TROPHIC ECOLOGY IN MARINE ECOSYSTEMS FROM THE BALEARIC SEA (WESTERN MEDITERRANEAN)

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TROPHIC ECOLOGY IN MARINE ECOSYSTEMS FROM THE BALEARIC SEA (WESTERN MEDITERRANEAN)

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Esta tesis te la dedico a ti mamá.
Tú me rodeaste de mar, me enseñaste a ser valiente y confiaste en mí.

Agradecimientos

List of papers

This thesis is based on the following papers:

I.   M. Valls, A. Quetglas, F. Ordines, J. Moranta (2011). Feeding ecology of demersal elasmobranchs from the shelf and slope off the Balearic Sea (western Mediterranean). Scientia Marina 75 (4), 633-639

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IV. M. Valls, M. Cabanellas-Reboredo, I. Uranga, A. Quetglas (2015). Feeding ecology of two squid species from the western Mediterranean. Marine Ecology Progress Series 531, 207-219

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V.   M. Valls, L. Rueda, A. Quetglas (2017). Feeding strategies and resource partitioning among elasmobranchs and cephalopods in Mediterranean deep-sea ecosystems. Deep Sea Research Part I, Accepted.

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Acronyms and abbreviations

A (%)	Appareance frequency
ANOVA	Analysis of Variance
AsB	Algerian sub-Basin
B	Biomass
BBL	Benthopelagic Boundary Layer
B_i	Levin's standardized index
BPC	Bentho-Pelagic Coupling
BsB	Balearic sub-Basin
C:N	Carbon Nitrogen ratio
CA/C	Cabrera
CD	Centroid Distance
CS	Coastal Shelf
DCF	Data Collection Framework
DR	Depth Range
DSL	Deep Scattering Layer
GSA	Geographical Sub-Area
H'	Trophic diversity
HC	Hyperbenthic Compartment
IDEADOS	Influencia de la estructura y dinámica oceanográfica sobre poblaciones demersales en aguas de las Islas Baleares
IKMT	Isaacs-Kidd Midwater Trawl Net
IRI	Index of Relative Importance
MC	Monte Carlo test
MDS	Multidimensional Scaling
MEDITS	Mediterranean International Trawl Survey
ML	Dorsal Mantle Length
MOCNESS	Multiple Opening/Closing Net and Environmental Sensing System
MS	Middle Slope
MSFD	Marine Strategy Framework Directive
N (%)	Percentage by Number of the food item
N	Total number of individuals
O (%)	Frequency of occurrence of the food item
PC	Pelagic Compartment
pCCA	partial Canonical Correspondence Analysis
PERMANOVA	Permutational Multivariate Analysis of Variance
POM	Particulate Organic Matter
R/V	Research Vessel
Reg	Regurgitated
RMT	Rectangular Midwater Trawl
SB	Shelf Break
SCA	Stomach Content Analysis
SEA	Standard Ellipse Area
SI	Schoener Index
SIA	Stable Isotope Analysis
SIAR	Stable Isotope Analysis in R
SIMPER	Similarity Percentage
SIMPROF	Similarity Profile analysis

SL	Standard Length
SO	Sóller
TA	Total convex hull Area
TDF	Trophic discrimination factor
TL	Total Length
T_{max}	Maximum size
T_{min}	Minimum size
TrG	Trophic Group
TrL	Trophic Level
TW	Total Weight
US	Upper Slope
V (%)	Percentage by Volume of the food item
v (%)	Vacuity index
V.E	Variance Explained
VG	Vertical Guild
WR	Weight Range
Δ	Isotopic discrimination
δ	Delta notation

Summary

Conservation of marine ecosystem structure and functioning is a priority target within the context of environmental management. For such a target is fundamental to know the array of predator-prey interactions as a basis to understand the food web structure and dynamics. In this Thesis, the trophic structure of deep-sea food webs off the Balearic Islands and the feeding ecology of different taxonomic groups playing a key role in the trophodynamics of marine ecosystems, were investigated.

Chapter 3 studied the trophic networks based on stable isotope analysis (SIA). A relatively large food web (89 species) encompassing both the pelagic and the hyperbenthic compartment from two geographical areas with contrasting hydrographic conditions were analysed. Although the food webs extended up to 4 trophic levels, both in the pelagic and hyperbenthic compartment, most species occupied intermediate levels. The wide range of isotopic values found suggested a high partitioning rate of trophic resources. Food webs of the two study areas showed similar close benthopelagic coupling decreasing with depth, although some location variability was found likely due to different hydrodynamism.

Chapter 4 investigated the trophic ecology of the most abundant demersal elasmobranchs from the continental shelf and slope using stomach content analysis (SCA). Results showed that batoids from the shelf preyed on decapod crustaceans and teleosts, whereas sharks from the slope fed upon mesopelagic prey. Diet overlap was found among most skates and between sharks from the upper and middle slope. *Raja clavata* and *Galeus melastomus* showed ontogenetic shifts in diet, whereas *Scyliorhinus canicula* diet changed both with size and depth.

In **Chapter 5** the diet and trophic ecology of the two co-generic squid species *Loligo vulgaris* and *L. forbesii* was examined for the first time in the Mediterranean. Although both species are piscivorous, the prey composition revealed a lack of diet overlap as a result of their bathymetric segregation. Both squids showed shifts in diet related to size and their reproduction period, but not to sex. The squid *L. vulgaris* displayed an ontogenetic shift in diet from small benthic prey to benthopelagic fish. During the reproduction period, *L. vulgaris* increased the consumption of the highly nutritive polychaetes, wherea the adults of *L. forbesii* carried out movements to deeper waters to feed on mesopelagic prey, which might help improving their individual body condition during such a period.

Chapter 6 focused on the trophodynamics of the mesopelagic fishes, owing to its importance as prey in marine foodwebs. Food sources (δ^{13}C) of zooplankton and mesopelagic fishes varied little over the spatial scales sampled but showed high seasonality, reflecting the intra-annual changes in species composition of the phytoplankton community. By contrast, spatio-temporal variations of trophic interactions (δ^{15}N) were minimal. Important niche segregation was observed between the non-migratory stomiiforms and some of the extensive migratory myctophids. There was little evidence of ontogenetic shifts in diet of the species analyzed, except for *Lampanyctus crocodilus*.

Finally, **Chapter 7** performed a comparative analysis of the trophic ecology of deep-sea cephalopods and elasmobranchs. The main aim was to know how their feeding strategies affect the role they play in the structure and dynamics of trophic networks. The combination of SCA and SIA showed that cephalopods and elasmobranchs displayed different feeding strategies with a clear resource partitioning between and within taxa. Results also revealed that squid and shark species identified as benthopelagic feeders, play a key role in the transport of energy from midwater regions to the benthos.

Resum

En el context de la gestió mediambiental és fonamental conèixer les interaccions predador-presa per poder entendre l'estructura i dinàmica dels ecosistemes marins. La present Tesi investiga l'estructura de les xarxes tròfiques d'aigües profundes del mar Balear i l'ecologia alimentària de diferents grups taxonòmics que juguen un paper clau en la trofodinàmica dels ecosistemes marins.

El **Capítol 3** investiga les xarxes tròfiques en base a l'anàlisi d'isòtops estables (AIE). Es va analitzar una cadena tròfica relativament llarga (89 espècies) cobrint els compartiments pelàgic i hiperbentònic en dues zones amb característiques hidrogràfiques diferents. Tot i que les cadenes tròfiques en els dos compartiments incloïen fins a 4 nivells tròfics, la majoria de les espècies es situaven en nivells intermedis. L'ampli rang de valors isotòpics trobat suggerí un alt nivell de partició dels recursos tròfics. Les cadenes tròfiques de les dues zones d'estudi van mostrar un acoblament bentopelàgic elevat i similar que va disminuir a l'estrat més profund, encara que amb alguna variació geogràfica, probablement atribuïda al seu diferent hidrodinamisme.

El **Capítol 4** investiga l'ecologia tròfica dels elasmobranquis demersals més abundants a la plataforma i talús a partir dels continguts estomacals (ACE). Els resultats van mostrar que els batoïdeus de la plataforma s'alimenten de crustacis decàpodes i teleostis, mentre que els taurons del talús mengen preses mesopelàgiques. Es va trobar solapament en la dieta de la majoria de les rajades i entre els taurons del talús mitjà i superior. *Raja clavata* i *Galeus melastomus* van mostrar canvis ontogènics en la seva dieta, mentre que la dieta de *Scyliorhinus canicula* va variar en funció de la talla i la profunditat.

En el **Capítol 5**, la dieta i l'ecologia tròfica de dos calamars congenèrics, *Loligo vulgaris* i *L. forbesii*, va ser examinada per primera vegada a la Mediterrània. Les dues espècies son piscívores, però les preses van revelar l'absència de solapament tròfic degut a la seva segregació batimètrica. Tots dos calamars van mostrar canvis en la dieta relacionats amb la talla i amb la reproducció, però no amb el sexe. Amb l'augment de la talla, la dieta de *L. vulgaris* va canviar de petites preses bentòniques a peixos bentopelàgics. Durant la reproducció, *L. vulgaris* va incrementar la ingesta de poliquets, mentre que els adults de *L. forbesii* van desplaçar-se a aigües més profundes per alimentar-se de preses mesopelàgiques, la qual cosa podria ajudar a millorar la condició individual durant aquest període.

Capítol 6 es centra en la trofodinàmica dels peixos mesopelàgics, per la seva importància com a preses en les xarxes tròfiques marines. Les fonts d'aliment (δ^{13}C), tant del zooplàncton com dels peixos mesopelàgics, va mostrar lleugeres variacions en l'escala espaial mostrejada, però en canvi mostrà una gran estacionalitat, reflectint els canvis intra-anuals en la composició específica del fitoplàncton. No obstant això, les variacions espai-temporals de les relacions tròfiques (δ^{15}N) foren mínimes. Es va observar una important segregació del nínxol tròfic entre els stomiiformes no migradors i alguns dels mictòfids altament migradors. Les espècies analitzades no van mostrar canvis ontogènics en la dieta, excepte en el cas de *Lampanyctus crocodilus*.

Per finalitzar, el **Capítol 7** portà a terme un anàlisi comparatiu de l'ecologia tròfica dels cefalòpodes i dels elasmobranquis del talús. La combinació d'ACE i AIE va permetre investigar les seves estratègies alimentàries, les relacions tròfiques, el solapament de dietes i els canvis ontogènics en la seva alimentació. Els cefalòpodes i els elasmobranquis van mostrar diferents estratègies alimentàries i una clara partició dels recursos alimentaris a nivell inter i intra-taxonòmic. Els resultats van desvelar que els calamars i els elasmobranquis categoritzats com a consumidors bentopelàgics, juguen un paper clau en el transport d'energia des de les capes superficials fins a la zona bentònica del talús.

Resumen

En el contexto de la gestión medioambiental es fundamental conocer las interacciones predador-presa para poder entender la estructura y dinámicas de los ecosistemas marinos. En la presente Tesis se investiga la estructura de las redes tróficas de aguas profundas del Mar Balear y la ecología alimentaria de diferentes taxones que juegan un papel clave en la trofodinámica de los ecosistemas marinos.

