %DW (Fig. 5F).

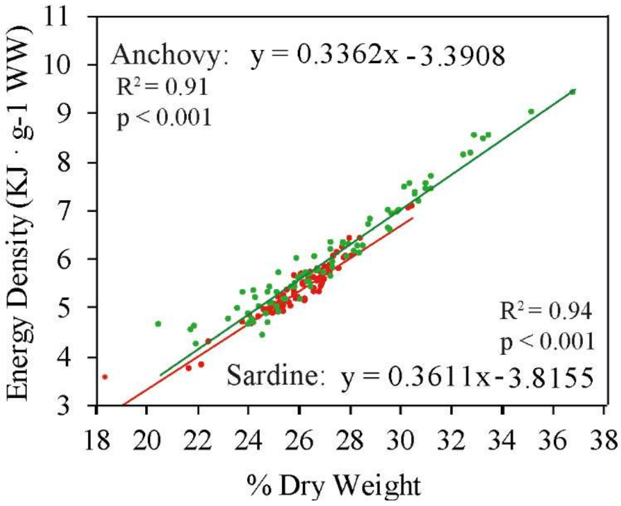

Fig. 4. Linear model relationships between energy density and per cent dry weight of European anchovy (red) and sardine (green).

The calorimetry of gonads revealed that anchovy and sardine had similar energy density values in the gonads (Pseudo-$F_{1,210}$=1.95, p=0.16). For both species, energy density of gonads varied between reproduction stages, with higher values of ED_{gonads} in actively spawning individuals (reproduction stage 3, 4 and 5) than for immature or resting individuals (reproduction stage 1, 2 and 6) (Pseudo-$F_{5,210}$=49.18, p<0.001; Table 1). No significant differences were detected between sexes in the ED_{gonads} of anchovy (Pseudo-$F_{1,101}$=7.30, p=0.79). Instead, sardine presented differences in ED_{gonads} between sexes (Pseudo-$F_{1,109}$=15.07, p<0.001).

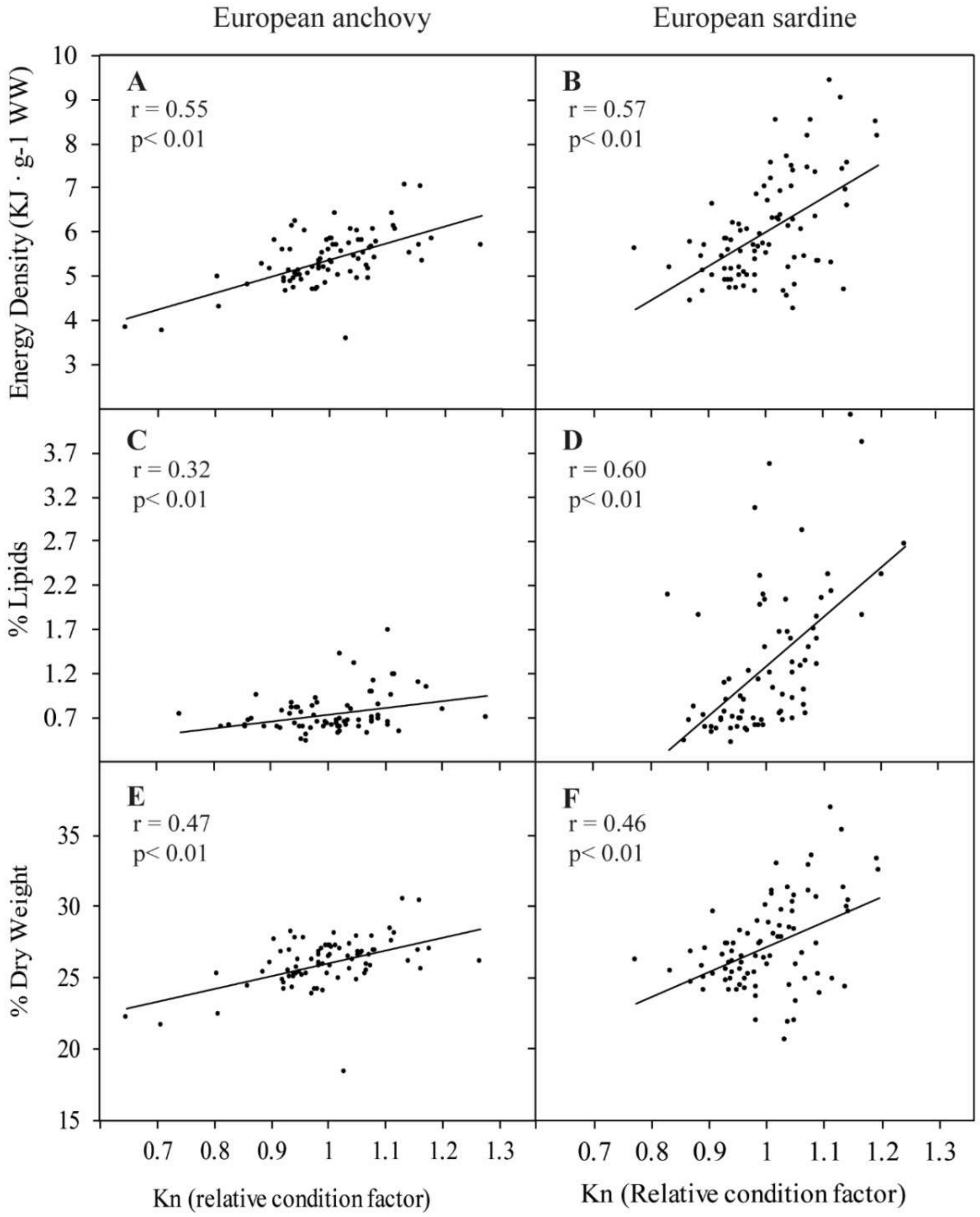

Fig. 5. Relationships between the relative condition factor (K_n) and energy density (ED; $KJ{\cdot}g^{-1}$ wet weight), lipid content (% Lipids) and dry weight content (ratio of dry and wet weight in %) for anchovy (A, C, E) and sardine (B, D, F), respectively. Spearman correlation and the level of significance are indicated (r; p). Lines indicate significant correlations.

The ED_{gonads} of anchovy and the GSI showed a strong positive correlation for both sexes (r_s=0.85, p<0.001; r_s=0.80, p<0.001, for females and males, respectively). The relationship fitted to a logarithmic regression explained 69% and 62% of the variance in anchovy females and males, respectively (Fig. 6A). Sardine also showed a positive correlation between ED_{gonads} of female and males and GSI (r_s=0.86, p< 0.001; r_s=0.64, p<0.001, for females and males, respectively). Instead, the logarithmic regression of sardine males explained only 25% of the variance, while the logarithmic regression of sardine females explained 72% of the variance (Fig. 6B).

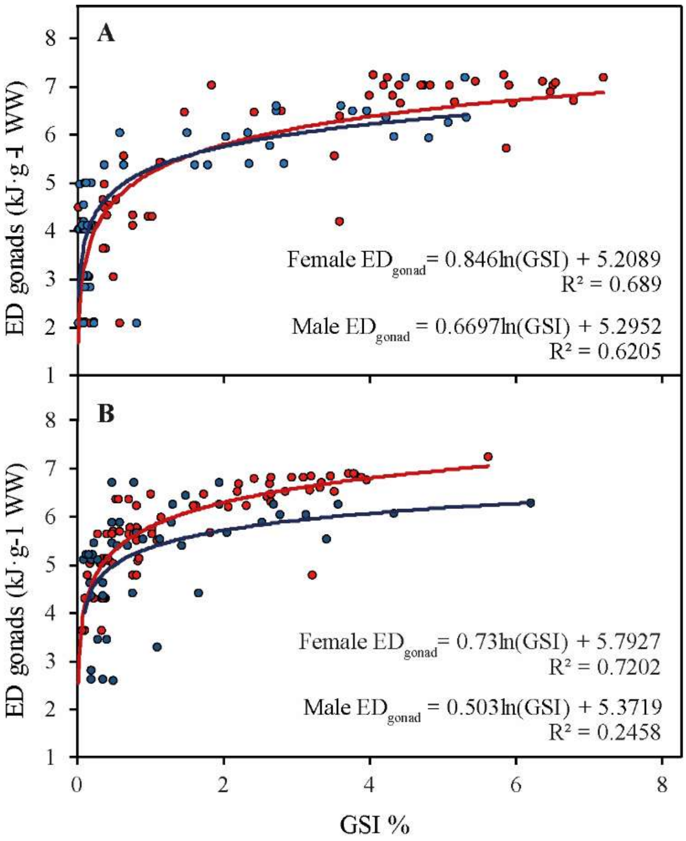

Fig. 6. Relationship between the gonadosomatic index (GSI %) and energy density of gonads (ED gonads; KJ·g^{-1} wet weight), for anchovy (A) and sardine (B). Females are represented in red and males in blue. Equation and logarithmic regression lines indicated are represented when the spearman correlations are significant.

4.4 Discussion

Annual body condition and energetic cycle of anchovy and sardine

Anchovy presented inter and intra-annual variability in the relative body condition index (Kn). In both years analysed, Kn was higher in spring, mainly after the peak in Chl-a and in synchrony with the increase of GSI. The higher values of Kn at the beginning of spring were in accordance with the higher lipid content and ED values observed for anchovy in spring. These results seem to indicate that anchovy relied in large proportion on current food intake for reproduction. Therefore, as described in previous studies, anchovy mainly exhibited an income breeder strategy (McBride et al., 2015; Brosset et al., 2017).

Anchovy ED, % lipids and Kn were the lowest immediately after the spawning season, suggesting that the final balance between energy intake and reproductive costs was negative and led to a deterioration of anchovy body condition. We observed a depletion in lipid content already in summer before the end of the reproduction activity, whereas the decline in ED was more progressive with minimum values after the reproduction period. This could indicate that lipids in the muscle were the first source of energy to be mobilized to the development of gonads in spring. In previous studies in the Gulf of Lions and the Strait of Sicily, body condition of small pelagic fish was positively related with river run-off, Chl-a, and diatoms and meso-zooplankton concentrations (Basilone et al. 2006, Brosset et al. 2015). Basilone et al. (2006) pointed out that the energy gained and stored before the spawning period might affect the reproductive output of anchovy in the Strait of Sicily. Therefore, the low lipid content and ED observed in our study at the end of the reproduction period might reflect unfavourable environmental conditions during the reproduction period in 2012-2014. Although in this study it is not possible to determine if the observed pattern is year-specific or is representative of other years, the low lipid and ED values observed are in line with the decline in body condition observed in anchovy in the last decade in the Mediterranean Sea (Van Beveren et al., 2014; Brosset et al., 2017; Albo-Puigserver et al., 2019).

On the contrary, all condition indeces (Kn, ED, Lipid) in sardine had a highly marked seasonality, with inverse patterns between Kn and GSI. The sardine spawning season covered the colder months of the year, peaking between December and February, as already described in previous studies (Palomera, 1992; Palomera & Olivar, 1996; Palomera et al., 2007). During the reproduction period, Kn, ED and lipids were at their lowest values. Rapidly after the end of the reproduction period, coinciding with the spring increase in zooplankton enhanced by strong riverine nutrient input at the Ebro Delta continental shelf (Salat et al. 2002, Lloret et al. 2004), a high increase in Kn, ED and lipids was observed for sardine. Similar to previous studies (Ganias et al., 2007; Nunes et al., 2011; Brosset et al., 2015b), sardine accumulated energy during the resting period and seemed to supply reproduction costs with stored resources, presenting a clear capital breeding strategy.

Kn in sardine peaked in June and in August 2013 was below 1. This imply that energy storage for reproduction was not maintained at high levels until the reproduction activity started (October), as it would be expected in a species with a capital breeding behaviour (McBride et al., 2015). In contrast, in previous studies of body condition, it has been observed how Kn is maintained high until the increase of GSI due to the mobilization of fat reserves for the development of gonads (Ganias et al., 2007; Nunes et al., 2011; Brosset et al., 2015a). Then, similar to the hypothesis proposed for anchovy, the decrease of Kn in sardine before the reproduction period could also be related with unfavourable environmental conditions that did not permit to accumulate enough energy reserves during spring and summer. A similar pattern of a decline in condition at the end of summer was described for sardine in the Gulf of Lions, and was attributed to a change in phenology of primary and secondary production (Brosset et al., 2015a). The low energy reserves observed at the beginning of the reproduction period, could also suggest that sardine may also rely on direct food intake towards the end of the in reproduction period. Therefore, sardine would be able to deploy both capital and income breeder strategies as was previously suggested for sardine of the eastern Mediterranean (Ganias, 2009) and Atlantic (Garrido et al., 2007).

The low amount of fat reserves that sardines accumulated prior to the spawning season during our study years could have had an important effect on the quality of eggs produced during the spawning season, as was demonstrated for the Iberian sardine in Portugal (Garrido et al., 2007). ED analyzed in female gonads of sardine was higher than males. Anchovy had similar ED_{gonads} between sexes and were similar to values observed in sardine. In both species ED_{gonads} was high during reproductive active stages with high GSI values when oocytes are hydrated, highlighting the energetic investment required by reproductive activity (Wang & Houde, 1994).