El **Capítulo 3** investiga las redes tróficas en base al análisis de los isótopos estables (AIE). Se analizó una cadena trófica relativamente larga (89 especies) abarcando los compartimentos pelágico e hiperbentónico en dos zonas con diferentes características hidrográficas. A pesar de que las cadenas tróficas en ambos compartimentos incluían hasta 4 niveles tróficos, la mayoría de las especies se situaban en niveles intermedios. El amplio rango de valores isotópicos encontrado sugirió un alto nivel de partición de los recursos tróficos. Las cadenas tróficas de las dos zonas de estudio mostraron un acoplamiento bentopelágico elevado y similar, que disminuyó en el estrato más profundo, aunque con alguna variación geográfica, probablemente atribuida a su diferente hidrodinamismo.

El **Capítulo 4** investiga la ecología trófica de los elasmobranquios demersales más abundantes de la plataforma y el talud a partir del análisis de contenidos estomacales (ACE). Los resultados mostraron que los batoideos de la plataforma se alimentan de crustáceos decápodos y teleósteos, mientras que los tiburones del talud basan su dieta en presas mesopelágicas. Se encontró solapamiento en la dieta de la mayoría de rayas y entre los tiburones del talud medio y superior. *Raja clavata* y *Galeus melastomus* mostraron cambios ontogénicos en su dieta, mientras que la dieta de *Scyliorhinus canicula* varió en función tanto de la talla como de la profundidad.

En el **Capítulo 5**, la dieta y ecología trófica de dos calamares congenéricos, *Loligo vulgaris* y *L. forbesii*, fue examinada por primera vez en el Mediterráneo. Aunque ambas especies son piscívoras, sus presas desvelaron la ausencia de solapamiento trófico debido a su segregación batimétrica. Ambos calamares mostraron cambios en la dieta relacionados con la talla y la reproducción, pero no con el sexo. Con el aumento de la talla, la dieta de *L. vulgaris* cambió de presas bentónicas a peces bentopelágicos. Durante la reproducción, *L. vulgaris* incrementó la ingesta de poliquetos, mientras que los adultos de *L. forbesii* se alimentaron de presas mesopelágicas, lo que ayudaría a mejorar su condición individual durante dicho período.

El **Capítulo 6** se centra en la trofodinámica de los peces mesopelágicos, debido a su importancia en las redes tróficas marinas. Las fuentes de alimento (δ^{13}C), tanto del zooplancton como de los peces mesopelágicos, mostró ligeras variaciones en la escala espacial muestreada, pero una gran estacionalidad, reflejando cambios intra-anuales en la comunidad del fitoplancton. No obstante, las variaciones espacio-temporales de las relaciones tróficas (δ^{15}N) fueron mínimas. Se observó una importante segregación del nicho trófico entre los stomiiformes no migradores y algunos de los mictófidos migradores. Las especies analizadas no mostraron cambios ontogénicos en la dieta, excepto en el caso de *Lampanyctus crocodilus*.

Para finalizar, el **Capítulo 7** llevó a cabo un análisis comparativo de la ecología trófica de los cefalópodos y los elasmobranquios del talud. La combinación de los datos de ACE y AIE permitió constatar diferentes estrategias alimentarias y una clara partición de los recursos alimentarios a nivel inter e intra-taxonómico. Los resultados revelaron que los calamares y los tiburones identificados como consumidores bentopelágicos, juegan un papel clave en el transporte de energía desde las capas superficiales hasta la zona bentónica del talud.
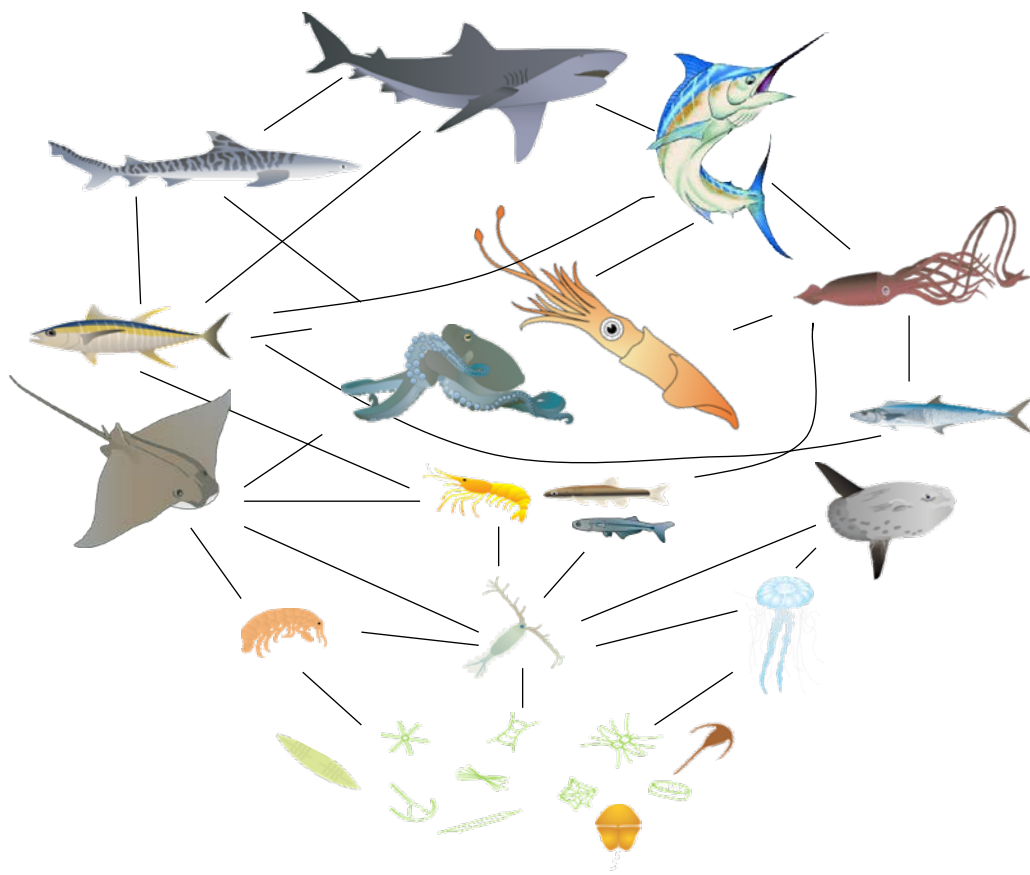
Chapter 1. Introduction

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1.1 Thesis motivation

Confined between Southern Europe, Middle East and North Africa, the Mediterranean is an enclosed basin connected to the wide ocean through the narrow Strait of Gibraltar. The Mediterranean Sea is already known to have a complex and multi-scale circulation, driven by wind, water flux, thermohaline and topographic features of its basins. The limited exchange of Atlantic and Mediterranean waters plays an important role in the circulation and productivity of the Mediterranean Sea. The Mediterranean act as a concentration basin: through the Strait of Gibraltar, relatively cool and low-salinity water from the Atlantic (AW) flows into the basin. Once in the Mediterranean, this water warms up to the east where it becomes saltier (due to evaporation) and then sinks before circulating west and leaving the Mediterranean through the Strait of Gibraltar below the lighter incoming AW (Bethoux and Gentili, 1999).

Its geological history, which has led to high rates of environmental change (i.e. Messinian salinity crisis), has produced a marine life that is unusually diverse for such a small enclosed sea (Lejeusne et al., 2010). The wide range of climate and hydrology have contributed to marine biota co-occurrence of both temperate and subtropical organisms, leading to an exceptional concentration of marine fauna and flora (4-18% of the world marine species depending on the phylum considered; (Bianchi and Morri, 2000) and endemisms (30.5%; Myers et al., 2000b). This way, the Mediterranean constitutes a biodiversity hotspot but also a climate change hotspot (Giorgi, 2006) based on results from global climate change projection scenarios which indicate warmer and drier conditions during the twenty-first century (IPCC, 2013).

Throughout the Mediterranean Sea, marine resources have been exploited since ancient times (Margalef, 1985), although fishing exploitation has generated a stronger pressure in the last decades (Colloca et al., 2013; Vasilakopoulos et al., 2014). It has suffered not only direct anthropogenic impacts (e.g. fishery, aquaculture) but also indirect effects of increased CO_2 emissions (e.g. changes in salinity, shifts in ocean currents, acidification), habitat loss and invasive species among others, which affected the abundance and distribution of marine populations (MerMex-Group, 2011). Such changes can reduce the ecosystem resilience (i.e. the ability of an ecosystem to persist despite disruption and change) to other man-induced pressures, leaving ecosystems increasingly sensitive to disruption. Sea level projections in the Mediterranean Sea for the 2070-2099 period indicate an increase in the sea surface temperature and sea level (Adloff et al., 2015; Ben Rais Lasram et al., 2010) which are predicted to have deleterious effects on marine flora and fauna (i.e. *Posidonia oceanica* meadows, Jordà et al., 2013; Pergent et al., 2014). Other predictions foresee a total modification of endemic assemblages on the 25% of the Mediterranean continental shelf by the end of the 21st century (Ben Rais Lasram et al., 2010). Predicted changes in western Mediterranean marine resources and ecosystems (Coll et al., 2008) were

found to be mainly driven by trophic interactions (37-53%), environmental factors (6-16%) and fishing activities (14%).

The need for a long-term sustainable development to protect the marine environment, endorsed by the Convention on Biological Diversity in 2000, demands an holistic point of view (Ecosystem Approach). After the recognition of the impacts that fishing imposes on marine ecosystems, an Ecosystem Approach to Fisheries (EAF) is being adopted by marine and fishery scientists (Marasco et al., 2007; Pikitch et al., 2004). Adopting such an approach requires an understanding of the ecological dynamics and processes operating in an ecosystem, such as trophic relationships among species in the food web. At a scientific level this implies a better understanding of the structure, functioning and processes of ecosystems (Krebs, 1989). Nevertheless, research is also relevant at lower levels through studies of the structure and dynamics of populations and communities (Christensen et al., 1996).

Trophic relationships are fundamental to understand biological interactions and ecosystem structure and dynamics. A food web is a representation of feeding relationships in a community that includes all the links revealed by dietary analysis (Pimm et al. 1991). Food webs are the outcome of dynamic interactions among organisms that acquire resources from the abiotic environment. Those trophic interactions define energy and material flows among species and provide a basic description of community structure. In turn, the structure of a food web (e.g. connectivity, length of food chains) critically affects the dynamics (e.g. resilience to perturbations) of the ecosystem (Pimm, 1982).

Data on species diet provide very useful information on trophic ecological issues such as pathways of energy flow (Lasalle et al. 2011), prey selection (Graeb et al., 2006), predator-prey size relationships (Johnson et al., 2012), intra and interspecific competition (Young et al., 2010), behaviour (Cartes et al., 2009), ontogenetic shifts in diet (Massutí et al., 1998), impact of species invasion (Golani, 1993), estimation of trophic levels (Stergiou and Karpouzi, 2002) and definition of trophic guilds (Cartes et al., 2002), among others.