Direct and indirect condition indeces in small pelagic fish

In sardine, both direct methods (% lipids and ED) were highly correlated with the indirect method Kn, and all of them successfully captured the variability in energy reserves between the reproduction and the resting period of sardine (spring-summer and fall-winter respectively). Instead, in anchovy ED was better correlated with Kn than %lipids, suggesting that ED and Kn better captured changes in body condition than lipid content. The %DW, that has also been positively related with lipid content in previous studies and used as an indirect condition index (Lloret et al., 2013), showed a significant positive correlation with Kn.

The better correlation of lipid content with Kn in sardine is explained by the higher variability of lipids in sardine than in anchovy due to their opposite breeding strategy (Albo-Puigserver et al. 2017). Sardine accumulates a high quantity of lipids in the muscle and mesenteric fat during the resting period that are then used for reproduction (Pethybridge et al., 2014; Brosset et al., 2015b; Albo-Puigserver et al., 2017). Instead, anchovy accumulates less energy, since the energy gain is used directly for reproduction and fewer energy is allocated to reserves (Albo-Puigserver et al., 2017; Gatti et al., 2018). While lipid content analysis only measures the bulk of lipids of the individual, direct calorimetry analysis also measures changes in other compounds such as proteins, which are usually mobilized when lipids are low. Then, in species that do not accumulate high quantities of lipids, like anchovy, direct calorimetry analysis to obtain ED measurements would be preferable than lipid content analysis. Instead, in species that store high quantities of energy, such as sardine, both lipid content and direct calorimetry are appropriate methods to study body condition variability.

In the case of samples that were used for direct calorimetry, another indirect condition method was calculated, the percentage of dry weight. According to our results, this index is highly related with ED, and linear regression equations presented in this study could be used to indirectly obtain measurements of ED in future studies, avoiding the use of direct calorimetry, a method that is time consuming. Strong positive linear relationships between ED and % DW have been reported in numerous studies targeting other fish species (Hartman & Brandt, 1995; Pedersen, 2001), and also in small pelagic fish from the Adriatic Sea (Tirelli et al., 2006) and the Bay of Biscay (Dubreuil &Petitgas, 2009; Gatti et al., 2018). The linear model obtained in the present study for anchovy and sardine differs from those of Tirelli et al (2006) and Dubreuil &

Petitgas (2009) suggesting this type of model is probably ecosystem-specific and should be used at a local scale to estimate ED from DW data.

In the case of the evaluation of ED in gonads, we observed the high variability in ED_{gonad} depending on the reproduction stage and sex. This was expected, since when the gonad has hydrated oocytes the lipid content increases (Garrido et al., 2007). For this reason, the correlation between the GSI and the ED_{gonad} was high for anchovy and sardine females and males. This is the first time that the ED of gonads is assessed in relation with the GSI, and the equation provided could be used for further studies and for bioenergetics models (Pethybridge et al., 2013). Yet, it is important to note that that the calculation of the energy invested in reproduction is difficult since sardine and anchovy are batch spawner species, and the energy measured at a certain point in time does not correspond to all the energy that will be invested. Moreover, the energy measured in the gonads correspond to energy invested in reproduction, but also, to a lesser extent, to gonad structure (Kooijman, 2010). Thus, gonad ED cannot be directly used as a measure of energy allocated to reproduction. However, variation in the ED_{gonad} can be used as an indirect measure of the energy invested, giving a starting point for the parameterization of bioenergetics models (Pethybridge et al., 2013; Gatti et al., 2017).

4.5 Conclusion

This study highlights the importance of understanding the seasonal energetic variation in small pelagic fish in order to understand population dynamics. The annual body condition and energetic cycle of both species were related to the temporal lag between spawning seasons and the late-winter early-spring phytoplankton bloom as has been described in other Mediterranean areas (Basilone et al., 2006; Pethybridge et al., 2014; Brosset et al., 2015a). Both studied species had low energy reserves in fall that could be related to changes in the phenology of plankton, but further studies analysing monthly variability of body condition of several years in relation to changes in environmental parameters are needed (Brosset et al., 2017; Albo-Puigserver et al., 2019).

Comparison between direct and indirect condition indeces revealed that ED and Kn are the preferable methods to capture the seasonal variability of condition for anchovy, while all direct and indirect methods are suitable for sardine. Since the analysis of calorimetry is highly time-consuming, the regression provided in this study validate the use of dry weight and GSI to obtain estimates of ED in whole individuals and in the gonads, respectively.

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PART III

Qualitative Ecosystem Model

Chapter 5

Who is to blame? Plausible pressures on small pelagic fish population changes in the northwestern Mediterranean Sea

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Abstract

Important changes have been observed in recent decades in small pelagic fish (SPF) populations of the NW Mediterranean Sea: declines in biomass and landings of European anchovy and sardine, and a geographical expansion of round sardinella. These changes have been linked to environmental factors directly influencing annual recruitment and growth. The role of climate change in affecting the composition of plankton has also been suggested to explain declines in SPF, while other causes could be the recovery of predators, competition with other pelagic organisms that prey on early life phases of SPF (i.e. gelatinous zooplankton), interspecific competition for food, or impacts from fisheries harvest. To test the role of these potential pressures, we developed qualitative mathematical models of a NW Mediterranean pelagic food web. We used analyses of sign directed graphs and Bayesian belief networks to compare alternative hypotheses about how SPF species may have responded to combinations of different pressures. Data documenting changes in SPF populations were used to test predicted directions of change from signed digraph models. An increase in sea surface temperature (SST) that had either a positive impact on round sardinella or on gelatinous zooplankton abundance was the pressure that alone provided the most plausible insights into observed changes. A combination of various pressures, including an increase in SST, an increase of exploitation and changes to zooplankton also delivered results matching current observations. Predators of SPF were identified as the most informative monitoring variable to discern between likely causes of perturbations to populations of SPF.

Keywords: Small pelagic fish; Population change; Food web; Qualitative model; Pressures; Cumulative impacts; Mediterranean Sea.

5.1 Introduction

Biomass and energy transfer in marine ecosystems from lower (e.g. plankton) to higher (e.g. predators) trophic levels is often modulated by organisms that occur at intermediate trophic levels, such as species of small pelagic fish (SPF) (Cury et al. 2000, Cury et al. 2011, Pikitch et al. 2013). SPF in the Mediterranean Sea are dominant in terms of biomass and are significant components of marine food webs (Palomera et al. 2007, Coll et al. 2008, Van Beveren et al. 2016a). For example, they are a key prey of important demersal and pelagic predators, such as European hake *Merluccius merluccius* (Mellon-Duval et al. 2017), tunas (Navarro et al. 2017), cetaceans (Gómez-Campos et al. 2011) and pelagic seabirds (Navarro et al. 2009). They are involved in essential processes to enhance primary productivity (Tudela & Palomera 1999) and are important in terms of fishery landings (Palomera et al. 2007, Van Beveren et al. 2016a).

SPF have short life spans, are sensitive to environmental fluctuations and are greatly impacted by climate variation (Cury & Roy 1989, Palomera et al. 2007). Some stocks of SPF are highly exploited or overexploited (Mullon et al. 2009, Pikitch et al. 2013, STECF 2016a). Due to the key role they play in marine ecosystems, changes in SPF populations can impact ecosystem dynamics across entire marine food webs, which can have significant consequences for commercial catch and profit (Cury et al. 2000, Pikitch et al. 2013). Taking into account that marine pelagic fisheries account for 26% of the world's fish and shellfish protein consumption (Tacon & Metian 2009), and that exploitation of SPF stocks is increasing (FAO 2016b), there is an urgent need to understand the ecological and socioeconomic consequences of how SPF will respond to the combined impact of human and environmental pressures.

In general, declines of SPF populations have been observed in the Mediterranean Sea, in parallel with an increase in fishing effort and a decline in primary productivity (Piroddi et al. 2017). Specifically, in recent decades, important changes have been observed in populations of SPF in the NW Mediterranean Sea: a decline in biomass and landings of European anchovy *Engraulis encrasicolus* and sardine *Sardina pilchardus*, and an observed spatial expansion of round sardinella *Sardinella aurita* (Sabatés et al. 2006, 2009, Palomera et al. 2007, Van Beveren et al. 2016a).

Although several hypotheses have been formulated to explain changes in SPF, factors that are controlling populations of SPF in the NW Mediterranean Sea are still unclear. Population fluctuations have been linked to environmental changes that can influence annual recruitment, growth and condition of SPF. Several explanatory hypotheses have been formulated (e.g. Palomera et al. 2007, Martín et al. 2008, Van Beveren et al. 2014, Brosset et al. 2017, Saraux et al. 2018), including the impact of higher sea surface temperature (SST) on the distribution of SPF populations that could negatively affect some species, such as sardine, and positively affect others, such as round sardinella (Sabatés et al. 2006, Maynou et al. 2014).

The role of climate change in affecting the composition of plankton has also been suggested to explain observed SPF population changes (Brosset et al. 2015, 2016, Saraux et al. 2018). Other potential causes could be the recent recovery of predators such as Atlantic bluefin tuna (Van Beveren et al. 2017), competition between pelagic organisms that feed on zooplankton and can also prey on early life phases of SPF (e.g. gelatinous zooplankton) (Purcell et al. 2014, Pascual 2016, Tilves et al. 2016, 2018), interspecific competition for food (e.g. between sardine juveniles and the expanding population of round sardinella) (Albo-Puigserver et al. 2017a) and the occurrence of pathogens and disease (Van Beveren et al. 2016b). Historical changes in anchovy and sardine landings have also been attributed to increases in fishing effort and currently high rates of exploitation (Palomera et al. 2007, FAO 2016a, STECF 2016a).

Understanding interspecific interactions in marine food webs and potential impacts of pressures is challenging, and precise quantitative measurements of these effects can be difficult to obtain (Stergiou & Karpouzi 2001). To address this scientific challenge, there is a growing need to develop and use novel methodologies of data integration and modelling that can account for ecological and environmental processes as well as uncertainties in data and knowledge of the system (Christensen et al. 2015, Parravicini et al. 2012). In order to understand the main patterns and trends, it is not always necessary to have precise quantitative measurements, as qualitative analyses of a system may help in predicting its general dynamics (Dambacher et al. 2009, Lassalle et al. 2014). In this context, qualitative mathematical modelling can be used to integrate available knowledge from different disciplines, and also account for complex dynamics driven by feedbacks in ecological systems, which can create counterintuitive results that often confound predictions and effective management. This approach provides a framework to consider alternative hypotheses about the structure and function of ecosystems and produce a general and realistic representation of the causal relationships that drive the system (Levins 1966, 1974). Qualitative mathematical modelling is especially useful when the basic relationships between variables are understood but where precise and detailed data is not available (Dambacher et al. 2009).

In this study, we applied qualitative mathematical modelling to depict a NW Mediterranean pelagic food web based on the available knowledge from the area and assess the likely role of potential pressures on SPF populations during the last 2 decades. We used the methodology of sign directed graphs in combination with an analysis of Bayesian belief networks (BBNs) (Dambacher et al. 2003a,b, Hosack et al. 2008). We considered alternative model structures and looked at the most plausible changes due to different pressures (fisheries, predators, bottom-up causes or temperature) that have been suggested as potential explanations for the decline of sardine *S. pilchardus* and anchovy *E. encrasicolus*, and for the increase of round sardinella *S. aurita*. The role of pressures to yield consistent results with observations were first investigated individually, and afterwards we considered combinations of multiple pressures (Folt et al. 1999, Crain et al. 2008). Finally, we identified which variables of the pelagic food web models were most information-rich and thus could be monitored in the future to obtain more knowledge about what is happening in the NW Mediterranean pelagic food web to reduce uncertainty from model predictions.

5.2 Materials and methods

Study area

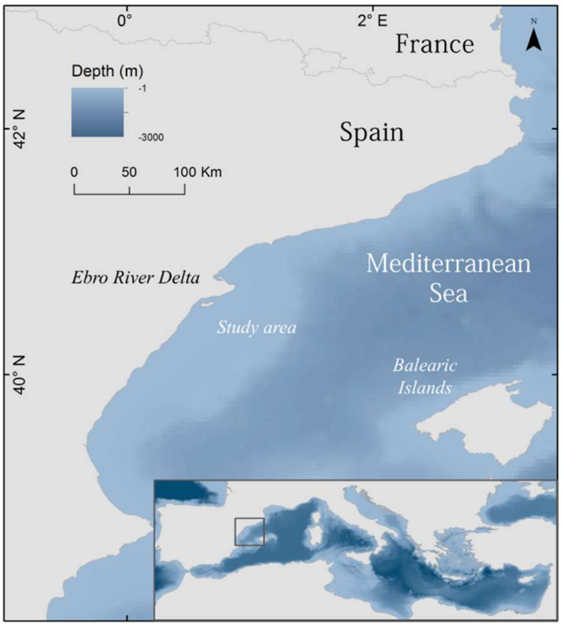


Fig. 1. Study area in the NW Mediterranean Sea (inset: Mediterranean Sea).