1.2 Bentho-pelagic coupling

Bentho-pelagic coupling (BPC) refers to the exchange of energy, mass or nutrients between benthic and pelagic habitats (Graff 1992). It plays a prominent role in aquatic ecosystems, and it is crucial to functions from nutrient cycling to energy transfer in food webs. BPC involves mechanisms of organism movement, trophic interactions, or biochemical cycling that connects the bottom and the water column and is ultimately influenced by physical forces of depth, temperature, light and mixing, and occurs multiple spatial and temporal scales (Baustian et al. 2014). However, the compartmentalization of both habitats in empirical studies and models often limits our understanding of the strength of interactions between them, their role in maintaining ecosystem function and their sensitivity to future change (Griffiths et al. 2017).

Physical proximity between pelagic and benthic species and weaker physical barriers such as thermoclines in continental shelf waters may allow a stronger benthic-pelagic coupling compared to deep-sea environments. Water masses transport, both vertically and horizontally, is generally a key determinant of local marine productivity and associated food webs (Polis et al., 1997). Traditionally, BPC in deep-sea has focused on the deposition of materials produced in the water column, which is a combination of phytodetritus (i.e. phytoplankton, faecal pellets, bacteria and other detritus) and zooplankton (Gage and Tyler 1991). The arrival of organic matter to the deep-sea is through two main pathways, the vertical and the advective fluxes of material. Advective fluxes (i.e. nepheloid layers) comprise fluvial inputs from the continent and shelf material (e.g. macroalgae, seagrass) that are transferred down the slope.

Efforts are increasing to describe and understand the diversity of process that couple benthic and pelagic habitats, especially those mediated by living organisms such as trophic relationships, ontogenetic shifts in habitat use or diel migrations (Baustian et al. 2014; Trueman et al. 2014; Smith et al. 2006). Advancing the knowledge of how habitat coupling processes function will improve our ability to predict ecosystem responses to environmental changes and to improve holistic approaches in marine ecosystem management.

1.3 Food webs as a basis for an ecosystem based management

The EU Marine Strategy Framework Directive adopted in 2008 to protect more effectively the marine environment across Europe (MSFD: 2008/56/EC), requires that each Member State takes the necessary measures to achieve or maintain Good Environmental Status in the marine realm by 2020. The MSFD is defined by eleven qualitative descriptors addressing topics such as biodiversity, contaminants, marine litter, commercially exploited fish and marine food webs. Regarding food webs, the MSFD aimes to monitor their status and trends in order to collect information needed to assess the current situation and implement the necessary preventive measures. There are three criteria and respective indicators based on productivity of key predator species, proportion of large fish and abundance of functionally important groups/species (COM Decision 2010/477/EU). Data for many species (e.g. habitat defining species, predators) and functional groups are needed for this purpose.

The MSFD also requires to implement a regular monitoring of stomach contents and of isotopic signatures of the food webs components on all marine areas to address two issues: i) to develop and test indicators of ecosystem structure to better monitor the ecological status of ecosystems; and ii) to highlight any changes in food web structure and their connectivity in the long term, and to try to link these changes to disturbances or other changes that occurred in the ecosystems.

Further, in the context of the ecosystem approach to fisheries management, increasingly sophisticated models have been developed to predict the response of marine ecosystems to environmental and anthropogenic changes. Knowledge of functional groups and species diet is essential for informing ecosystem models (e.g.

Ecopath with Ecosim; Christensen and Walters, 2004). The structure of these models is mainly based on functional groups constituted by many species, some of them with ecological and biological traits that are still poorly known in each specific area; alternatively they are taken from other areas or estimated using empirical equations. However, it has been recognized that this information is often ecosystem-specific (Hanson and Chouinard, 2002). Deficiencies and scarcity of biological data, such as information on diet composition, functional groups, trophic niche or ontogenetic shifts, have already been identified in the western Mediterranean (Bănaru et al., 2013; Coll et al., 2013, 2006; Navarro et al., 2013). Data quality must then be improved for a sound marine management in the Mediterranean.

1.4 The study area: the Balearic Sea

The Balearic Islands (western Mediterranean) are the emergent areas of the Balearic promontory. This archipelago delimits the Balearic sub-basin (BsB) in the north, from the Algerian sub-basin (AsB) in the south. These sub-basins are characterized by contrasting oceanographic conditions (EUROMODEL Group, 1995). The BsB is more influenced by atmospheric forcing and Mediterranean waters, which are colder and more saline, whereas the AsB is affected basically by forcing due to the density gradients and receives warmer and less saline Atlantic waters (Pinot *et al.*, 2002).
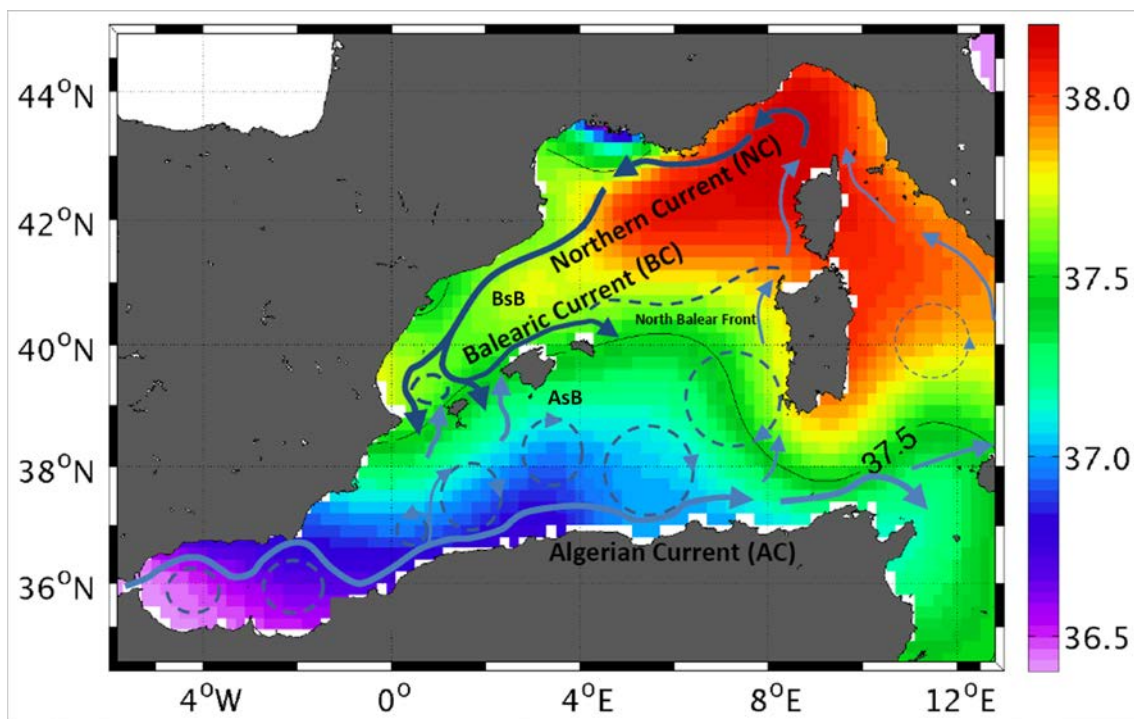
Figure 1.1 Average July salinity and major currents characterizing the western Mediterranean circulation (map adapted from Reglero et al., 2012). The isohaline 37.5 approximately separates the fresh Atlantic waters to the south from the resident Atlantic waters to the north. Gyres (dashed lines) are also shown. BsB: Balearic sub-basin; AsB; Algerian sub-basin.

The hydrographic conditions of the Balearic Islands have been extensively studied and the existing channels have been shown to control the regional circulation around the Archipelago (Pinot *et al.*, 2002). The regional circulation in the area is dominated by the Northern Current (NC) which carries down Atlantic waters (AW) from the Gulf of Lions along the continental slope of the Iberian Peninsula into the BsB (Font *et al.*, 1988) (Fig. 1.1). This current bifurcates when reaches the Ibiza Channel; one significant part crosses the channel transporting waters from the Mediterranean into the AsB, while the other part cyclonically returns to the northeast forming the Balearic Current (BC) along the northern coasts of the Balearic Islands. This general pattern is the one expected for late spring–summer after relatively mild winter in the western Mediterranean, but fluctuates considerably in mesoscale, seasonal and interannual time scales, changing dramatically after a cold winter (García Lafuente *et al.*, 1995; Pinot *et al.*, 2002).

On the shallow shelf, the algal beds develop down to 90–100 m depth (Ballesteros, 1992, 1994), forming the facies of soft red algae and calcareous red algae beds (maërl) (Massutí and Reñones, 2005). On the other hand, the deep shelf is characterized by detritic sandy-mud bottoms mainly structured by echinoderms (crinoid beds) (Ordines and Massutí 2008). Both habitats are known to enhance habitat heterogeneity by developing three-dimensional communities (Gili and Coma, 1998), providing shelter from predators and food availability (Rozas and Odum, 1988). These habitats are known to be highly productive areas and play a major role in the production of the main demersal resources (Ordines and Massutí 2009). Muddy bottoms of biogenic origin dominate the deeper areas (Acosta *et al.*, 2003). Organic matter on sediments increases with depth from the shelf-slope break to bathyal bottoms parallel to the increase in the proportion of mud (Cartes et al. 2008).

In 2009, the General Fisheries Commission for the Mediterranean established 30 Geographical Sub-Areas (RES-GFCM/33/2009/2) (Fig. 1.2) for the assessment and management of living resources (resolution GFCM/31/2007/2). The Balearic Islands constitute the geographical sub-area number 5 (GSA05). A comprehensive comparison including different aspects such as geomorphology, habitats, fisheries and exploitation state of resources and ecosystems between the Balearic Islands and the adjacent coast of the Iberian Peninsula, concluded that the Archipelago should be maintained as an independent unit for assessment and management purposes in the western Mediterranean (Quetglas et al., 2012).

01 - Northern Alboran Sea	07 - Gulf of Lions	13 - Gulf of Hammamet	19 - Western Ionian Sea	25 - Cyprus Island
02 - Alboran Island	08 - Corsica Island	14 - Gulf of Gabes	20 - Eastern Ionian Sea	26 - South Levant
03 - Southern Alboran Sea	09 - Ligurian and North Tyrrhenian Sea	15 - Malta Island	21 - Southern Ionian Sea	27 - Levant
04 - Algeria	10 - South and Central Tyrrhenian Sea	16 - South of Sicily	22 - Aegean Sea	28 - Marmara Sea
05 - Balearic Island	11.1 - Sardinia (west) 11.2 - Sardinia (east)	17 - Northern Adriatic	23 - Crete Island	29 - Black Sea
06 - Northern Spain	12 - Northern Tunisia	18 - Southern Adriatic Sea	24 - North Levant	30 - Azov Sea

Figure 1.2 The 30 Geographical Sub-Areas (GSAs) established by the General Fisheries Commission for the Mediterranean for management purposes in the region (GFCM, 2009).

1.5 Marine food webs from the Balearic Islands

Globally, the Mediterranean Sea is considered an oligotrophic region. The exchange of water at Gibraltar may be one of the causes of the low nutrient content of deep Mediterranean waters as compared with those of the Atlantic (Estrada, 1996). Based on satellite imagery data, the mean annual surface primary production, as indicated by the chlorophyll-a concentration, ranges between 1-2 mg·m^{-3}·year^{-1} in the most productive areas of the western basin, such as the Gulf of Lions or the Alboran Sea, and 0.02-0.03 mg·m^{-3}·year^{-1} in the most eastern oligotrophic areas in the south of Crete and Cyprus (Baillie et al., 2004).

Owing to the high oligotrophy of the eastern Mediterranean (Danovaro et al., 1999), its food webs are generally sustained by mesopelagic resources (Madurell and Cartes, 2005a; Madurell et al., 2004), a feature that also characterizes some insular slopes (Blaber and Bulman, 1987; Haedrich and Merrett, 1992). By contrast, ecosystems from the western basin, with higher net primary production that generates greater phytodetritus deposition on the bottom, depend mainly on benthic resources (Cartes and Maynou, 1998).

Owing to the lack of land inputs from rivers run-off, the waters around the Balearic Islands show a pronounced oligotrophy (0.1-0.2 mg·m^{-3}·year^{-1}) compared to adjacent areas off the Iberian coast and the Gulf of Lions (Estrada, 1996), where the presence of rivers and large submarine canyons enhance productivity. Particle fluxes on the continental slope from the Balearic Islands range between the highest

values reported in adjacent areas of the western Mediterranean (Pasqual et al., 2015) and the lowest values found in the Adriatic (Miserocchi et al., 1999) and Ionian (Boldrin et al., 2002) Seas.

Differences between continental slope ecosystems from the Mediterranean coast of the Iberian Peninsula and the Balearic Islands, in terms of the communities (Massutí et al., 2004; Maynou and Cartes, 2000; Moranta et al., 1998) and the bathymetric distribution of some species (Cartes et al., 2004), have been linked to trophic web structure and energy flow (Fanelli et al., 2013a; Maynou and Cartes, 2000). Deep-sea megafauna food webs structure over the insular slope have shorter food chains, lower benthopelagic biomass and lower individual sizes compared with those from the Catalan slope (Fanelli et al., 2013a, 2013b; Papiol, 2013). Further, mesopelagic resources are more abundant in the diets of species inhabiting the insular slope than in other adjacent areas (Cartes et al., 2009, 2008b; Fanelli and Cartes, 2008).

1.6 Trophic studies in the Balearic Sea

The Laboratory of Marine Biology was established in Mallorca (Porto Pi) in 1906. Trophic relationships received little attention during the first half of the 20th century. Within the context of stocks assessment, the first studies dealt with exploited pelagic fish from the Balearic Islands. In the years 1926 and 1927, Navarro included some data on stomach contents of sardine (*Sardina pilchardus*) and round sardinella (*Sardinella aurita*) respectively. Sardine diet was further investigated, together with prey availability, by Massutí and Oliver (1948) in waters off Menorca. The common labrid *Xyrichtys novacula* (raó), which supports an important recreational fishery, was also studied by Oliver and Massutí (1952), who provided a general description of the taxa found in their stomachs.

Diet studies of marine fauna from the Balearic Islands, however, expanded fifty years later. Massutí et al. (1998) described the diet of the dolphinfish *Coryphaena hyppurus*, an important target species of the small-scale fishery in Mallorca. The stomach contents of the benthic fish *Trigla lyra* were analysed by Pons-Moyà et al. (1998). The first specific diet study of a cephalopod species in the area, investigated the feeding ecology of the squid *Todarodes sagittatus* (Quetglas et al., 1999). Several works of the same author described the diet of some demersal cephalopod species, together with other biological traits: *Octopus vulgaris* (Quetglas et al., 1998), *Bathypolypus sponsalis* (Quetglas et al., 2001), *Octopus salutii* (Quetglas et al., 2005), *Pteroctopus tetracirrhus* (Quetglas et al., 2009) and the Histioteutidae family (Quetglas et al., 2010).

In recent years, stable isotope analysis have been used to get new insights into deep-sea trophic webs from the Balearics. The first community level approach of deep-sea megafauna (fishes and crustaceans) food web structure was done by Polunin et al. (2001) at the south of Ibiza. Results indicated a single primary source material (e.g. marine snow) for this assemblage and very few differences in the isotopic ratios of the taxa analyzed among depths.

Later on (2003-2006), within the framework of project IDEA (Influence of oceanographic structure and dynamics on demersal populations in waters of the Balearic Islands, see Massutí et al. 2008 for details; http://www.ba.ieo.es/idea/) several works were conducted regarding the trophodynamics of different species/assemblages inhabiting the slope of Mallorca. Madurell et al. (2008) and Fanelli et al. (2009) analyzed the food web structure of low trophic level taxa (suprabenthos and near-bottom zooplankton) revealing a wide spectrum of feeding guilds and high resource partitioning. Suprabenthos dynamics was found to correlate with the sediment variables (e.g. total organic matter content), whereas zooplankton was almost exclusively dependent on primary production, which suggested two different food sources for suprabenthos and zooplankton (Cartes et al., 2008a). Seasonal and spatial fluctuations of pelagic resources, which are highly coupled with primary production, were found to be larger than those of benthic resources (Cartes et al., 2008a; Hidalgo et al., 2008). Such fluctuations were, in turn, reflected in their predators diet (Fanelli and Cartes, 2010, 2008). In terms of diet, two target species of the bottom trawl fleet, the red shrimp (*Aristeus antennatus*) (Cartes et al. 2008) and the European hake (*Merluccius merluccius*) (Cartes et al. 2009), were examined. Their prey composition reflected the importance of the mesopelagic community sustaining the trophic requirements of both species.

The physical processes were found to play a key role in structuring faunal communities. Contrasting hydrodynamic scenarios were found between the north (Balearic sub-basin: BsB) and south (Algerian sub-basin: AsB) of Mallorca (Lopez-Jurado et al., 2008), which would explain the differences observed in these two areas with respect to zooplankton-suprabenthos communities (Cartes et al., 2011, 2010, 2008a), megafaunal assemblages (Fanelli et al. 2013a; Guijarro et al. 2012 ; Moranta et al., 2008), and species trophodynamics (Cartes et al., 2009, 2008b). Although the demersal megafauna from the BsB was found to be mainly linked to primary production (Fanelli et al., 2013b), it seems more influenced by processes taking place on the continental margin of the Iberian Peninsula and Gulf of Lions. This results in a greater availability of benthic prey in the BsB compared to the AsB, particularly at bathyal depths (Cartes et al., 2008a). On the contrary, demersal food webs from the AsB are mainly supported by planktonic biomass rather than benthos (Cartes et al. 2001; Fanelli et al. 2009).

Despite the IDEA project increased the knowledge of deep-sea ecosystem structure and functioning, of the Balearic Islands, many topics remain largely unexplored. Regarding trophic interactions, an integral approach relating trophic energy flow through the water column (involving pelagic and benthic taxa) encompassing different trophic levels (from food source to predators) was lacking. How the hydrodynamic variability (i.e. BsB *vs* AsB) influences benthic-pelagic connections remained far from being fully investigated. Whit this aim, the IDEADOS project (Structure and dynamics of the bentho-pelagic slope ecosystem in two oligotrophic zones of the western Mediterranean: a multidisciplinary approach at different spatio-temporal scales in the Balearic Islands; http://www.ba.ieo.es/ideados/) was conducted.

On the coastal waters, the trophic relationships of its fauna are poorly studied. Jennings et al. (1997) and Deudero et al. (2004) highligthed the isotopic spatial

variability among islands likely reflecting feeding plasticity and the importance of benthic pathway supporting rocky fishes. The diet of few macrocarnivorous fishes have been studied in the Balearics. Morales-Nin et al. (1997) described the biology and fishery of *Dentex dentex*, including a general description of its diet. Reñones et al. (2002) and Linde et al. (2004) investigated the trophic habits of the grouper *Epinephelus marginatus* revealing that individuals older than 1 year old relied on the benthic food web.

A key ecological feature of the Balearic shelf bottoms are extensive meadows of seagrass (e.g. *Posidonia oceanica*) (Procaccini et al. 2003) and macroalgae (i.e. maërl) (Massutí and Reñones, 2005) developping up to 100 m depth (Ballesteros, 1992, 1994). Little is known about the benthic producers contribution to the carbon entering the trophic chain. Cardona et al. (2007) assessed the relevance of different primary carbon sources for consumers inhabiting the coastline and the pelagic ecosystem from Menorca, highligthing the low contribution of *P. oceanica*. The effect of invasive macroalga on the contribution of food sources for the black seabream (*Spondyliosoma cantharus*) Box et al. (2009) and the fan mussel (*Pinna nobilis)* (Cabanellas-Reboredo et al. 2010) diet have also been evaluated.

1.7 Study species

Three different taxonomic groups were used as case studies in this Thesis: cephalopods (Mollusca), elasmobranchs (Chondrichthyes), and mesopelagic fishes (Osteichthyes). All of them play a key role in marine food webs (see references below) but knowledge on their trophic ecology is still scarce in the Mediterranean and especially in the study area.

1.7.1 Elasmobranchs

Elasmobranchs are long lived, slow growing and invest in few well-developed young, whereby they are a classical example of slow life-history strategy (Jeschke and Kokko, 2009). In general, elasmobranchs play an important role as predators in marine food webs (Heupel et al., 2014; Rosenblatt et al., 2013). They present a high diversity of trophic levels displaying a broad range of feeding strategies from zooplanktivors to top predators (Hussey et al. 2014). As most species are opportunistic predators with a wide trophic spectrum elasmobranchs are naturally linked to a wide variety of prey species (Wetherbee et al., 2012), connecting ecologically distinct food webs in some cases. They have low population growth rate and thus low potential recovery, being highly sensitive to human impacts such as fishing activity, pollution and habitat degradation (Dulvy et al., 2014; Stevens, 2000). Given the ongoing changes in many elasmobranch populations worldwide and the potential impacts on their prey and communities, developing our understanding of the trophic relationships of sharks and other elasmobranchs is crucial to our knowledge of how marine systems function (Cortes, 1999; Stergiou and Karpouzi, 2002).

The Balearic Islands show one of the highest values of diversity and abundance of demersal elasmobranchs in the western Mediterranean (Massutí and Moranta, 2003) mainly due to the presence of many skate species on the coastal shelf (Ramírez-Amaro et al., 2015). The sediment type, the presence of sensitive habitats (e.g. soft red algae, maërl and crinoids beds; Ordines and Massutí, 2009) and a lower intensity of fishing exploitation (Quetglas et al., 2012) in the area could explain such high biodiversity and abundance (Ordines et al., 2011). A total of nine different elasmobranch species (6 batoids, 3 selachians) have been analyzed in this Thesis. Altoghether, these nine species account for up to 86% of the total biomass of the elasmobranchs taken in the study area (Table 1.1).

Table 1.1 Demersal elasmobranch species (batoids and selachians) coming from MEDITS (2007-2016) surveys analyzed in this Thesis. B: mean biomass ± sd and percentage of biomass (B%); A: percentage of appareance in their depth range (Ordines et al., 2011).