Our study area represents the pelagic marine ecosystem of the NW Mediterranean Sea, with special emphasis on the marine ecosystem of the Ebro River Delta continental shelf in the NW Mediterranean (Fig. 1), from where most of the information included in this study is available (see Table 1). As a consequence of particular oceanographic conditions, such as wind conditions, vertical mixing and river discharges (Salat 1996, Palomera et al. 2007), this area is an important spawning habitat and fishing ground of sardine and anchovy in the Mediterranean Sea (Tugores et al. 2011, Giannoulaki et al. 2013) and has been identified as a priority area for conservation (Coll et al. 2015, Piante & Ody 2015). Important landings of SPF, mainly anchovy and sardine, have been observed in the region since the 1970s, with maximum catch in the early 1990s (Fig. 2). Since then, landings of SPF have declined, and from the mid-2000s, the catch of sardine is lower than that coming from anchovy. While round sardinella is not a primary commercial species, levels of catch have increased in the last few decades, with large fluctuations due to its low commercial value (Fig. 2), highlighting an increase of this species in the NW Mediterranean Sea and a northern expansion of its distribution (Sabatés et al. 2006, 2009). It is important to note that while *Sardina pilchardus* (from hereon referred to as sardine) reproduces in winter, *Sardinella aurita* (from hereon referred to as round sardinella) and *Engraulis encrasicolus* (from hereon referred to as anchovy) do this during the summer; therefore, these 3 species are differently affected by environmental conditions (Palomera et al. 2007, Maynou et al. 2014).

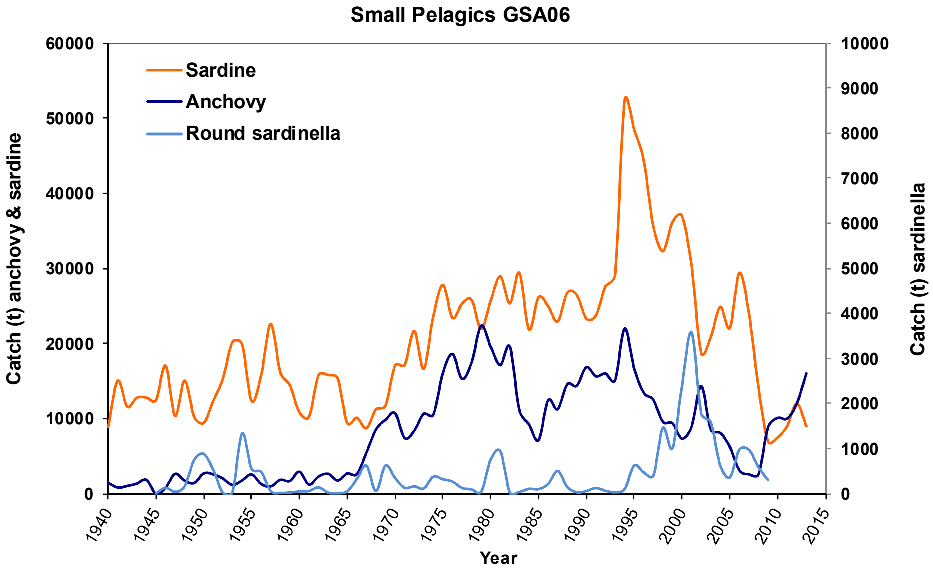


Fig. 2. Anchovy and sardine landings from 1940 to 2014 and round sardinella landings from 1945 to 2009 in the geographical subarea 6 (GSA06; as defined by the General Fisheries Commission for the Mediterranean [GFCM]) region in the Mediterranean Sea. Landings data from 1940 to 1986 are from annual fishing reports of Spanish fishermen organizations. From 1987 onwards, data are from the Spanish Ministry of Agriculture and Fisheries recovered by the Spanish Oceanographic Institute and from Regional Autonomic Fisheries statistics.

Sign directed graphs

Qualitative mathematical modelling is based on a general understanding of the relationships that connect ecosystem variables, and can be represented by either equations, matrices or graphs (Puccia & Levins 1985, Dambacher et al. 2009). In this study, we used the modelling methodology of sign directed graphs, or signed digraphs, to assess model stability as an indication of the likelihood of the modelled system to exist and persist in nature, and to predict how the system could possibly respond to an array of possible pressures (Dambacher et al. 2003a,b). Functional groups in signed digraph models and their pressures are depicted as nodes and the signs of the direct effects among them are represented by directed links between the nodes. The graph links contain the signs (+, –, 0) of the direct effects. A signed digraph has an equivalent representation in a community matrix, where each *aij* element represents the direct effect of variable *j* on variable *i*, and can be used to perform algebraic operations.

From the structure of signed digraphs and the community matrix, the feedback properties of a system can be examined to understand its dynamics and identify processes and interactions that play a key role in maintaining a state of equilibrium. Model stability is assessed to determine whether or not a model is a feasible representation of a real system, such that the model possesses the virtue of persisting despite a shock or perturbation and whether it can exhibit familiar dynamics (Dambacher et al. 2015). The system feedback is calculated by the product of links in the system; while negative feedback (such as a predator–prey relationship) returns the opposite effect to an initial change in a variable and acts to maintain equilibrium, positive feedback can magnify changes in a variable and drives the system away from its previous state through un checked growth or collapse (Puccia & Levins 1985). Therefore, the qualitative analysis of feedback permits an evaluation of system stability and an understanding of the role of pressures. Analysis of model stability entails an assessment of a system's response to a pulse perturbation, which is an instantaneous and temporary increase or decrease in the abundance or level of 1 or more variables, where stable systems have the ability to return to their former equilibrium levels and unstable systems do not (Dambacher et al. 2003b).

All signed digraph models in this study were obtained using the digraph editor software PowerPlay version 2.0. Stability and perturbation analyses were developed using a Maple software program for qualitative and symbolic analyses of the community matrix. PowerPlay and the Maple program are available as downloads in Supplement Revisions 1 and 2 from esapubs. org/ archive/ ecol/E083/022.

Model formulation of NW Mediterranean pelagic food web

To develop the food web models of the NW Mediterranean pelagic system, we represented a 'relevant subsystem' (Dambacher et al. 2009, 2015) that included the essential dynamics of SPF in the NW Mediterranean Sea, and which tailored the model to the specific problem to be investigated. The goal was to provide a conceptual framework that integrated knowledge of various disciplines, but was constrained to the context of the management problems at hand and the local environment. Therefore, we included those ecological groups that were relevant for sardine, anchovy and round sardinella population dynamics (Palomera et al. 2007, Coll et al. 2008, Albo-Puigserver et al. 2016). The functional groups to depict the food web were chosen based on existing ecological knowledge and literature of the pelagic food web of the NW Mediterranean Sea (see Table 1), with special emphasis on the marine ecosystem of the Ebro River Delta continental shelf in the NW Mediterranean (Fig. 1). We also considered relevant information from adjacent ecosystems when available (such as the Gulf of Lions located in the most northern part of the Mediterranean Sea basin).

Table 1. Summary of direct effects between variables and factors on the core model. Numbers in the 'From' and 'To' columns and numbers in parentheses in the 'Mechanism' column represent graph nodes in **Fig. 3**

Direct effect			Mechanism
Sign	From	To	
+	1;2;3	7	Sardine (1), anchovy (2) and round sardinella (3) are the most important prey for tuna, little tunny and swordfish (7)(Navarro et al. 2017, Van Beveren et al. 2017)
-	1;2;3	5	Sardine (1), anchovy (2) and round sardinella (3) prey on zooplankton (5) (Tudela & Palomera 1997, Plounevez & Champalbert 2000, Lomiri et al. 2008, Costalago et al. 2012, Costalago & Palomera 2014)
-	1	4	Sardine (1) prey also on phytoplankton (4) at adult stages (Lomiri et al. 2008, Costalago & Palomera 2014)
-	3	6	Gelatinous plankton (6) is an important trophic resource for round sardinella (3)(Albo-Puigserver et al. 2017a)
-	4	6	Phytoplankton (4) is preyed on by gelatinous zooplankton (6), but only in high quantities during bloom events. Therefore, gelatinous zooplankton does not have a negative effect on phytoplankton maintained throughout the year (Canepa et al. 2014)
-	6	2;5	Different stages of jellyfish (6) prey on anchovy larvae (2) and zooplankton (5) (Tilves et al. 2016, 2018)
-	8	1;2	Purse-seiners (8) harvest on sardine (1) and anchovy (2) with effort that is independent of amount of catch; thus, no positive links from (1) and (2) back to (8)

Firstly, we developed a core food web model (M_0) with 8 variables or nodes (Table 1, Fig. 3). M_0 represented the base configuration of the pelagic food web associated with SPF in the Ebro River Delta (NW Mediterranean) and incorporated the main established knowledge about ecological interactions (Fig. 3). Links between nodes or functional groups and their pressures were based on the available scientific knowledge of the system (Table 1). In Fig. 3, links ending in an arrow represent a positive direct effect, such as births due to consumption of prey, whereas links ending in a filled circle represent negative direct effects, such as mortality due to predation. Self-effects, such as intraspecific resource limitation, are depicted as links that start and end at the same node. M_0 included direct effects between predators and prey, and fisheries. All variables included negative self-effects, thus assuming that they are, to some degree, self-limiting. In our case, this was used to represent intraspecific density-dependent processes. Based on historical evidence from the study area (Coll et al. 2008), the role of SPF in the core model was represented as having the role of wasp-waist control (Cury et al. 2000), impacting both on their prey and their predators (e.g. large demersal and pelagic fish, seabirds and marine mammals).

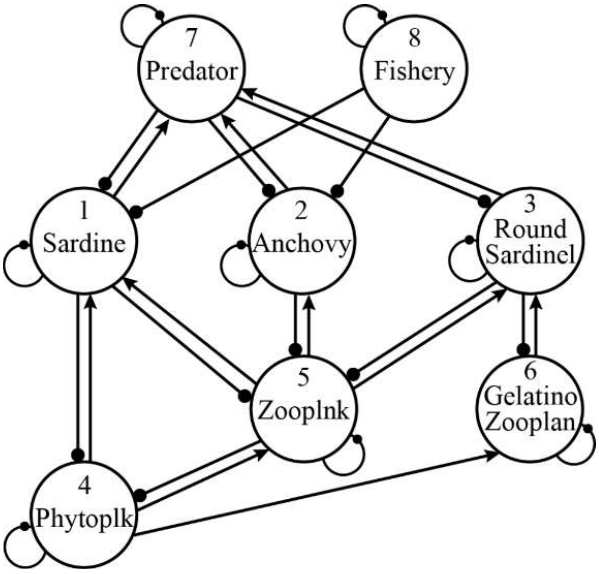
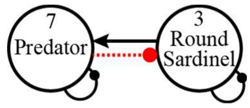
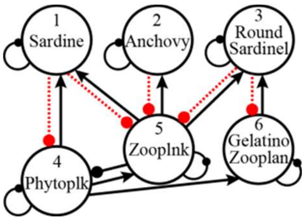
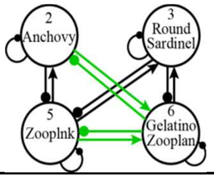
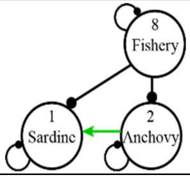
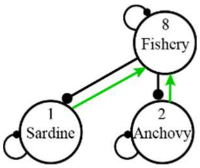
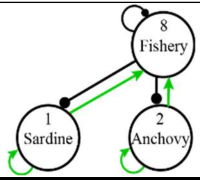


Fig. 3. Signed digraph of the pelagic food web core model M_0 of the NW Mediterranean Sea (Table 1). Positive effects are denoted by links terminating in an arrow, and negative effects by links terminating in a filled circle. 1: sardine, 2: anchovy, 3: round sardinella, 4: phytoplankton, 5: zooplankton, 6: gelatinous zooplankton, 7: predators (tuna), 8: purse seine fisheries

Secondly, to address the uncertainties in some relationships between model variables, we compared alternative model structures to M_0 by building 4 alternative food web models (M_1, M_2, M_3 and M_4; Table 2a, Fig. 4). These alternative models included relationships that had scarce information or ambiguity in the response, thus alternative links of the core model were proposed: Model M1 was modified to represent that predators do not limit round sardinella due to its recent expansion (Sabatés et al. 2006, 2009); Model M_2 incorporated the modification of SPF not limiting their trophic resources and therefore not competing for food due to partial difference in their diets (Albo-Puigserver et al. 2016); Model M_3 incorporated significant predation of gelatinous plankton on anchovy larvae and zooplankton, as has been recently described to occur in the study area (Tilves et al. 2016, 2018); and M_4 incorporated all additional links of M_1, M_2 and M_3 to the core model M_0.

Table 2. (a) Alternative models to the core model M0 (Table 1, Fig. 3) showing the structural changes to the core model configuration. (b) Hypotheses regarding how fisheries interact with small pelagic fish. Numbers in parentheses represent graph nodes in Fig. 3. Links deleted from the core model (M0) are in red, those added are in green

(a) Alternative models to M₀	**Diagram**
M1. predators (7) do not limit round sardinella (3) (remove link 7:3)	
M2. sardine, anchovy and round sardinella do not limit their trophic resources and do not compete for food: phytoplankton (4) and zooplankton (5,6) (remove links 1:4, 1:5, 2:5, 3:5, 3:6)	
M3. jellyfish (gelatinous plankton 6) significantly prey on anchovy larvae (2) and zooplankton (5) (add links 2:6, 6:2, 5:6, 6:5)	
(b) Fisheries hypothesis	**Diagram**
H1. fisheries (8) prefer and overexploit anchovy (2) and switch to sardine (1) when anchovy (2) is low (add link 2:1)	
H2. sardine (1) and anchovy (2) recruitment highly fished (8) (add link 2:8, 1:8)	
H3. sardine (1) and anchovy (2) recruitment overfished (8) (add link 2:8, 1:8, 1:1, 2:2)	

Thirdly, 3 hypotheses regarding how fisheries interact with SPF were tested (H_1 to H_3, Table 2b) (Palomera et al. 2007, SAC-GFCM 2015): H_1: Overall fishing effort is unresponsive to stock abundance; thus, there is no positive link from fish stocks to fishery, but fisheries preferably target anchovy and switch to sardine when anchovy is low (observed from M. Coll's personal experience interacting with the fishery's operations); here, anchovy abundance effectively suppresses the intensity of fishing effort on sardine, which constitutes a modified interaction (Dambacher & Ramos-Jiliberto 2007) and is represented in the signed digraph by a positive link from anchovy to sardine; H_2: Sardine and anchovy are both highly fished, such that the level of fishing effort on both stocks increases as a function of combined stock abundance, which is represented by a positive link from both the sardine and anchovy populations back to fisheries; and H3: Sardine and anchovy recruitment is overfished, which destabilizes the stock's population dynamics and imposes a positive self-effect on sardine and anchovy population (Dambacher et al. 2015). Finally, combinations of H_1 with the 2 other fisheries hypotheses was tested (H_1 & H_2 and H_1 & H_3).

Structural stability of food web models

Assessing the potential for a signed digraph model to be stable is important because it indicates whether the model is a feasible representation of a real system that can persist despite everyday disturbances, and also whether such a system could exhibit predictable dynamics. Model stability is based on the system's feedback cycles, both in terms of the balance of positive and negative cycles, and the balance of short versus long feedback cycles (Dambacher et al. 2003b). System feedback is defined at different levels of the system depending on the number of interactions that form a feedback cycle (level 1, or self-effects; level 2 resulting from pairwise interactions such as predator–prey interactions; and higher levels involving any number $n - 1$ of links with n variables). There are 2 criteria for stability: (i) feedback at any level of the system is not dominated by positive feedback, and (ii) the system is not dominated by higher-level feedback or cycles with long path lengths. Based on their structural characteristics, signed digraph models can be assessed *a priori* to determine their potential to be stable and if they are prone to failing stability Criterion (i) or (ii) (Dambacher et al. 2003b).

The potential for a signed digraph model to pass or fail Criterion (i) is determined by its maximum weighted feedback, *wFn*, which follows from an accounting of all feedback cycles (positive and negative) at the highest level of the system and calculating the ratio of the net to absolute sums (or prediction weight) (Dambacher et al. 2003b). Values of *wFn* close to –1.0 imply a limited amount of positive feedback and thus a relatively high potential for stability, values of *wFn* close to +1.0 imply a relatively large amount of positive feedback and an unstable system, while a value of *wFn* near zero indicates a roughly equal chance for the system to be stable or unstable (Dambacher et al. 2003b). When a system is destabilized due to excessive positive feedback, a pulse-type disturbance to the system kicks off exponential growth or decay in 1 or more variables, and thereafter it is unable to return to the former equilibrium by its own internal dynamics or processes.

Models that fail Criterion (ii) are destabilized by systemic overcompensation, which is due to feedback at higher levels in the system overwhelming feedback at lower levels, leading to undamped oscillations (Dambacher et al. 2003b). The potential for a model to pass or fail stability criterion (ii) is assessed by the relative balance of feedback at higher versus lower levels of the system through a weighted determinant *(wD)*. Positive values of *wD* imply a high potential for stability, while those close to, or less than, zero imply a low potential for stability. The degree to which a weighted determinant is 'close' to zero is complicated by the fact that values of *wD* naturally decrease as the size, or number of variables, in a system increases. Determination of the relative value of a weighted determinant for a given signed digraph model or community matrix is made possible by comparison to a standard 'model c-type system' (i.e. model with the same number of variables constructed as a strain-chain system with predator–prey interactions between each successive trophic level, Dambacher et al. 2003b). For a given signed digraph model, a value of *wD* that is less than that for a corresponding model c-type system indicates that it has a high potential for failing stability Criterion (ii). A useful stability metric can thus be the ratio of a model's value for *wD* with respect to that of a model c-type system, with a ratio less (greater) than 1 indicating a high (low) potential for failing Criterion (ii).

Dambacher et al. (2003b) tested the utility of the above 2 stability metrics through quantitative simulations in a random and evenly distributed parameter space. These metrics proved a robust means to assess potential stability of signed digraph models of any size or level of complexity. Here, we apply their results to assess the potential stability of the 5 models (M_0, M_1, M_2, M_3 and M_4) and their combinations with the 3 hypotheses (H_1, H_2 and H_3). Accordingly, we apply a threshold value of $wFn \geq 0$ and a wD ratio to model c < 1 to distinguish model structures with a low potential for stability from those with a moderate to high potential.

Perturbation scenarios

Prior to undertaking an analysis of a system's expected response to a permanent shift in its equilibrium (i.e., a press perturbation), one must first determine if it has the potential to be stable during the shift from the old to the new equilibrium state. Once this condition is established, then predicting the direction of change for the system variables proceeds from an analysis of all the direct and indirect effects formed by the pathways of interaction that lead from the input variable to each response variable. When there are both positive and negative effects influencing the response of a variable, then its prediction is qualitatively ambiguous. To resolve this ambiguity, knowledge of the relative strength of interactions involved in the pathways can be used to determine whether the positive or negative effects will prevail (Puccia & Levins 1985). Alternatively, the relative balance of positive versus negative effects in a response prediction can be used to assign a probability of sign determinacy to response predictions (Dambacher et al. 2015).

Previous work tested the sign determinacy of qualitative response predictions across a wide array of signed digraph models (Dambacher et al. 2003a, Hosack et al. 2008). Numerical simulations were previously used to randomly allocate interaction strengths within signed digraph models and examined the frequency with which qualitative predictions of perturbation response matched the sign of responses in quantitatively specified systems. For example, if there are 4 pathways of interaction leading from an input variable to a response variable, with 2 positive and 2 negative in the sign of their effect, then in numerical simulations that randomly assign interactions strengths, the probability of either a positive or negative shift in the response variable will be 50% either way, with probability of sign determinacy being no better than a coin toss. If, however, there are 3 pathways with a positive effect and

1 with a negative effect, then the frequency of a positive response will exceed 90%, with the probability that the 1 single negative effect could overwhelm the other 3 positive effects being less than 10%.

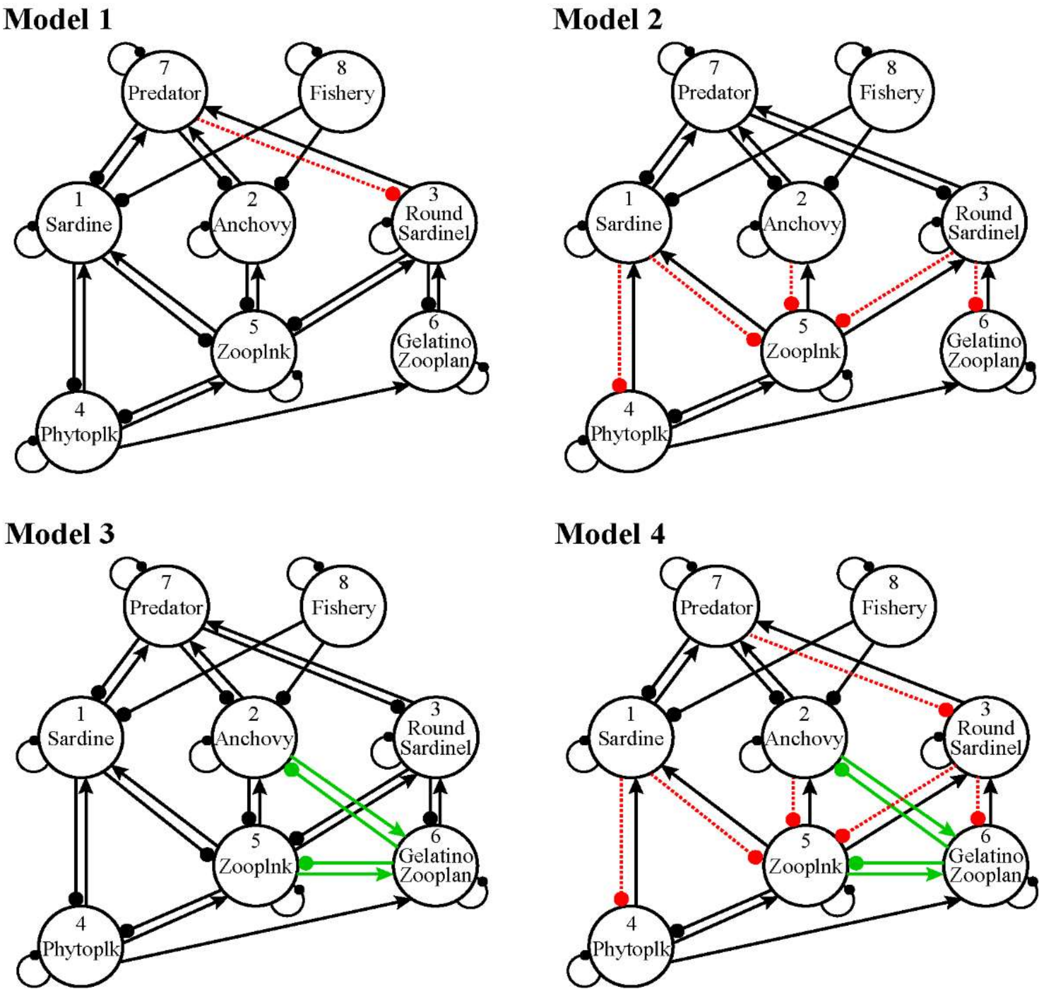


Fig. 4. Signed digraphs of the alternative food web models M1 to M4 of the NW Mediterranean Sea (Table 2a). Positive effects are denoted by links terminating in an arrow, and negative effects by links terminating in a filled circle. Node numbers are the same as in Fig. 3. Links deleted from the core model (M0; Fig. 3) are in red, those added are in green.

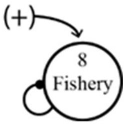
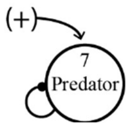
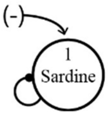
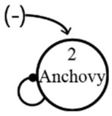
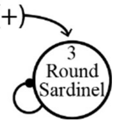
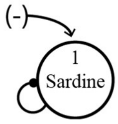
Hosack et al. (2008) developed the means to incorporate probabilities of sign determinacy for qualitative model predictions within BBNs as conditional probabilities, and we used this method in our study (i.e. Eqs. 5 and 6 of Hosack et al. 2008). These conditional probabilities were derived directly from the sign directed graphs based on the number of positive and negative effects that make up any given response prediction. Representing qualitative models within a BBN has a number of advantages, including (1) a probabilistic representation of qualitative model predictions, including the means to assess multiple perturbations simultaneously, (2) the ability to validate a qualitative model against observed perturbation responses, (3) diagnosis of most likely source of a perturbation, and (4) a sensitivity analysis that can identify the most informative monitoring variables for diagnosis of perturbation sources or for model validation. The first 3 of these functions (i.e. prediction, validation, diagnosis) are reported in the BBN as probabilities, while the 4th (i.e. sensitivity analysis) is reported as mutual information, which is a measure of the mutual dependence between 2 variables.

In this study, we used only those models with a moderate to high potential for stability to develop BBNs to analyse the outputs of perturbation scenarios. Since there are various possible explanations for the decline of SPF in the study area, we calculated the probability of sign determinacy of each model's predictions when applying different pressures to input variables of the pelagic food web of the NW Mediterranean Sea (Figs. 3 & 4), and selected models with predictions that were most consistent with field observations.

Specifically, we applied 6 pressures scenarios to each model based on existing hypothesis of change. Individual perturbations were developed to reproduce sustained changes in main pressures on SPF, mainly related to sustained changes in fisheries, predators, zooplankton composition and climate conditions (i.e. SST). Pressures were developed by considering inputs to the following variables and pressures (Table 3): P_1: an increase of purse-seine fishery, the main fisheries for SPF (thus increasing fishing mortality) (Palomera et al. 2007); P_2: a recovery of SPF predators that could have a negative impact on the abundance of SPF (thus increasing predation mortality) (Navarro et al. 2009, 2017, Gómez-Campos et al. 2011, Mellon-Duval et al. 2017); P_3: a change in zooplankton that affects its com position (thus resulting in lower quality of food for sardine and anchovy, with the ultimate consequence of a reduction of sardine and anchovy abundance) (Brosset et al. 2015, 2016, Saraux et al. 2018); P_4: an increase of SST that increases the abundance of round sardinella (thus resulting in a spatial expansion and higher abundance) (Sabatés et al. 2006, 2009); P_5: an increase of SST that reduces the reproduction rate of sardine (thus increasing natural mortality) (Palomera et al. 2007); and P_6: an increase of SST that increases the abundance of gelatinous zooplankton (thus resulting in a benefit on its growth and persistence in the water column) (Licandro et al. 2010).