Species	Taxa	B (kg·km^{-2})	B (%)	A (%)
Scyliorhinus canicula	Selachii	111.49 ± 19.28	39.6	85
Raja clavata	Batoidea	70.77 ± 24.30	25.1	50
Galeus melastomus	Selachii	28.31 ± 9.84	10.1	91
Myliobatis aquila	Batoidea	8.02 ± 3.56	2.9	100
Dipturus oxyrinchus	Batoidea	8.05 ± 2.68	2.9	24
Raja polystigma	Batoidea	6.33 ± 3.57	2.2	13
Leucoraja naevus	Batoidea	6.01 ± 2.26	2.1	31
Raja miraletus	Batoidea	3.70 ± 0.93	1.3	63
Etmopterus spinax	Selachii	0.90 ± 0.81	0.3	75

1.7.2 Cephalopods

In general, cephalopods have short life spans, high population growth rates and high fecundity, which are characteristics of typical fast life-history strategists (Jeschke and Kokko, 2009). It is well known that cephalopods, particularly squid, are a central component of marine food webs worldwide (Coll et al., 2013). Due to their high consumption and production rates, squid transfer large amounts of energy from lower to higher trophic levels (Nixon, 1987; O'Dor and Wells, 1987). They are important prey of apical predators (Battaglia et al., 2013; Blanco et al., 2006; Massutí et al., 1998) and opportunist consumers in variable environments (Rodhouse and Nigmatullin, 1996). Owing to their fast life-history strategy, cephalopods are very sensitive to changing environmental conditions (Pierce et al., 2008; Quetglas et al., 2016), specially at local scale (Puerta et al., 2015). The relevance of squid in marine food webs may change in response to fisheries induced alterations of trophic structure (Caddy and Rodhouse, 1998; Piatkowski et al., 2001), climate oscillations (Field et al., 2007) and invassive species (Nader et al., 2012).

The Iberian-Lions bioregion is characterized by high richness values of cephalopod species, similar to the Tyrrhenian and the Ionian Sea (Keller et al., 2016). A total of 30 cephalopod species have been reported in the Balearic Sea (Quetglas et al., 2014, 2000), which accounted for about 40% of the 66 species currently reported in the Mediterranean (Bello, 2008). This estimate is higher than the species reported in some studies conducted in the adjacent Catalan Sea: 15 to 22 species (Fanelli et al., 2012; Sanchez, 1986;). Such results may be related to differences in biogeographic factors, the lower fishing intensity in the archipelago compared to the Iberian peninsula (Quetglas et al., 2012) or the close coupling between primary and secondary producers in oligotrophic areas (Rosa et al., 2008) such as the Balearic Sea. A total of 19 cephalopod species have been analyzed in this Thesis, which represents up to 35% of the total biomass of cephalopods taken in the study area (Table 1.2). A single species, the common octopus *Octopus vulgaris*, accounts for the 60% of the cephalopod biomass; however, this species has not been included in this work because its trophic ecology has already been analysed in the study area (Quetglas et al., 1998).

1.7.3 Mesopelagic fishes

Mesopelagic fishes refer to the fish species inhabiting the portion of the water column between 200 and 1000 m (Gartner et al., 1987), although many species perform extensive vertical displacements reaching the surface at night for feeding. These mesopelagic fishes are important components in terms of biomass in the oceanic waters of temperate and tropical regions, and probably the most abundant vertebrates worldwide (Gjøsaeter and Kawaguchi, 1980; Irigoien et al., 2014). Myctophid (and gonostomatid) species are the dominant mesopelagic fish of the oceanic ecosystem worldwide, whereby they play a key role in marine food webs. They have been reported to remove up to 10% of the zooplankton biomass per night (Watanabe et al., 2002). In turn, they are consumed by a range of predators, including fish, squid, seabirds, and marine mammals (Connan et al., 2007; Rodhouse and Nigmatullin, 1996). As many mesopelagic fishes are strong vertical migrators, feeding at night in the upper layers and excreting and respiring at greater depths during the day, they play a pivotal role in energy transfers from the surface to the mesopelagic domain.

Owing to the pronounced oligotrophy of the Balearic Sea, mesopelagic fishes (e.g. myctophids) likely constitute an important food resource for demersal fauna, as already found for some commercially relevant species such as the European hake (Cartes et al., 2009) and the red shrimp (Cartes et al., 2008b). Determining their feeding habits and trophic positions are thus essential for a better understanding of the functioning of the marine ecosystems from the Balearic Islands.

Table 1.2 Cephalopod species coming from MEDITS surveys (2007-2016) analyzed in this Thesis. B: mean biomass ± sd and percentage of biomass (B%); A: percentage of appareance in shelf (a) (50-200 m) (Quetglas et al. 2000) and slope (b) (200-900m) (Quetglas et al., 2014) bathymetric strata.

Species	Order	B (kg/km^2)	B (%)	A (%)
Loligo vulgaris [a]	Teuthoidea	5.25 ± 2.41	4.1	65.4
Illex coindetii [b]	Teuthoidea	18.17 ± 18.26	14.3	40.
Eledone cirrhosa [b]	Octopoda	8.13 ± 5.47	6.4	24.3
Loligo forbesii [b]	Teuthoidea	4.69 ± 3.24	3.7	20
Todarodes sagittatus [b]	Teuthoidea	3.1 ± 2.10	3.7	47.1
Sepietta oweniana [b]	Sepiodea	1.04 ± 0.93	0.8	22.9
Pteroctopus tetracirrhus [b]	Octopoda	0.85 ± 0.72	0.7	10.0
Octopus salutii [b]	Octopoda	0.83 ± 0.35	0.6	10.0
Sepia orbignyana [b]	Sepiodea	0.82 ± 0.93	0.6	10.0
Scaeurgus unicirrhus [b]	Octopoda	0.61 ± 0.40	0.5	4.3
Histioteuthis reversa [b]	Teuthoidea	0.38 ± 0.21	0.3	31.4
Bathypolypus sponsalis [b]	Octopoda	0.37 ± 0.30	0.3	17.1
Histioteuthis bonnellii [b]	Teuthoidea	0.28 ± 0.35	0.2	2.9
Todaropsis eblanae b	Teuthoidea	0.19 ± 0.33	0.1	2.9
Rossia macrosoma [b]	Sepiodea	0.09 ± 0.10	0.1	15.7
Abralia veranyi [b]	Teuthoidea	0.05 ± 0.04	0.04	41.4
Ancistroteuthis lischtensteinii [b]	Teuthoidea	0.04 ± 0.03	0.03	5.7
Heteroteuthis dispar	Sepiodea	0.03 ± 0.08	0.02	15.7
Rondeletiola minor [b]	Sepiodea	0.002 ± 0.002	0.001	14.3

1.8 Methodological approaches

Different approaches exist to study trophic interactions. Traditional methods include stomach content analysis (SCA), scat analysis or direct observation of foraging. SCA has both pros and cons. It provides a detailed estimation of feeding links and valuable taxonomic information on the prey consumed by a predator. However, it only gives information on the most recent meal consumed and large samples are required for reliable estimation of spatial, temporal and ontogenetic variations. Collecting comprehensive gut content data is time consuming and requires extensive taxonomic skills. It also underestimates, and in some cases ignores, certain types of dietary materials (Hyslop, 1980).

More recently, biochemical tracer techniques (e.g. fatty acid profiles, stable isotope methods-in particular those of carbon and nitrogen) have been developed for investigating trophic relationships. The use of stable isotope analysis (SIA) relies on the fact that the carbon isotope ratio ($\delta^{13}C$) of consumers reflects that of their food sources, with minimal discrimination (Δ) lower than 1‰ (DeNiro and Epstein, 1978; Peterson and Fry, 1987), while the heavy nitrogen isotope ^{15}N ($\delta^{15}N$) displays a stepwise enrichment at around 3‰ with each trophic level (Post, 2002; Sweeting et al., 2007). Variations in $\delta^{13}C$ values provide useful information

on foraging locations as it is possible to determine, for instance, whether a consumer feeds on benthic or pelagic prey (Cherel et al., 2009; Miller et al., 2010b). By contrast, the δ^{15}N value provides information on the trophic level of both consumers and prey. The stepwise enrichment of ^{15}N between prey and consumer allows estimating the trophic level of any species relative to other species. The trophic level may be computed if there is an estimate of the isotopic value of the food at the base of the trophic web (isotopic baseline) which is needed for comparisons across food webs (Vander Zanden et al., 1997).

The SIA can also be used to follow changes of the trophic structure over spatial or temporal gradients (Layman et al., 2007; Post et al., 2000). Because of the indirect nature of the data, there are various sources of potential ambiguity in the interpretation of isotope values. Stable isotope values are not only driven by trophic interactions but also by biochemical processes. In this respect, issues such as the relative incorporation rates of the elements, tissue turnover rates (from days to months), or δ^{15}N discrimination factors for each trophic transfer (Martínez Del Rio et al., 2009; Post, 2002), among others, should be considered. Both δ^{15}N and δ^{13}C values can vary among seasons in relation to changes in primary production and seasonal shifts in diet (Vander Zanden et al., 1999).
Finally, SIA generally does not allow a detailed evaluation of differences among diets of ecologically similar species or estimate the species composition present in the diet.

Given that all techniques possess both strengths and weaknesses, the greatest potential lies in using them in combination to provide a detailed and more complete picture of trophic relationships and food web interactions. When applied together, SIA and SCA reveal a more detailed and accurate trophic structure of the community, including greater taxonomic, temporal and spatial resolution (Winemiller et al., 2007), sized-based interactions (Hussey et al., 2010) and niche overlap (Vaudo and Heithaus, 2011). Therefore, both SCA and SIA were used in this thesis to provide a better description of the structure and dynamics of marine food webs from the Balearic Islands.

1.9 Aims

The main aim of the present Thesis is to determine the structure and dynamics of marine ecosystems from the Balearic Islands (western Mediterranean) analysing the trophic ecology of three key taxonomical groups in food web networks: cephalopods, elasmobranchs and mesopelagic fishes.

The work combines different study levels, from single species dietary analyses (Chapter 5) to community analyses of single (Chapter 4, 6) and combined (Chapters 3, 7) taxonomical groups. Furthermore, the work also takes advantage of the currently most used methodological approaches to investigate food web networks: stomach content analysis (SCA) and stable isotope analysis (SIA). The combined use of these two approaches would allow obtaining a more comprehensive picture of the trophic relationships by means of exploiting the pros and cons of each methodology.