A total of 280 perturbation scenarios were developed by applying the individual or multiple perturbations (P_1 to P_6) with the different alternative models (M_0 to M_4) and structural hypotheses (H_1 and H_2). Results of perturbation scenarios were analysed considering available observations that describe a decline in sardine *S. pilchardus* and anchovy *E. encrasicolus*, and an increase in round sardinella *S. aurita* in the study area (Table 4). Predictions for each perturbation scenario were classified as being highly sign-determined when the probability of decline or increase for a variables was ≥90%, moderately sign-determined with a probability between 80 and 90%, or ambiguous with a probability <80%.

Table 3. Pressures P1 to P6 applied in perturbation scenarios analysed in Bayesian belief networks. Numbers in parentheses represent graph nodes in Fig. 3

Pressure		Input variables
Fisheries P_1: increase of purse-seine fishery effort (8)		Positive input to fisheries
Predator P_2: recovery and increase of the abundance of predators (7)		Positive input to predators
Bottom-up P_3: changes in zooplankton (5) composition with lower quality of food for sardine (1) and anchovy (2)	 	Negative input to sardine and anchovy
Temperature P_4: increase in temperature increases the abundance of round sardinella (3)		Positive input to round sardinella
P_5: increase in temperature reduces reproduction rate of sardine (1)		Negative input to sardine
P_6: increase in temperature increases abundance of gelatinous zooplankton (6)		Positive input to gelatinous zooplankton

Sensitivity analyses

Perturbation scenarios that provided predictions that were highly consistent (i.e. >90% probability) with observations (Table 4) were retained for sensitivity analyses (Hosack et al. 2008). A sensitivity analysis within the BBN shows how the probabilities of observing 1 node are affected by changes in another node, and can be used to identify which variables of a model are most informative. Specifically, sensitivity analyses use the change in mutual information between 2 nodes due to

the reduction of entropy in node X because of a finding at node Y, where the expected reduction in entropy of X due to a finding at Y is zero if X is independent of Y (Hosack et al. 2008). For our purpose, variables identified as being most informative were highlighted as interesting to be monitored in order to detect the most likely source of input to the food web and most plausible pressure(s) on SPF. BBN analyses were performed using the software NETICA v.4.08 following Hosack et al. (2008).

5.3 Results

Structure stability

The stability analyses revealed a moderate to high potential for stability for the core model and most of the alternative model structures analysed (Table 5). The exception was model structures that included recruitment overfishing of anchovy and sardine by purse-seine fisheries (structural hypothesis H_3), all of which had a low potential for stability. Ten models that were formulated with H_3 alone or combining H_3 with H_1 had values of $wF_n \geq 0$ or wD ratio to model c < 1. These models were judged to have little to no potential for stability, and thus unlikely to represent viable responses for press perturbations, and were subsequently excluded from BBN analyses.

Table 4. Available knowledge documenting small pelagic fish population changes in the NW Mediterranean Sea. GSA06: geographical subarea 6 (as defined by the General Fisheries Commission for the Mediterranean [GFCM])

Node Fig. 3	Variable name	Observation	Narratives and references
1	Sardine *Sardina pilchardus*	Decrease of biomass	Decrease in body size and condition during last decade maintaining the same high recruitment with disappearance of the older age classes (Palomera et al. 2017, Van Beveren et al. 2014)
		Decrease of abundance	Decline in abundance in GSA06 between 2003 and 2006 by acoustic survey data, consistent with the decrease in stock sizes in the area since the 1990s (Tugores et al. 2010)
		Decrease of landings	Stock status depleted (SAC-GFCM 2015) and decreasing of landings (this study)
2	Anchovy *Engraulis encrasicolus*	Decrease of biomass	Decrease in body size and condition during last decade maintaining the same high recruitment (Palomera et al. 2007, Van Beveren et al. 2014, Brosset et al. 2017)
		Decrease of abundance	Decline in abundance in GSA06 between 2003 and 2006 by acoustic survey data, consistent with the decrease in stock sizes in the area since the 1990s (Tugores et al. 2010)
		Decrease of landings	Since 2001, landings decreased to the lowest levels of the time series available, with a slight recovery since 2010 (Martin et al. 2012, STECF 2016b)
3	Round sardinella *Sardinella aurita*	Expansion of distribution	Expansion to more northern areas of the Mediterranean Sea and higher abundance (Aegean Sea: Tsikliras 2008; Adriatic Sea: Sinovčić et al. 2004; NW Mediterranean Sea: Sabatés et al. 2006, 2009)
		Increase of landings	Overall increase in landings was observed over the period 1950–2010 in the western Mediterranean Sea, with a maximum in 2003 (Sabatés et al. 2006, Alheit et al. 2014, Stergiou et al. 2016)

Table 5. Stability properties of core and alternative models (Figs. 3 & 4); models with low potential for stability in italics (i.e. wFn ≥ 0 or wD ratio to model c < 1). See 'Materials and methods' for explanations of model c-type system, Models M0 to M4, Hypotheses H1 to H3, and Criteria (i) and (ii). BBN: Bayesian belief network, wD: weighted determinant, wFn: maximum weighted feedback

Model and hypothesis	Criterion (i) wF_n	Criterion (ii) wD	*wD* ratio to model c	Included in BBN
M_0	–0.32	0.000021	8800	Yes
M_1	–0.32	0.000024	10000	Yes
M_2	–1.0	0.000026	100000	Yes
M_3	–0.27	0.53×10^{-5}	2200	Yes
M_4	–0.57	0.000053	22000	Yes
M_0–H_1	–0.33	0.000017	7200	Yes
M_0–H_2	–0.25	0.000011	4600	Yes
M_0–H_3	*0.074*	*0.49×10^{-11}*	*0.002*	*No*
M_0–H_1 & H_2	–0.28	0.99×10^{-5}	41000	Yes
M_0–H_1 & H_3	*0*	*0.11×10^{-10}*	*0.0046*	*No*
M_1–H_1	–0.32	0.00002	8300	Yes
M_1–H_2	–0.24	0.000013	5300	Yes
M_1–H_3	*0.069*	*0.25×10^{-11}*	*0.001*	*No*
M_1–H_1 & H_2	–0.26	0.000012	4900	Yes
M_1–H_1 & H_3	*0*	*-0.45×10^{-11}*	*–0.0018*	*No*
M_2–H_1	–1	0.0002	82000	Yes
M_2–H_2	–0.67	0.000088	36000	Yes
M_2–H_3	*0.33*	*0*	*0*	*No*
M_2–H_1 & H_2	–0.73	0.000069	28000	Yes
M_2–H_1 & H_3	0.67	-0.14×10^{-10}	–0.0058	No
M_3–H_1	–0.23	0.39×10^{-5}	1600	Yes
M_3–H_2	–0.22	0.31×10^{-5}	1300	Yes
M_3–H_3	*–0.016*	*-0.29×10^{-11}*	*–0.0012*	*No*
M_3–H_1 & H_2	–0.21	0.26×10^{-5}	1100	Yes
M_3–H_1 & H_3	*–0.035*	*0.42×10^{-11}*	*0.0017*	*No*
M_4–H_1	–0.56	0.000045	19000	Yes
M_4–H_2	–0.34	0.000025	10000	Yes
M_4–H_3	*0.069*	*0.23×10^{-12}*	*0.000096*	*No*
M_4–H_1 & H_2	–0.36	0.000023	9500	Yes
M_4–H_1 & H_3	*0*	*-0.30×10^{-10}*	*–0.012*	*No*

Perturbation scenarios with single pressures

Table 6. Predictions of Bayesian belief networks from perturbation scenarios considering a single pressure at a time (P 1 to P6; see Table 3) for alternative model M2 in combination with structural hypotheses H1, H1 & H2, or the core model. Only predictions of decline or increase of ≥80% probability of sign determinacy (classified as moderate) are shown; scenarios where prediction probabilities exceed 90% (classified as high) are in **bold**; ✓ indicates inclusion of a pressure within a perturbation scenario.

Structural hypothesis	Pressure						Prediction probability		
	P_1: increase in purse-seine	P_2: increase in predators	P_3: decrease in zooplankton composition	P_4: increase in round sardinella	P_5: decrease of sardine	P_6: increase in gelatinous zooplankton	Decrease in sardine	Decrease in anchovy	Increase in round sardinella
H₁				✓			**High**	**High**	**High**
H₁						✓	**High**	**High**	**High**
Core				✓			**High**	**High**	**High**
Core						✓	**High**	**High**	**High**
H₁			✓				High	Moderate	High
H₁	✓						High	Moderate	High
H₁ & H₂	✓						High	Moderate	High
Core			✓				Moderate	Moderate	High
Core	✓						Moderate	Moderate	High

Perturbation scenarios that combined alternative model M2 with either P4 or P6 as single pressures were highly consistent with observed changes in populations of sardine, anchovy and round sardinella, with all predictions having a high (≥90%) probability of sign determinacy (Table 6). All these models included 4 perturbation scenarios that incorporated the modification of SPF not limiting their trophic resources, and therefore not competing for food due to partial difference in SPF diets, in combination with 2 structural hypotheses: H_1 (fisheries preferably target anchovy and switch to sardine when anchovy is low) or the core hypothesis (fisheries target anchovy and sardine independently of their abundance). P4, which is a positive input to round sardinella, and P6, which is a positive input to gelatinous zooplankton, both resulted from an increase in SST. Five scenarios with predictions that had a moderate (80–90%) probability of sign determinacy included pressures based on an increase in the purse-seine fishery (P_1) or a decrease in the composition of zooplankton (P_3). These scenarios included alternative model M2 in combination with structural hypotheses H_1, H_1 & H2, or the core model.

Perturbation scenarios with multiple pressures

Table 7. Predictions of Bayesian belief networks from perturbation scenarios considering combinations of multiple pressures (P_1 to P_6; see Table 3), for alternative model M2 in combination with structural hypothesis H 1 or the core model, or alternative model M 3 in combination with structural hypotheses H1, H1 & H2, or the core model. Only predictions of decline or increase with ≥80% probability of sign determinacy (classified as moderate) are shown; scenarios where all prediction probabilities exceed 90% (classified as high) are in **bold**; ✓ indicates inclusion of pressure within a perturbation scenario

		------------------------------Pressure------------------------------						---------Prediction probability---		
Alternative model	Structural hypothesis	P_1: increase of purse-seine	P_2: increase in predators	P_3: decrease in zooplankton composition	P_4: increase of round sardinella	P_5: decrease of sardine	P_6: increase in gelatinous zooplankton	Decrease in sardine	Decrease in anchovy	Increase in round sardinella
M_2	**H_1**	✓	✓	✓	✓		✓	**High**	**High**	**High**
M_3	H_1	✓	✓	✓	✓		✓	High	Moderate	High
M_3	H_1 & H_2	✓	✓	✓	✓		✓	High	Moderate	High
M_2	**Core**	✓	✓	✓	✓		✓	**High**	**High**	**High**
M_3	Core	✓	✓	✓	✓		✓	High	Moderate	High
M_2	H_1		✓		✓	✓	✓	High	Moderate	High
M_2	Core		✓		✓	✓	✓	High	Moderate	High
M_2	**H_1**			✓	✓		✓	**High**	**High**	**High**
M_2	**Core**			✓	✓		✓	**High**	**High**	**High**
M_2	H_1	✓		✓				High	Moderate	High

Results from the perturbation scenarios including multiple pressures yielding predictions that were highly consistent with observations were also based on alternative model M_2 in combination with structural hypothesis H_1 or the core model (Table 7). These included scenarios that considered 5 or 3 simultaneous pressures.

Considering predictions with a moderate (80–90%) probability of sign determinacy, model configuration M_3, which incorporated significant predation of gelatinous zooplankton on anchovy larvae and zooplankton, was included in 3 scenarios (Table 7). In these 3 scenarios, model M_3 was combined with structural hypotheses H_1, H_1 & H_2, or the core model.

In perturbation scenarios with multiple pressures, it was always the same combination of pressures that gave prediction probabilities with a moderate to high level of sign determinacy (>80%) (Table 7). The greatest number of combined pressures, 5, included the same 5 pressures in each perturbation scenario (i.e. P_1, P_2, P_3, P_4 and P_6). Scenarios with 4 pressures always included P_2, P_4, P_5 and P_6, those with

3 pressures always included P_3, P_4 and P_6, while a combination with 2 pressures, P_1 and P_3, occurred in only 1 perturbation scenario.

Most informative indicators

A sensitivity analysis on model configuration M_2 within the BBNs was used to further discriminate between which inputs, and thus which source of a perturbation, were most likely to provide high probabilities of predicting field observations (i.e. a decline in anchovy and sardine and increase in round sardinella).