A list of more specific objectives of the present Thesis follows:

a) Characterize the diet and feeding strategies of individual species of the three taxonomical groups targeted in this Thesis: cephalopods (19 species), elasmobranchs (9 species) and mesopelagic fishes (18 species).

b) Determine potential trophic niche overlap and resource partitioning of coexisting species of those three case study taxonomical groups.

c) Identify the effect of biotic (size and sex) and abiotic (season, depth and location) drivers determining intraspecific differences in the diets of the three case study taxonomical groups.

d) Define the trophic web structure and trophodynamics of the pelagic and hyperbenthic megafaunal communities along the continental slope.

e) Define the trophic web structure and dynamics of the mesopelagic fish community along the water column of the continental shelf and slope.

f) Investigate food web bathymetric (shelf-break, upper and middle slope) and spatial (Balearic *vs* Algerian sub-basins) differences in faunal assemblages.
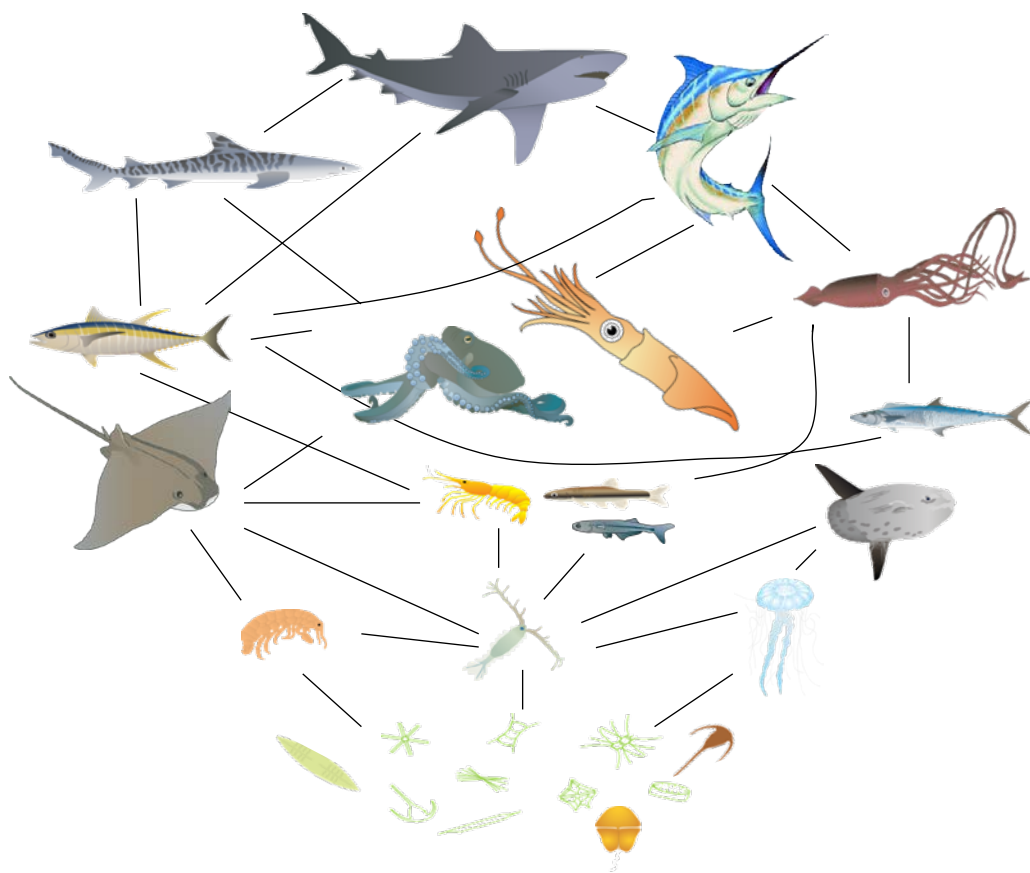
Chapter 2. Material and methods

Chapter 2. Material and methods

2.1 Datasets

The thesis was carried out in waters around the Balearic Islands (see Chapter 1 section 1.2) using two different data sources: 1) scientific surveys (MEDITS and IDEADOS); 2) biological sampling at the laboratory within the Data Collection Framework (DCF).

2.1.1 Scientific surveys

2.1.1.1 MEDITS program

In 1980 the Spanish Institute of Oceanography (IEO) started annual research bottom trawl surveys in the Atlantic area, in order to evaluate demersal ecosystems and their marine resources. Owing to the increasing importance of trophic studies and to better understand changes in marine ecosystems, the IEO implemented simultaneously SCA. Assessment of demersal resources by means of bottom trawl surveys started many years later in Mediterranean waters (1994) (GSA01: Alboran Sea and GSA06: Northern Spain) within the Mediterranean International Trawl Survey (MEDITS) programme. In the Balearic Islands (GSA05) the first surveys began in 2001 as BALAR surveys (Massutí and Reñones, 2005), which were eventually included into the MEDITS programme in 2007. The MEDITS programme is based on a standardized (a common sampling methodology and protocol) bottom trawl survey carried out in most EU riparian Mediterranean countries to produce basic information on macro-benthic and demersal species, in terms of distribution, density and population structure, at a regional scale (Bertrand *et al.*, 2002). The surveys take place between May and July, depending on the country. The sampling stations follow a depth stratified sampling scheme with random drawing of the positions within each stratum. The number of hauls in each stratum is proportional to the area of these strata and they are made in the same position from year to year.

Figure 2.1 Location of the sampling stations around the Balearic Islands (western Mediterranean) sampled during the MEDITS surveys.

The following depths are fixed in all GSA areas as strata limits: A (10-50 m), B (51-100 m), C (101-200 m), D (201-500 m) and E (501-800 m). The sampling gear is the experimental bottom trawl GOC 73, with a 20 mm mesh codend and average horizontal and vertical net openings of 16.0 and 2.7-3.2 m, respectively. The towing speed is around 2.7-3.0 knots to ensure the best trawl geometry, and the effective trawling duration varies between 20 and 60 min depending on the depth-strata.

Trophic studies in the Balearic Islands (GSA05), using both SCA and SIA, were introduced in 2007. The target species of these studies were selected based on their abundance, biomass or ecological importance at each prospected bathymetric strata. Between 20 and 30 species (teleosts and elasmobranchs) are regularly sampled each year. Until now up, to 17.000 stomachs (Table 2.1) have been analyzed and 2200 samples processed for SIA (Table 2.3), during the MEDITS surveys from 2007 to 2016.

2.1.1.2 IDEADOS project

The project "Structure and dynamics of the bentho-pelagic slope ecosystem in two oligotrophic zones of the western Mediterranean: a multidisciplinary approach at different spatio-temporal scales in the Balearic Islands" (IDEADOS) hypothesized that the differences observed between the nekto-benthic communities of the Balearic sub- (BsB) and the Algerian (AsB) sub-basins could be due to a different trophic structure associated with their contrasting oligotrophy. The project simultaneously approached, from a multidisciplinary perspective, the study of the pelagic communities along the water column and the nekto-benthic communities. One specific objective was to characterize the trophic dynamics of the nekto-benthic communities and their coupling with the pelagic domain. In order to do so, the feeding ecology of abundant demersal and mesopelagic species, as well as the characterization of the trophic structure, was tackled through SCA (Table 2.2) and SIA (Table 2.4).

Surveys were conducted on the southern (CA: Cabrera) and northwestern (SO: Sóller) continental shelf (shelf break, SB = 250 m) and slope (upper slope, US = 650 m and middle slope, MS = 850 m) of Mallorca (Balearic Islands) (Fig. 2.2). These two sites (located in the AsB and BsB, respectively) are separated by a distance of about 120 km. The surveys were carried out during late autumn (December 2009) and summer (July 2010) with both pelagic and benthic sampling performed simultaneously by two vessels: 1) a commercial fishing boat to sample the nekto-benthic and using a commercial "huelvano"-type bottom trawl with a 20 mm diamond mesh cod-end; and 2) a an oceanographic vessel over continental slope grounds from 200 to 900 m depth to sample the pelagic community. In this case, samples were collected in the main sound scattering layers (near the surface at 0–80 m depths; in the 400–600 m deep scattering layer; and less than 50 m above the bottom in the benthic boundary layer) using a double-warp modified commercial mid-water trawl with 10-mm diamond mesh cod-end.

In addition, several smaller nets were also used (IKMT, RMT, and MOCNESS) to collect smaller specimens (Olivar et al., 2012). The zooplankton samples were collected by vertical hauls in the 0–200 m water column at two bathymetric strata (200 and 900 m) within each study area. A double Calvet net (53 μm mesh size) was used to collect microzooplankton and a triple WP2 net (200 μm mesh size) was used for the collection of meso- and macrozooplankton. The nekton-benthic communities were sampled using a bottom trawl (20 mm diamond mesh cod-end and mean horizontal and vertical net openings of 25 and 2 m, respectively). A beam trawl (10 mm diamond mesh cod-end and horizontal and vertical openings of 3.5 and 0.6 m, respectively) and a suprabenthic sledge (rectangular net of 1.25 and 0.3 m as well as 500 μm and 1 mm mesh size used during the 2009 and 2010 surveys, respectively) were used to sample the epi- and supra-benthic communities, respectively.

Particulate organic matter (POM) samples were collected using a 1-year-round moored time-series sediment traps (800 m water depth and 30 m above the bottom) at both locations. The sediment trap samples were wet-sieved through a 1-mm nylon mesh to retain the largest organisms. Swimmers smaller than 1 mm were manually removed.
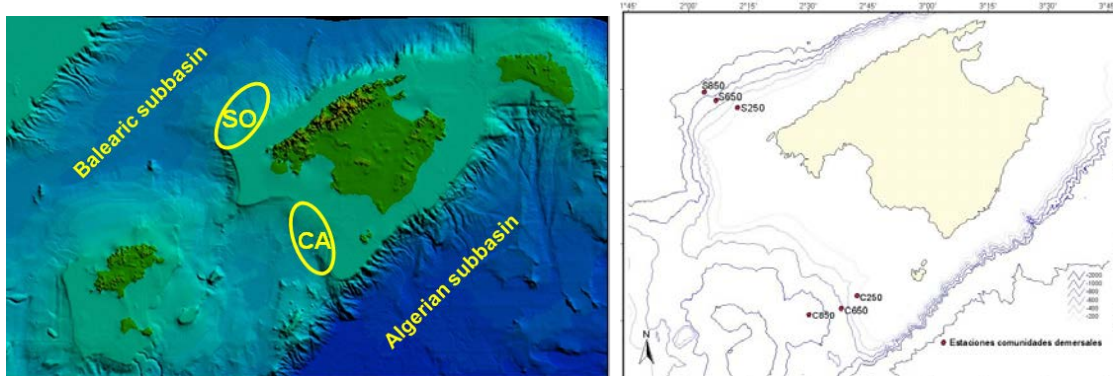
Figure 2.2 Map of the study area and depth strata (250, 650 and 850 m) sampled during the IDEADOS surveys. SO: Sóller; CA: Cabrera

2.1.1.3 Data Collection Framework (DCF)

Since 2000, an EU framework for the collection and management of fisheries data is in place. This framework was last reformed in 2008 resulting in the Data Collection Framework (DCF), regulated by means of the Council Regulation (EC) 199/2008 concerning the establishment of a Community framework for the collection, management and use of data in the fisheries sector and support for scientific advice regarding the Common Fisheries Policy. Under this framework the Member States collect, manage and make available a wide range of fisheries data (biological, technical, environmental and socio-economic) needed for scientific advice. Among others, the DCF entails a regular biological sampling of commercially important stocks. Given that SCA of cephalopods is very time

consuming and logistically difficult on board (most prey remains are reduced to very small pieces by the cephalopod beaks), stomachs of some species (i.e. *Sepia officinalis*, *Loligo vulgaris*) were obtained from monthly biological sampling at the laboratory carried out within the DCF.

2.2 Sampling

2.2.1 Stomach contents analysis

Data from stomach contents were obtained according to the predator's taxon. Elasmobranchs and osteichthyes were sampled on board (A) (Fig. 2.3), whereas cephalopods were stored for latter processing at the laboratory (B).

Entire organisms and body parts of some species (e.g. otoliths, cephalopod beaks and crustacean mandibles) were collected during the surveys and stored and catalogued to build our own reference collection of putative preys of the studied predators. Each reference material was placed in a small vial filled with ethanol solution and labelled with the species prey name, size, depth and survey. Once in the laboratory, each specimen and/or part was also photographed (stereomicroscope imaging software) and stored in a database.

Figure 2.3 Stomach sampling on board the R/V Cornide de Saavedra during the Medits surveys in waters around the Balearic Islands (western Mediterranean).

A) Elasmobranchs sampling: whenever possible, ten individuals per species from each haul were taken and their total length (TL, to the nearest mm), sex (male, female and unknown) and maturity stage (immature, maturing and mature) noted. Prey items were sorted out into individual taxonomic categories and the percentage of these categories in relation to the total stomach content volume (in cc) estimated with device known as a *trophometre* (Olaso *et al.*, 1998) which is a calibrated instrument consisting of several different-sized half cylinders built into a tray (Fig 2.4). All prey items were counted and measured whenever possible (to the nearest mm). The degree of digestion was also noted (1: fresh, 2: partially digested, 3: digested). Due to time constraints or troubles identifying a particular prey item, some stomach contents were stored and labelled for later identification in the laboratory. Whenever possible all data recorded during the survey are daily entered in a computer on board, as data is validated and potential errors corrected in an easy way.

B) Cephalopods sampling: for each individual, the following measurements were taken: dorsal mantle length (ML, to the nearest mm), total weight (TW, to the nearest 0.1 g), sex (male, female and unknown) and maturity stage (immature, maturing and mature). Individual stomachs were preserved in ethanol for later processing. Stomach contents were analysed under a binocular microscope and the prey items were identified to the lowest possible taxon and counted. Most prey categories, including all unidentified parts, were photographed for posterior checking or identification.

Figure 2.4 Stomach sampling on board with a trophometre and a half cylinder detail.

The diet of elasmobranch species was quantified using the following indices: 1) Frequency of occurrence (%O), the percentage of stomachs with a specific type of prey referred to the total number of stomachs containing food; 2) Numerical (%N) and volumetric (%V) composition, expressed as the percentage contribution of each prey, in number or volume respectively, to the whole content; 3) Index of

Relative Importance (IRI=%F(%N+%V) which was standardized as %IRI=(IRI/∑IRI)×100 (Cortes, 1997); and 4) vacuity index (%v), the percentage of empty stomachs.

Cephalopods diet composition was quantified by calculating the percent frequency of occurrence (%O) and percent number (%N) of each prey item in the stomach contents. As cephalopods use the beak to grind their prey, it is difficult to quantify their prey composition by volume or mass percent.

2.2.2 Stable isotope analysis

The individuals collected for stable isotope analysis (SIA) were first measured (to the nearest mm) using the following indices: total length (most of the bony fishes and elasmobranchs), standard length (mesopelagic fishes), anal length (macrourids), mantle length (cephalopods), and cephalothorax length (decapod crustaceans). Then, tissue samples were excised based on the taxon of the samples: dorsal white muscle of fish, mantle in cephalopods, caudal muscle in decapods crustaceans, mysiids, and euphausiids, and whole body for amphipods and small species (e.g. *Cyclothone braueri*). The samples were placed in a vial and frozen on board at −20°C for later processing.

The tissues were dried at 60°C during 24 h and ground to a fine powder using pestle and mortar. The samples generally consisted of just one individual, except for zooplankton and a few small organisms (e.g. *Boreomysis artica*), for which several individuals were pooled together. Prior to analysis, a subsample from the whole individuals and POM were tested for carbonates with 0.1 M HCl. If the sample effervesced, it was acidified further by drop-wise addition of acid until the effervescence ceased. The samples were then re-dried at 50°C during 24 h. Buffered formaldehyde in filtered seawater was used as a preservative for POM samples. The δ^{13}C and δ^{15}N POM values are reported with caution because formalin fixation can have a variable effect on stable isotope values (Bosley and Wainright, 1999; Bicknell et al, 2011).

The analyses of carbon and nitrogen stable isotopes were performed at the Scientific-Technical Services of the University of the Balearic Islands (http://www.uib.eu). The homogeneous dried powder of each individual (1–2 mg) and POM (10–20 mg) sample was placed into cadmium tin cups and then combusted by continuous flow isotope ratio mass spectrometry (CF-IRMS) using a THERMO DELTA X-PLUS mass spectrometer. In addition, three conditioners, one blank, and one internal reference material (Peach Leave Standard-PLS-NIST 1547; Bovine Liver Standard-BL- NIST 1577c) were analyzed at the beginning of each sequence, and one PLS/BL was analyzed after every five samples. The raw data obtained in IRMS measurements are then corrected and calibrated to compensate for the drift over time. The data were expressed in δ notation as parts per thousand relative to the global standard CO_2 for δ^{13}C and atmospheric nitrogen for δ^{15}N. The analytical precision based on the standard deviation of replicates of the internal standard was ≤0.25‰ for the biological samples and 0.05‰ for the POM samples for both δ^{13}C and δ^{15}N.

Table 2.1 Species sampled during the Medits surveys (2007-2016). Total number of stomach sampled (N); minimum (T_{min}) and maximum (T_{max}) species size (in mm); number of regurgitated stomachs (Reg); and vacuity index (%v).

	Species	N	T_{min}	T_{max}	Reg	%v
Elasmobranchii	*Dasyatis pastinaca*	28	359	1004	0	17.9
	Dipturus oxyrinchus	69	170	1080	0	27.5
	Etmopterus spinax	285	42	481	0	30.5
	Galeus melastomus	608	94	682	0	23.2
	Leucoraja circularis	1	602	602	0	0
	Leucoraja naevus	85	137	535	0	35.3
	Mustelus mustelus	3	485	494	0	100
	Myliobatis aquila	38	510	1420	0	7.9
	Pteromylaeus bovinus	1	762	762	0	0
	Raja brachyura	52	191	1190	0	13.5
	Raja clavata	493	105	918	0	8.5
	Raja miraletus	82	170	430	0	3.7
	Raja montagui	41	190	770	0	19.5
	Raja polystigma	93	186	1000	0	7.5
	Raja radula	77	305	847	0	3.9
	Rostroraja alba	2	720	1160	0	50.0
	Scyliorhinus canicula	1095	100	530	4	18.8
	Squalus acanthias	1	262	262	0	0
	Squalus blainville	5	280	483	0	20.0
	Torpedo marmorata	4	156	391	0	75.0
Osteichthyes	*Alepocephalus rostratus*	2	246	425	0	100
	Arnoglossus laterna	2	70	100	0	0
	Capros aper	10	60	90	0	60.0
	Chauliodus sloani	70	135	458	0	61.4
	Chelidonichthys cuculus	941	70	300	4	22.1
	Coelorinchus caelorhincus	86	22	220	3	9.3
	Conger conger	51	78	1350	0	52.9
	Eutrigla gurnardus	11	143	260	0	45.5
	Gadiculus argenteus	12	80	130	2	50.0
	Glossanodon leioglossus	20	90	150	0	20.0
	Gnathophis mystax	1	250	250	0	100
	Helicolenus dactylopterus	558	39	325	0	39.2
	Hymenocephalus italicus	15	20	210	0	40.0
	Lepidorhombus boscii	709	62	333	0	28.2
	Lepidorhombus whiffiagonis	8	190	300	0	25.0
	Lepidotrigla cavillone	409	70	146	3	33.0
	Lepidotrigla dieuzeidei	226	66	226	2	28.8
	Lophius budegassa	204	60	578	2	71.1
	Lophius piscatorius	208	95	1430	0	64.4
	Merluccius merluccius	2347	54	550	475	53.6
	Micromesistius poutassou	445	70	457	21	36.0
	Mullus barbatus barbatus	21	120	210	0	76.2
	Mullus surmuletus	707	120	290	0	32.8
	Nezumia aequalis	34	10	50	2	14.7
	Pagellus acarne	71	109	257	0	77.5
	Pagellus bogaraveo	5	140	190	0	60.0
	Pagellus erythrinus	278	100	270	0	43.5
	Peristedion cataphractum	160	95	323	5	61.9
	Phycis blennoides	830	20	600	275	48.1
	Scorpaena elongata	32	75	424	0	56.3
	Scorpaena loppei	88	64	128	0	32.0
	Scorpaena notata	556	70	999	0	31.3
	Scorpaena porcus	29	130	193	0	37.9
	Scorpaena scrofa	519	35	506	0	50.5
	Serranus cabrilla	807	80	244	78	44.9
	Spicara smaris	33	110	190	0	90.9
	Synchiropus phaeton	31	90	210	0	74.2
	Trachinus draco	1296	90	353	1	49.1
	Trachinus radiatus	17	230	455	0	47.1
	Trigla lyra	596	50	502	6	24.8
	Trigloporus lastoviza	827	73	265	14	11.1
	Trisopterus minutus	46	90	190	5	30.4
	Uranoscopus scaber	44	143	305	0	50.0
	Zeus faber	523	50	590	1	39.0

Table 2.2 Stomachs sampled during the IDEADOS project. Total number of stomachs sampled (N); minimum (T_{min}) and maximum (T_{max}) species size (in mm); number of regurgitated stomachs (Reg); and vacuity index (%v).

	Specie	N	T_{min}	T_{max}	Reg	%V
Elasmobranchii	*Dalatias licha*	2	915	1009	0	50.0
	Etmopterus spinax	78	103	483	0	61.5
	Galeus melastomus	319	96	630	1	29.2
	Raja clavata	94	130	882	0	6.4
	Scyliorhinus canicula	99	132	491	1	27.3
Osteichthyes	*Alepocephalus rostratus*	53	124	440	4	94.3
	Arctozemus risso	2	122	126	0	0
	Argentina sphyraena	29	108	201	2	37.9
	Capros aper	41	35	115	5	82.9
	Centrolophus niger	1	721	721	0	0
	Chauliodus sloani	2	227	278	0	50.0
	Coelorinchus caelorhincus	40	33	81	1	32.5
	Conger conger	47	330	1150	0	72.3
	Gadiculus argenteus	21	48	104	0	4.8
	Glossanodon leioglossus	34	112	141	1	38.2
	Helicolenus dactylopterus	55	74	215	1	38.2
	Hoplostethus mediterraneus	15	88	179	0	33.3
	Hymenocephalus italicus	2	35	39	0	100
	Lampanyctus crocodilus	128	78	327	10	64.8
	Lepidion lepidion	25	94	280	2	80.0
	Lepidorhombus boscii	126	74	298	0	35.7
	Lepidorhombus whiffiagonis	29	199	410	1	41.4
	Lepidotrigla cavillone	10	118	132	0	50.0
	Lophius budegassa	86	66	528	0	68.6
	Lophius piscatorius	4	180	1145	0	50.0
	Merluccius merluccius	397	82	582	190	71.3
	Micromesistius poutassou	73	172	365	0	58.9
	Mora moro	77	62	469	9	100
	Nettastoma melanurum	21	444	710	0	57.1
	Nezumia aequalis	37	17	60	7	48.6
	Notacanthus bonaparte	4	175	241	0	100
	Phycis blennoides	176	89	483	75	85.8
	Scorpaena elongata	1	200	200	0	100
	Trigla lyra	82	88	437	2	36.6
Cephalopods	*Abralia veranyi*	101	8	43	0	42.6
	Bathypolypus sponsalis	25	25	95	0	12.0
	Eledone cirrhosa	72	25	145	0	11.1
	Heteroteuthis dispar	21	10	25	0	28.6
	Histioteuthis bonnellii	3	15	150	0	33.3
	Histioteuthis reversa	74	11	120	0	24.3
	Illex coindetii	259	24	245	0	36.3
	Loligo forbesii	84	140	451	0	3.6
	Octopus salutii	10	40	165	0	10.0
	Pteroctopus tetracirrhus	7	45	140	0	0
	Rondeletiola minor	45	15	70	0	4.4
	Rossia macrosoma	56	10	21	0	44.6
	Sepia orbignyana	14	26	92	0	35.7
	Sepietta oweniana	99	13	33	0	3.0
	Todarodes sagittatus	41	110	268	0	9.8

2.2.2.1 Lipid content

Lipids are depleted in δ^{13}C relative to proteins and carbohydrates (Sweeting et al., 2006). The lipid content can bias ecological interpretations through three mechanisms: i) systematic patterns (e.g. seasonal patterns in nutrition or reproduction); ii) sample variance and consequent considerations for statistical power; and iii) treatment or correction mechanisms that may induce alternative errors. In the present study, the potential for lipid bias was explored based on the C:N ratios from percent element by weight. As the dataset contained diverse taxa, but generally low C:N ratio, the recommendation of Post et al. (2007) by which a C:N ratio higher than 3.5 indicated potential for notable lipid bias, was adopted.