Table 8. Sensitivity analysis results from Bayesian belief networks, for model configuration M2 under the core model hypothesis, structural hypothesis H1, or a combination of both of core and H1, given an input to small pelagic fish, with probability of occurrence attributed to each model hypothesis. For each input variable (horizontal row), the 2 variables with the largest mutual information are in **bold**.

Model and hypothesis	Input to	----------------Mutual information-------------			
		Sardine (%)	Anchovy (%)	Round sardinella (%)	Predators (%)
M_2 50% Core; 50% H_1	Sardine	**16.1**	2.31	1.01	**4.5**
M_2 50% Core; 50% H_1	Anchovy	0.1	**21.7**	2.0	**9.7**
M_2 50% Core; 50% H_1	Round sardinella	3.4	2.3	**12.0**	**4.5**
M_2 Core (100%)	Sardine	**21.7**	2.3	1.3	**5.8**
M_2 Core (100%)	Anchovy	2.3	**21.7**	1.3	**5.8**
M_2 Core (100%)	Round sardinella	2.3	2.3	**12.3**	**5.8**
M_2 H_1 (100%)	Sardine	**11.5**	2.3	0.8	**3.5**
M_2 H_1 (100%)	Anchovy	1.1	**21.7**	2.8	**14.8**
M_2 H_1 (100%)	Round sardinella	**4.9**	2.3	**11.8**	3.5

Sensitivity analysis under the core model hypothesis (fisheries target anchovy and sardine independently of their abundance), structural hypothesis H_1 (fisheries preferably target anchovy and switch to sardine when anchovy is low), or a combination of both of them (core and H_1, where both were given the same weight of 50% probability of occurrence) yielded similar results (Table 8). To distinguish between likely inputs to sardine, anchovy, and round sardinella, the most informative model variable, after excluding their own input variable, was, in all but 1 instance, the variable 'predators'. Therefore, information about predators in the ecosystem (e.g. if they have declined or increased) would allow in the future to further decrease the uncertainty in the results of the perturbation scenarios performed in this study.

The one exception to these results was observed under the structural hypothesis H1. In this case, the most informative variable in the model to distinguish which is the likely perturbation source to round sardinella was ‘sardine’ (Table 8). Therefore, further information on sardine dynamics would allow in the future decreasing the uncertainty in those perturbation scenarios performed in relation to round sardinella.

5.4 Discussion

Structure of NW Mediterranean pelagic food web

This study provides insights about plausible causes of change in the NW Mediterranean pelagic food web, highlights some uncertainties and identifies guidance for future research. Testing of the alternative model structures found the configuration most frequently selected was model M2, which represents the modification of SPF not limiting their trophic resources and therefore not competing for food (for phytoplankton and zooplankton) due to partial difference in their trophic niche (Costalago et al. 2012, Costalago & Palomera 2014, Albo-Puigserver et al. 2016). This suggests an ecosystem structure where there is no wasp-waist control of SPF (as previously suggested in true upwelling systems, Cury et al. 2000), but only control to the predators and not to the prey, which is in agreement with findings in other regions (Koehn et al. 2016). These results would suggest that population changes of SPF could have an impact on their predators. On the contrary, in previous studies in the study area covering the 1970s to the early 2000s, we described sardine as an important species exerting wasp-waist control in the system (Coll et al. 2008), thus also exerting control on its prey. These new results may indicate a loss of wasp- waist control of SPF due to a decline of their biomass and the current low levels of the stock (Table 4).

They also highlight that a further development of this work should focus on testing individually the role of sardine as a wasp-waist organism. The rest of the other model structures, models M_1, M_3 and M_4, did not produce predictions that were as highly consistent with observations as model M_2 for either single or multiple pressures, and thus are considered to be less likely to represent observed dynamics of the system. This suggests that the hypo thesis that predators do not limit round sardinella due to its larger abundance and recent expansion (Sabatés et al. 2006) (M_1), the significant predation of gelatinous plankton on anchovy larvae and zooplankton (Tilves et al. 2016) (M_3), and a combination of all the alternative hypotheses are less able to lead to model predictions consistent with observed changes in SPF.

Our results also demonstrate that to understand the structure of the pelagic marine food web, it is important to consider how fisheries affect SPF populations. In our analyses, the structural hypothesis H_1 gave predictions that were highly consistent with observations several times. Hence, our results support the possibility that fisheries preferably target anchovy and switch to sardine when anchovy is low as a plausible mechanism governing purse-seine fisheries (as has been already observed when interacting with fishermen in the study area). This mechanism, however, is ruled by market and price behaviours, which highlights the need to consider fisheries dynamics within the ecosystem dynamics. Future development of this work should include a socioeconomic model linked with the biological processes modelled in this study, as has been done elsewhere (Dambacher et al. 2009, 2015). Our results also showed that the structural hypothesis considering that sardine and anchovy recruitment is highly fished (Palomera et al. 2007) (H_2) in combination with H_1 could be potentially viable. An explicit socioeconomic model of the fishery could help distinguish between these plausible options.

Models that included the structural hypothesis that sardine and anchovy recruitment was overfished (H_3) had a high potential to be unstable, and hence we did not incorporate these models into our analyses to predict change in equilibrium. The dynamics of these models are dominated by excessive amounts of higher-level feedback and thus are prone to exhibit undamped oscillations. Since both stocks of sardine and anchovy have been, or are, highly fished and could be overfished (Palomera et al. 2007, STECF 2016a), these ecosystems could be near to or at an unstable state, and the system could shift to another equilibrium or become highly oscillatory if recruitment overfishing were to persist. Modelling the dynamics of unstable systems exceeds the scope of traditional approaches to ecosystem modelling (qualitative or quantitative) and requires precise knowledge of system thresh olds and extra caution in interpretation and application of modelling results.

Plausible pressures on SPF change

Perturbation scenarios with only a single pressure yielded results that were consistent with observations. An increase in SST that produces a positive impact on round sardinella or on gelatinous zooplankton was selected as a pressure that could alone describe field observations, i.e. a decline in anchovy and sardine, and an increase in round sardinella. In fact, there is strong evidence of an overall increase of SST in the area, and biological impacts on several organisms spanning different habitats have already been documented (Calvo et al. 2011), which includes impacts on anchovy and sardine (Checkley et al. 2017). These results complement previous knowledge highlighting that an increase in SST has probably already impacted the ecosystem functioning of the pelagic food web of the NW Mediterranean Sea. Therefore, the geographic expansion of round sardinella and the increase in gelatinous zooplankton (Sabatés et al. 2006, 2009, Maynou et al. 2014, Tilves et al. 2016), which are linked to changes in SST, may have had important effects on other compartments of the pelagic system, such as the abundance of sardine and anchovy through, for example, mechanisms of competition for food between SPF. These results are in line with a previous study where the analysis of long time series of SPF landings from the Atlantic and Pacific Oceans highlighted that the hypothesis with broadest support regarding SPF changes was process noise dependence (Hosack et al. 2013). This hypothesis states that environmental parameters may cause one species to predominate over the other due to changes in environmental conditions, such as temperature, acting directly on SPF, or acting through their food abundance or quality.

An increase of fisheries impacts alone could also have led to important changes in the pelagic food web, but considering our results, the probability that this is the main driver of change is lower than an increase of SST. This is in line with previous consideration in the Gulf of Lions pelagic ecosystem (Van Beveren 2015, Saraux et al. 2018). Fisheries statistics and stock assessment models, however, suggest that sardine and anchovy have been highly fished in the study area (SAC-GFCM 2015, STECF 2016b,c). Therefore, our results could also reflect the fact that the role of fisheries has been important in the past, but once the stock is relatively low, as in the current case, then environmental factors such as SST can dominate SPF dynamics (Planque et al. 2010, Essington et al. 2015).

Bottom-up changes of the food web could also be part of the drivers of SPF changes, due to changes in zooplankton abundance, composition or quality, and could have strong effects on the reproduction, growth and mortality of SPF, as has been proposed by Brosset et al. (2015, 2016). These changes could explain the low body condition that sardine and anchovy have shown in the Mediterranean Sea in recent years (Albo-Puigserver et al. 2017b, Brosset et al. 2017). Changes in plankton composition and quality need to be further analysed (Saraux et al. 2018), and if confirmed, new results could be tested in our models.

The increase of predators as a potential explanation for the decline in SPF did not yield predictions that were consistent with observations. This suggests that it is unlikely that the recovery of predators of SPF will have an effect on SPF populations under current food web structure. Previous studies have already suggested that predation mortality of Atlantic bluefin tuna *Thunnus thynnus* on SPF populations has a very low impact on their abundance (Van Beveren et al. 2017), and that SPF species are not the only prey or dominant source of food for pelagic predators (Navarro et al. 2017).

With respect to cumulative impacts, it is important to highlight that perturbation scenarios combining multiple pressures showed several combination sets of pressures that had moderate to high probabilities of producing observed results, though in general they all had lower probabilities of occurrence than a single pressure alone. Interestingly, the same sets of multiple pressures gave predictions with moderate to high levels of sign determinacy in combination with various alternative models and structural hypotheses, suggesting perhaps that idiosyncratic sets of pressures can produce similar results across different model structures. Even though a more parsimonious response is preferred, a combination of drivers that yield a decline of sardine and anchovy and an increase of round sardinella cannot be disregarded, as multiple pressures often spatially overlap in the ocean worldwide (Halpern et al. 2015), and in the Mediterranean Sea in particular (Coll et al. 2012, Micheli et al. 2013). The most frequent set of multiple pressures considered included an increase of SST positively affecting round sardinella and gelatinous zooplankton, a decrease of zooplankton quality, an increase of fishing impact and an increase of predators. Therefore, we cannot falsify the possibility that cumulative impacts of pressures and their synergistic effects may be important when trying to understand the causes of change of SPF populations in the NW Mediterranean Sea.

Our study provides a first means to compare different hypotheses that have been proposed to explain SPF changes in the region but have not been previously tested together, and can be the basis for future work. Quantitative dynamic modelling approaches could help further evaluate these alternative hypo theses (e.g. Fulton 2010, Fulton et al. 2015). Another promising approach will be to test some of the key aspects of our more plausible model structures using analysis of time series data by state-space modelling (Hosack et al. 2013). Furthermore, as more information is collected, additional models based on refined or new hypotheses can be considered and current models can be modified to address aspects of the problem not considered so far (Dambacher et al. 2015).

Complementing monitoring strategies

Even though several combinations of drivers and structural hypotheses were considered in this study, further work needs to be done to reduce uncertainty in modelling predictions and consider new hypotheses. Sensitivity analyses allowed us to identify key monitoring variables to reduce model structural uncertainty. One important monitoring variable identified in our models was ‘predators’. The importance of this variable can be linked to the large bottom-up control that SPF play in the ecosystem and the dependency of predators on their populations (Cury et al. 2011). This suggests that it is important to distinguish which predators base their diet on anchovy and sardine and could be monitored in order to indirectly observe any effects of change in populations of SPF. The breeding success and fitness of predators can be highly affected by the quantity (food availability) and quality (energy intake per unit) of their prey (Österblom et al. 2008). Thus, predators that rely mainly on SPF would be highly sensitive to changes in SPF populations, as has been previously reported for seabirds such as Audouin’s gull *Larus audouinii* of the Ebro Delta (Arcos & Oro 2002, Payo-Payo et al. 2016), Steller sea lions *Eumetopias jubatus* in the Gulf of Alaska (Rosen & Trites 2000) or African penguin *Spheniscus demersus* in South Africa (Robinson et al. 2015).

To increase the knowledge of which pressures are driving round sardinella, our analyses suggest that sardine populations are a good monitoring target and research investment. The importance of this variable is probably related to the divergent trends that both species have shown in the ecosystem historically, with a decline of sardines and an increase of round sardinella (Palomera et al. 2007). This result is relevant since there is a lack of information about sardine biology and ecology in the Mediterranean Sea (Palomera et al. 2007), and further studies could be beneficial.

Complexity when predicting and managing change

While there were 19 perturbation scenarios that gave predictions moderately or highly consistent with observations, the vast majority of the 285 scenarios considered yielded low levels of sign determinacy. This highlights the challenge of predicting complex systems such as marine food webs and the need to use complementary modelling techniques that quantify the strength of the interactions (Fulton 2010, Fulton et al. 2015). Results from this study provide insight into model structure that can inform future quantitative modelling endeavours in the study area such as the ones developed for SPF in other marine ecosystems (e.g. Shannon et al. 2004, Koehn et al. 2016, Punt et al. 2016, Kaplan et al. 2017).

Of all the pressures that were tested, managing for pressures such as SST or changes in plankton abundance is difficult at local or regional scales. Here, we can only aim to manage fisheries in a sustainable and adaptive way, with all the challenges implied in the process. Future work should focus on testing which management alternatives can ensure the sustainability of the marine ecosystems of the NW Mediterranean and are also robust to a changing climate.

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General Discussion

To advance on our understanding about ecosystem functioning and progress towards a sustainable management of marine resources from an ecosystem-based approach, it is necessary to gain detailed knowledge on the biology, ecological interactions and the transfer of energy of key marine species (Pauly et al. 2002).

Current declines of small pelagic fish (SPF) populations in the northwestern Mediterranean Sea have generated socio-economic and ecological impacts. As a consequence, different hypotheses to potentially explain these changes have been proposed and tested (Saraux et al. 2019; Coll and Belllido, 2019). In this context, this thesis has contributed to fill important gaps in the trophodynamic aspects of the most important species of the pelagic compartment. Specifically, I have provided robust scientific findings on the trophic ecology and ecological energetics of small and medium pelagic fish from the study area by combining different methodological approaches. I have also contributed to the understanding of the structure of the pelagic compartment, providing insights about plausible causes of change in the northwestern Mediterranean pelagic food web.

Main findings on trophodynamic of SPF and their ecological implications

Trophic interactions between species are dynamic rather than fixed and may vary through the year depending on several biotic and abiotic factors (França et al. 2011). Biological processes (i.e. growth, maintenance or reproduction) and external factors (i.e. food availability) affect energy storage and nutritional condition of fish that ultimately, and in combination with the trophic interactions, affects their ecological energetics. In the northwestern Mediterranean, information on trophic interactions and energy allocation at seasonal scale is very limited, impairing our capacity to evaluate the ecosystem dynamics through the year.

In Chapter 1, the seasonal study of trophic interaction between small, medium pelagic fish and potential predators or competitors, revealed that the structure of the pelagic compartment does not present a seasonal variability at community level.

However, when looking at species level, changes in the trophic overlap between pairs of species were observed. Specifically, in summer, there was less trophic overlap than in the rest of the year, while in winter, when biological productivity was low, the proportion of species with overlapping niches was higher. This is the case of sardine and anchovy, whose trophic niches were segregated in summer and partially overlapped their niches in the other seasons. Instead, the hypothesis of potential competition of round sardinella with sardine and anchovy was not confirmed for adults (Tsikliras et al. 2005, Sabatés et al. 2006). The higher trophic level observed in round sardinella suggested that this species was probably feeding on different and higher trophic level zooplankton than sardine and anchovy. However, information on the feeding ecology of adults of round sardinella in the western Mediterranean was not available previous to this thesis to fully understand the trophic relationships between SPF.

Therefore, in Chapter 2 we studied the seasonal dietary habits of round sardinella using stomach content and stable isotopes analyses. In this chapter, juveniles were also included in the analysis, as well as adult individuals. While round sardinella did not overlap at adult stages (Chapter 1), juvenile individuals of round sardinella had a wider trophic overlap with juvenile of sardine and anchovy and adults of anchovy. Ontogenetic shifts in diets have been widely described in many species, in general trophic level increases with ontogenetic stage, as observed in anchovy and round sardinella. Instead, in sardine there was a decrease in the trophic level of adults due to the increase of consumption of diatoms (Costalago & Palomera, 2014; Chapter 2). These results highlight the importance of taking into account not only the temporal variability in diet but also the ontogenetic stage of investigated individuals when looking at interactions between species.

Stomach content analysis helped to understand that the higher trophic levels observed in adults of round sardinella and the overlap at juvenile stages with sardine and anchovy was due to differences in prey size. Moreover, for the first time in the Mediterranean Sea, we described gelatinous zooplankton, and more specifically salps, as a main food resource for a small pelagic fish (Chapter 2). In other areas, such as the Pacific Ocean, evidences of the increase in consumption of gelatinous zooplankton by forage pelagic fish has been recently found for periods with high SST and it was related with lower body condition of forage fish (Brodeur et al. 2019). Gelatinous zooplankton is generally classified as a poor nutritional prey (Doyle et al. 2007), but recent studies have seen how certain marine predators might achieve the energy demands through high consumption of gelatinous zooplankton (Dubischar et al. 2012). In a context of climate change where gelatinous zooplankton is more abundant (Grémillet et al. 2017), it is highly important to assess how this can affect the trophic web and ultimately the ecological energetics of pelagic species. In our case, salps in stomach of round sardinella were observed in spring, when the species accumulates energy just before the spawning period (Chapter 3). Thus, the consumption of gelatinous zooplankton in certain seasons might influence the accumulation of energy during this period and compromise the different biological processes (i.e. maintenance, growth or reproduction) of the following seasons.

In Chapter 3, the energy dynamics of round sardinella and other six forage fish were studied. Seasonal variability was observed in most of the species, with a general pattern of higher accumulation of energy in spring, regardless of their breeding strategy. As we observed for the seasonal variation in trophic relationships, the higher energy density (ED) values in spring were probably related to the late-winter early-spring phytoplankton bloom that led to higher zooplankton availability with higher lipid content, as observed in the area of study by Barroeta et al. (2017). Another factor that determined the energy storage in SMPF was spawning season since it is a highly consuming process energetically.

When looking at the trophic level and energy density of small and medium sized pelagic fish, I observed how medium pelagic fish (MPF) had higher trophic level and energy density (ED) values than SPF (Chapter 1 and 3). Trophic level and body size are often correlated, especially in pelagic fish, since larger sized fish may be able to feed on larger prey with higher energy content (Cohen et al., 1993; Jennings et al., 2008; Bachiller and Irigoien, 2013). In the case of MPF, other features, such as the accumulation of fat reserves for migration may also contribute to the high ED values (Chapter 3). Within SPF, sardine was an exception and had mean annual ED values similar to those of MPF. This is mainly explained by the capital breeding strategy of sardine. The accumulation of fat reserves during the resting period (spring and summer), when zooplankton biomass is high (Sabatés et al. 2006), allowed sardine to have ED values similar to those of MPF.

Energy variability of forage species that are highly consumed by marine predators has not been traditionally taken into account in bioenergetic models of predators or food-web models (Van Beveren et al. 2017). For instance, in the case of sardine that is a highly consumed forage fish (Stergiou & Karpouzi, 2002), the difference in energy gain per gram consumed between feeding on sardine in spring or winter would be of 1.9 kJ per gram. Feeding in spring on round sardinella instead of sardine would imply to gain 0.6 kJ less per gram ingested. These differences in ED between seasons and species should be incorporated when evaluating the ecosystem consequence of the observed declines in sardine and anchovy biomass and body condition in the northwestern Mediterranean Sea (Brosset et al. 2017, Coll and Bellido et al. 2019). For instance, the "Junk food hypothesis" has been proposed in different areas were there has been a shift in diet of predators from high energy prey to low energey prey (Österblom et al. 2008). In the Gulf of Alaska or the North Sea the reduction of fattier pelagic fish translated in a decline in Steller sea lions and common guillemots, respectively (Rosen & Trites 2000, Wanless et al. 2005). Thenrefore, with the observed decline in biomass and body condition of sardine and anchovy, we could expect to observe "junk-food" effects in predators specialized in SPF in the northwestern Mediterranean. In fact, in Chapter 5, predators of SPF were identified as a good monitoring variable to track changes in SPF. Few studies on body condition or changes in food preference of predators have include information on prey quality in the Mediterranean Sea. Arcos & Oro (2002) found that nutritional requirements of audouin's gull (*Ichthyaetus audouinii)* were mainly covered by clupeid fish from purse-seiners in the Ebro Delta. Also, Vivien et al. (2012) proposed a potential relation between prey quality and body condition of hake (*Merluccius merluccius*). However, the recent declines in body condition and abundance of sardine and anchovy have not been directly associated with declines in predators yet since for many species, predators and preys, temporal biological information on body condition and feeding habits is limited in the northwestern Mediterranean Sea.

The evaluation of the nutritional status of populations has proved to be a good indicator of ecosystem health and has been recommended to be included in the

Integrated Ecosystem Assessment (IEA; ICES 2017). To evaluate the physiological status of fishes and measure the stored energy, different condition indeces are available (Lloret et al., 2013). In Chapter 3 and 4, with the used of indirect and direct condition indeces we observed the seasonal variability in body condition of sardine and anchovy. The energetic cycle of both species was closely related to the temporal lag between the spawning seasons and late-winter early spring phytoplankton bloom (Basilone et al. 2006, Pethybridge et al. 2014). Although sardine and anchovy had different energy allocation strategies, capital and income breeder respectively, in both species, an early decline in energy reserves was observed in late summer and early fall, that may be related with unfavorable environmental conditions during spring and summer. The low energy reserves observed were in line with the decline in body condition reported for sardine and anchovy in the last decade in most of the Mediterranean (Brosset et al. 2017). Similar to what we observed from the results in Chapter 1, 2 and 3, data analysis at the seasonal scale in Chapter 4 allowed us to better understand intra-annual processes that could ultimately affect inter-annual variability.

Sustained declines in body condition can be a symptom of changes in food quality and quantity (Rosen & Trites 2000). In the northwestern Mediterranean Sea, one of the main hypotheses that has been proposed to explain the decline in body condition is the change in plankton phenology (Saraux et al. 2019). However, the lack of time-series of plankton composition has impeded the evaluation of this hypothesis until now. Indirectly, changes in stomach content analysis of SPF have partially corroborated this hypothesis (Brosset et al. 2016) and the observation of salps in stomachs of round sardinella could also be an indicator of a change in plankton composition (Chapter 2).

When analyzing different model structures of the pelagic food web (Chapter 5), it was observed how the best model structure selected to explain observed changes in SPF did not include a control of SPF on lower trophic level, contrary to the wasp-waist control exerted by sardine observed in the past by quantitative food-web models from the same area (Coll et al. 2008). These new results may indicate a loss of wasp- waist control of SPF due to a decline of their biomass and the current low levels of the stock (Coll and Bellido 2019).

Many other hypotheses have been proposed to explain the decline in abundance, biomass, body condition and growth (Van Beveren et al. 2014, Brosset et al. 2017), not only by the scientific community but also from different stakeholders that are involved in the fishing activity. Whether single pressures or cumulative pressures could better explain the decline of sardine and anchovy and the increase in biomass of round sardinella it is not clear (Chapter 5). The increase in SST having either a positive impact on round sardinella or on gelatinous zooplankton abundance was the pressure that alone provided the most plausible insights into observed changes. Instead, when looking to the combination of various pressures, an increase in SST, an increase of fisheries exploitation and changes in zooplankton also delivered results matching current observations. From these results I can conclude that environmental changes and bottom up changes including changes in zooplankton are likely to play an important role, but that also other factors such as fishing pressure are very likely involved in the explanation of the current situation. One of the main challenges in other fisheries collapses studied has been to disentangle the impact of fishing vs. natural processes on population dynamics (Essington et al. 2015, Quattrocchi 2017). Since natural processes are difficult to control at the local scale, one can only aim to manage fisheries in a sustainable and adaptive way, reducing fishing pressure when populations may be at risk to protect the food web from collapse and avoid long-term consequences on catches (Essington et al. 2015, Ramírez et al. 2018; Figure 16).

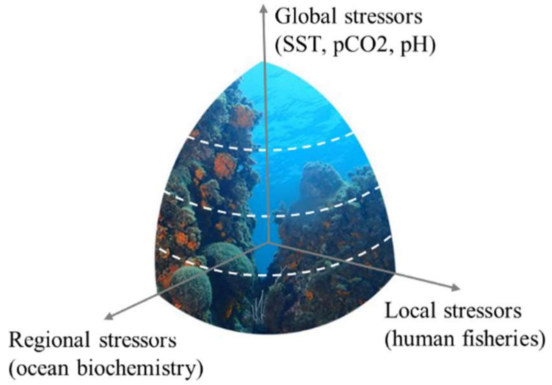

Figure 16. Three dimensions of the safe operating space (SOS) for the Mediterranean Sea. Source: Ramírez et al. 2018.

Complementary methodological approach

In this thesis I have used different methodologies in order to gain a better comprehensive understanding of SPF trophodynamics. All methodologies have their advantages and disadvantages, and the combination of several complementary methodologies can contribute to obtain more accurate information.

In the case of the study of trophic relationship and the structure of the food web, stomach content analysis (SCA) has been traditionally used. However, in the last decades, new methodologies such as stable isotope analysis (SIA) has been widely implemented and more recently, fatty acids, compound specific stable isotopes and DNA metabarcoding are also being used in the study of trophic interactions (Nielsen et al. 2018). Although the use of stable isotopes of $\delta^{15}N$ and $\delta^{13}C$ allowed me to characterize the seasonal structure of the pelagic community, the low taxonomic resolution provided by SIA means that information on the feeding ecology at species or group level is necessary in order to better interpret the SIA results. Therefore, when information on the feeding ecology of the species is not available from literature, I recommend to use both analysis, SIA and SCA, whenever possible as illustrated in Chapter 2.

The study of ecological energetics and physiological condition in sardine and anchovy was conducted using direct calorimeter and relative condition indeces (Le Cren, 1951). Both measures were highly correlated and reflected seasonal variability in energy content for both species. Instead, lipid content in muscle seemed to be more suitable for species like sardine, which accumulate a lot of lipids in certain periods of the year, than for those species in which lipid content is lower and with les intra-annual variability such as anchovy (Chapter 4). The advantage of using direct calorimetry to measure changes in energy is that it is a direct measure that gives comparable values through different studies and integrates changes in proximate composition, including lipids but also proteins. However, it is a highly time consuming technique and difficult to use for rapid assessments. Instead, the indirect condition index (Le Cren 1951) has been widely used and validated for the species as it is a rapid and cost-effective method easy to implement in long-term monitoring programs (ICES 2008). Other new techniques and devices such as the fatmeter have not been evaluated in this thesis, but previous research has demonstrated the utility of this indirect method to rapidly evaluate the body condition and energy content of SPF (Brosset, Fromentin, et al. 2015). In this thesis, the relationship between percentage of dry weight and energy density of the entire individual is provided, as well as between the gonadosomatic index and energy density of gonads for anchovy and sardine (Chapter 4). These relationships might be of use in the future for indirect measurement of ED of anchovy and sardine in the northwestern Mediterranean Sea.

In order to integrate available biological and ecological data with the final objective to obtain an overall picture of how ecosystems are structured and how they function, food-web models have emerged as a suitable tool (Christensen et al. 2015). In order to understand the main patterns and trends, it is not always necessary to wait until we have precise quantitative measurements of all interactions when the direction of the interaction is known. Thus, qualitative analyses of a system may help in predicting its general dynamics (Dambacher et al. 2009). In Chapter 5, the qualitative models allowed to analyses the stability of different model configuration and determine the most likely structure of the pelagic food web related to small pelagic fish. Moreover, it was a useful tool to perform the assessment of different press perturbation scenarios and determine the most likely explanation to the decline of sardine and anchovy and increase of round sardinella (Chapter 5). Results obtained from the qualitative modelling could be used to inform future quantitative models. Furthermore, as more information become available, additional models based on refined or new hypotheses can be considered and current models can be modified to address aspects of the problem not considered so far (Dambacher et al. 2015).

Data gaps, limitations and future research

In this thesis I focused on the study of intra-annual variability in trophic relationships and ecological energetics of pelagic fish with monthly and seasonal samplings that allowed me to study processes at small temporal scale in depth. This information is key to later interpret processes at longer temporal scales. However, in order to study inter-annual processes and quantify the relationship of biological parameters with environmental variables, the analysis of time-series data of several years would be needed (Van Beveren et al. 2014). All the data presented in this thesis can be used as a baseline for comparison with data collected in future projects.

Findings of each chapter also generated new research questions and highlighted gaps of knowledge. In Chapter 1 the use of stable isotope analysis allowed me to characterize the structure of the pelagic food web. However, some of the variability found between congeneric species such as mackerels and horse mackerels could not be fully interpreted since data on feeding habits, distribution and migration of these species are limited in the study area. Moreover, in future research it will be necessary to obtain reference values of stable isotopes for the baseline (i.e. phytoplankton) and for prey (i.e. copepods, cladocerans, salps) in each season, in order to go one step further and develop isotopic mixing models to estimate diets of SMPF.

In Chapters 2 and 5 results pointed out that gelatinous zooplankton might be playing an important role in SPF dynamics. However, evidence of predation of gelatinous zooplankton by sardine and anchovy is still lacking. In future research it would be necessary to determine if sardine and anchovy are also able to prey on salps and if the predation on gelatinous zooplankton might affect the physiological state of SPF (Brodeur et al. 2019). Gelatinous zooplankton is not always easy to detect due to its rapid digestion. Therefore, novel techniques such as DNA metabarcoding could be combined with SCA and SIA (Nielsen et al. 2018). Salps are mainly present in high abundance in spring and in certain years when blooms occur (Henschke et al. 2016). Thus, in order to test if round sardinella and the other SPF have a preference for feeding on salps or if they feed on salps when other preferred resources (i.e. copepods) are low (Mianzan et al. 2001, Brodeur et al. 2019), it would be necessary also to have data on plankton composition.

The decline in body condition of SPF in the western Mediterranean Sea in the last decade has been reported in previous studies (Brosset et al. 2015, 2017). In Chapter 3 and 4, I observed that there is a high seasonal variability in energy density that is highly related to the environmental variables and primary productivity. The energetic data obtained in combination with other life history parameters (i.e. growth, feeding rate) could be used in future bioenergetics models with the aim of evaluating the potential consequences of environmental changes in the energy allocation of SPF (Pethybridge et al. 2013, Gatti et al. 2017).

In the qualitative network model developed (Chapter 5), the variable “predators” was the most informative variable to monitor changes in small pelagic fish. However, nowadays there is scarce information on stomach content analysis of predators in order to use them as a monitoring variable. Consequences of the decline of SPF have not been yet observed in higher trophic levels due to the lack of information on predators that will need to be addressed in the future. On the other hand, the other type of “predators” included in the model were fisheries. How fisheries behavior affect SPF populations was important to determine the structure of the pelagic marine food web. Hence, future research on the functioning of the pelagic food web should consider the fisheries dynamics interactions including socioeconomic processes and the participation of the local ecological knowledge to better represent the real interaction between fisheries and SPF resources (Dambacher et al 2009, 2015).

In addition to the hypotheses tested in Chapter 5, there are other hypotheses on why SPF have substantially changed in the northwestern Mediterranean Sea in recent decades that have not been included in this thesis and need to be addressed in the future (Figure 17; Saraux et al. 2019). For example, the increase of diseases has been reported as a potential causes of the decline in sardine and anchovy, but no evidence has been found until now (Ferrer-Maza et al. 2016, Van Beveren et al. 2016). In addition, how contaminants and plastic pollution in the seas could influence the physiology of SPF is still unknown and as new techniques become available this issue should be properly addressed (Compa et al. 2018, Sala et al. 2019). Future success in predicting and evaluating species and ecosystem change will depend on how successful we are at integrating the physiological, evolutionary, ecological and environmental processes that happen at cellular, individual and supra-individual levels of organization without losing perspective (McKenzie et al. 2016). A compromise between generality, realism and precisions is necessary to navigate this challenge (Larocque 2012, Weathers et al. 2016).

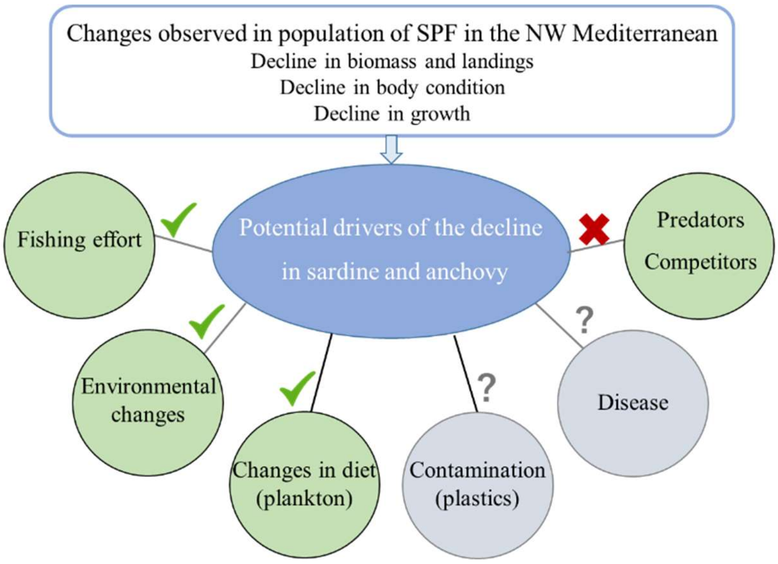

Figure 17. Summary of the potential drivers of the decline of sardine and anchovy populations. Green circles represent potential drivers explored in this thesis, while grey circles represent potential drivers not included in this thesis. When evidence of relationship of the driver with the decline of sardine and anchovy have been confirmed, it is indicated with the symbol "√", while evidence have not been found the symbol is an "X". The relationship that have not yet been confirmed are indicated with the symbol "?". Adapted from: Saraux et al. 2019.

Final Conclusions

Main conclusions derived from this thesis are presented below (see also Figure 18):

1. Despite intraspecific seasonal variability for some species and higher trophic overlap in winter than in summer, overall community trophic structure appeared relatively stable throughout the year.

2. Stable isotope analysis revealed a clear trophic segregation and higher trophic position of adult round sardinella from adult sardine and anchovy, which was mainly explained by the preference of round sardinella for larger zooplankton. Instead, the trophic niche of juvenile round sardinella partially overlapped with that of juvenile sardine and anchovy. Therefore, the range expansion of round sardinella would affect sardine and anchovy populations at certain ontogenetic stages in a situation of food limitation.

3. Variation in seasonal dietary habits was found in the stomach contents of round sardinella, with copepods, tunicates and diatoms dominating the diet in winter, cladocerans and copepods in summer and in spring the main prey were salps. This is the first time that gelatinous zooplankton has been identified as an important prey for a small pelagic fish in the Mediterranean Sea. The trophic link between gelatinous zooplankton and SPF should be incorporated in future scenarios.

4. The ecological energetics of seven forage fish species revealed that the inter-specific differences in energy density were linked to spawning period, energy allocation strategies for reproduction and growth, and feeding ecology. In general, higher energy density was observed in spring for all species, regardless of their breeding strategy, probably as a consequence of the late-winter phytoplankton bloom.

5. The high energy density observed in sardine during spring and summer, and similar to the medium pelagic fish was due to its capital breeding strategy. The

changes in energy between seasons should be included in the understanding of predator dynamics, since the energy gain per unit of prey will differ not only depending on the prey but also depending on the season.

6. Sardine and anchovy presented opposite breeding strategies. The use of direct and indirect condition indexes revealed how the use of Kn adequately represented the changes in body condition, as well as in ED. Instead lipids in anchovy were only partially reflecting the changes in anchovy body condition.

7. The decline in body condition at the end of summer and early fall in sardine and anchovy are probably related to a decrease of the energy obtained during spring and early summer. This could be linked to the previously reported decline in body condition of the last decade.

8. Among all the potential pressures that have been suggested as drivers of the observed declines in landings and body condition of sardine and anchovy, the increase in sea surface temperature that had a positive impact on both round sardinella and on gelatinous zooplankton abundance and the increase of fisheries exploitation were the pressures that provided the most plausible insights into observed changes.

9. Predators of SPF were identified as the most informative monitoring variable to discern between likely causes of perturbations to populations of SPF and further monitoring programs collecting data on high trophic levels are needed in order to assess how the observed changes in small pelagic fish are affecting higher TL.

10. It has been seen how both fisheries and environmental variability play a key role in the understanding of the food-web structure and changes. Since the species target in this PhD thesis are of high commercial interest, in future integrated ecosystem analyses it will be necessary to deeply amplify the socio-economical component.

11. The combination of stable isotope analysis, stomach content, direct calorimetry and body condition indeces allowed us to understand the trophodynamics of small and medium pelagic fish at species and community level and use this information to understand the structure of the pelagic community in a qualitative model.

12. This PhD thesis was mainly focused on one year of sampling that allowed us to characterize the seasonal changes in the pelagic community in depth. In future research it is necessary to incorporate the seasonality in over multiple years longer term assessments covering not only seasonal changes but also the spatio-temporal distribution of these changes.

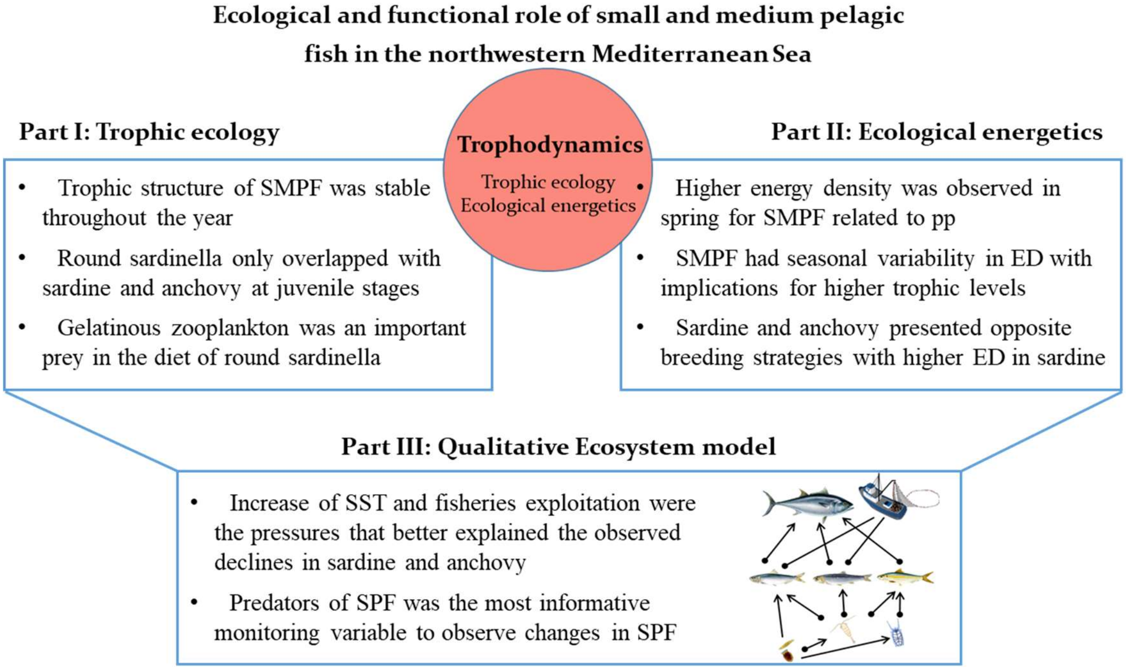

Figure 18. Summary of the main results of the present thesis. “pp” stand for primary production.

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** General Introduction and Discussion references*

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