Elasmobranchs exhibited low lipid-free C:N ratio of less than 2.5, while zooplankton frequently exhibited higher lipid-free C:N ratio of more than 3.5. Only a minority of the samples exhibited a C:N ratio of more than 3.5; however, these ratios appeared to be mostly independent of lipids (e.g. echinoderms or thaliaceans sampled as a whole). Only some mesopelagic fishes exhibited C:N dynamics consistent with relatively high lipid contents (C:N from 3 to 5). Therefore, the analysis dealing with the whole food web (Chapter 3) was conducted on uncorrected δ^{13}C, consistent with the generally low lipid levels noted in the Mediterranean species (Fanelli et al., 2011a, 2013). Whereas δ^{13}C data regarding the trophic structure of mesopelagic fish species (Chapter 6) were lipid normalized according to (Post et al., 2007):

δ^{13}C'=δ^{13}C$_{bulk}$-3.32+0.99*C:N$_{bulk}$,

where bulk values are those observed in the untreated sample and δ^{13}C' is arithmetically corrected lipid free δ^{13}C.

Table 2.3 Number of species collected during the MEDITS surveys (2007-2015) and processed for stable isotope analysis. Total number of individuals sampled (N); minimum (T_{min}) and maximum (T_{max}) individuals size (in mm).

	Species	N	T_{min}	T_{max}
Elasmobranchii	Dipturus oxyrhinchus	7	400	1112
	Etmopterus spinax	12	143	462
	Galeus melastomus	38	190	605
	Leucoraja naevus	9	250	506
	Myliobatis aquila	3	740	950
	Raja clavata	63	240	910
	Raja miraletus	10	280	397
	Scyliorhinus canicula	85	210	490
Osteichthyes	Boops boops	12	14	222
	Caelorhynchus caelorhynchus	3	55	55
	Capros aper	10	53	95
	Centracanthus cirrus	8	100	115
	Ceratoscopelus maderensis	6	60	73
	Chelidonichthys cuculus	33	130	240
	Chelidonichthys lastoviza	21	135	215
	Coelorhynchus caelorhynchus	6	40	90
	Conger conger	8	410	870
	Deldentosteus quadrimaculatus	6	65	87
	Engraulis encrasicolus	12		
	Gadiculus argenteus	6	64	95
	Glossanodon leioglossus	17	50	131
	Helicolenus dactylopterus	58	95	273
	Himenocephalus italicus	3	40	41
	Lampanyctus crocodilus	12	75	202
	Lepidorhombus boscii	66	130	330
	Lepidorhombus whiffiagonis	13	221	321
	Lepidotrigla cavillone	5	121	133
	Lophius budegassa	65	180	570
	Lophius piscatorius	31	122	899
	Merluccius merluccius	468	0	540
	Micromesistius poutassou	27	230	320
	Mullus surmuletus	58	140	250
	Nezumia aequalis	9	32	50
	Pagellus erythrinus	47	110	259
	Phycis blennoides	49	90	510
	Sardina pilchardus	9	126	170
	Scorpaena notata	12	120	160
	Serranus cabrilla	33	110	210
	Serranus hepatus	8	69	98
	Sinchiropus phaeton	7	145	205
	Spicara smaris	6	99	187
	Tachinus draco	51	130	290
	Trachurus picturatus	11	90	266
	Trachurus mediterraneus	6	116	185
	Trachurus trachurus	12	104	221
	Trisopterus minutus	6	140	190
	Zeus faber	99	90	580

	Species	N	T_{min}	T_{max}
Mollusca	Abralia veranyi	9	31	44
	Alloteuthis media	37	36	92
	Ancistroteuthis lichtensteinii	5	40	146
	Bathypolypus sponsalis	24	20	700
	Eledone cirrhosa	29	25	140
	Eledone moschata	23	35	148
	Heteroteuthis dispar	7	15	23
	Histioteuthis bonnellii	9	19	140
	Histioteuthis reversa	26	38	85
	Illex coindetii	35	55	230
	Loligo forbesii	36	44	279
	Loligo vulgaris	26	55	245
	Octopus salutii	22	40	857
	Octopus vulgaris	35	50	130
	Pteroctopus tetracirrhus	10	80	112
	Rondeletiola minor	4	12	20
	Rossia macrosoma	16	22	67
	Scaeurgus unicirrhus	25	30	90
	Sepia elegans	36	15	47
	Sepia officinalis	31	85	163
	Sepia orbignyana	33	21	92
	Sepietta oweniana	21	15	30
	Todarodes sagittatus	47	114	374
	Todaropsis eblanae	6	80	200
	Cymbulia peronii	10		
	Aequipecten opercularis	4	29	35
Crustacea	Geryon longipes	2	56	56
	Aristeus antennatus	4	21	35
	Nephrops norvegicus	5	44	47
	Parapenaeus longirostris	8	22	22
	Pasiphaea multidentata	11	29	29
	Pasiphaea sivado	5		
	Plesionika antigai	3		
	Processa canaliculata	3		
	Meganyctiphanes norvegica	6	8	10
	Misidaceos	21		
Thaliacea	Pyrosoma atlanticum	3	18	20

Table 2.4 Number of species collected during the IDEADOS surveys and processed for stable isotope analysis. Total number of individuals sampled (N); minimum (T_{min}) and maximum (T_{max}) individuals size (in mm).

	Species	N	T_{min}	T_{max}
	Galeus melastomus	46	123	584
	Raja clavata	17	200	815
Osteichthyes	*Alepocephalus rostratus*	10	228	363
	Argyropelecus hemigymnus	7	13	39
	Benthosema glaciale	9	35	42
	Cyclothone braueri	6		
	Capros aper	18	63	113
	Ceratoscopelus maderensis	20	38	59
	Diaphus holti	6	25	49
	Electrona risoi	4	43	50
	Glossanodon leioglossus	3	126	136
	Helicolenus dactylopterus	12	140	209
	Hygophum benoiti	4	46	56
	Hygophum hygomii	5	41	58
	Lampanyctus crocodilus	30	55	210
	Lampanyctus pusillus	4	37	41
	Lepidion lepidion	20	79	239
	Lepidorhombus boscii	22	74	269
	Lepidorhombus whiffiagonis	7	187	410
	Lobianchia dofleini	6	32	37
	Lophius budegassa	29	86	446
	Maurolicus muelleri	6	36	39
	Merluccius merluccius	47	85	551
	Micromesistius poutassou	15	172	339
	Mora moro	19	80	436
	Myctophum punctatum	6	41	60
	Nezumia aequalis	19	26	54
	Notolepis rissoi	5	132	193
	Notoscopelus elongatus	14	39	95
	Phycis blennoides	33	114	463
	Stomias boa	5	76	125
	Symbolophorus veranyi	7	45	130
	Trachurus mediterraneus	2	30	40
	Trachurus trachurus	1	37	37
	Vinciguerria attenuata	6	34	37
Mollusca	*Cymbulia peroni*	6		
	Abralia veranyi	4	30	35
	Ancistrocheirus lesuerii	1	165	165
	Ancistroteuthis lichtensteinii	3	13	155
	Bathypolypus sponsalis	8	51	271
	Heteroteuthis dispar	9	16	25
	Histioteuthis bonnellii	2	22	43
	Histioteuthis reversa	22	21	145
	Illex coindetii	9	169	206

	Species	N	T_{min}	T_{max}
Mollusca	*Loligo forbesii*	25	150	475
	Neorossia caroli	1	51	51
	Octopus salutii	2	45	90
	Opistoteuthis calypso	2	30	40
	Pteroctopus tetracirrhus	7	85	140
	Rondeletiola minor	16	11	18
	Rossia macrosoma	16	25	50
	Scaeurgus unicirrhus	2	34	34
	Sepietta oweniana	9	13	28
	Taonius pavo	1	153	153
	Todarodes sagittatus	14	207	400
Crustacea	*Anapagurus laevis*	1		
	Munida tenuimana	3	12	18
	Pagurus alatus	3		
	Geryon longipes	11	37	55
	Macropipus tuberculatus	12	13	35
	Monodaeus couchii	1		
	Calocaris macandrae	2		
	Alpheus glaber	6	25	30
	Aristeus antennatus	24	20	59
	Chlorotocus crassicornis	1	16	16
	Pandalina profunda	2		
	Parapaneus longirostris	15	11	27
	Pasiphaea multidentata	11	9	36
	Pasiphaea sivado	1	18	18
	Philocheras echinulatus	18	7	48
	Plessionika acanthonotus	9	7	15
	Plessionika antigai	3	32	41
	Plessionika heterocarpus	11	10	13
	Plessionika martia	8	15	17
	Processa canaliculata	7	6	12
	Richardina sp.	4		
	Sergestes arcticus	4	34	40
	Solenocera membranacea	4	8	14
	Meganycthiphanes norvegica	8	7	8
	Boreomysis artica	3		
	Eucopia unguiculata	1		
	Lophogaster typicus	1	11	11
Other	*Pyrosoma atlanticum*	5		
	Salpa maxima	1		
	Gryphus vitreus	19		

Chapter 3. Structure and dynamics of food webs in the water column on shelf and slope grounds of the western Mediterranean

Chapter 3. Structure and dynamics of food webs in the water column on shelf and slope grounds of the western Mediterranean

3.1. Introduction

The connections between ecological processes in the water column and seafloor are called bentho-pelagic coupling (BPC) (Graf, 1992; Smith et al., 2006). In marine systems, BPC is important for the flow of nutrients, propagules, and predators (Boero et al., 1996; Meyer et al., 1983). Previous studies have suggested that around 30% of global fisheries yield (Pauly and Christensen, 1995) and most of the coastal biomass of organisms as diverse as suspension feeders and marine mammals are dependent on BPC (Dunton et al., 1989; Smith et al., 2006). BPC is presumed to be weaker and indirect at abyssal depths, where production is almost exclusively sustained by marine snow, i.e. the settling of organic matter produced in surface water (Gooday, 1988; Iken et al., 2005; Miquel et al., 1994). However, the BPC in intermediate depths, e.g. those of the shelf edge and continental slope, is less clear.

An empirical understanding of the magnitude and direction of benthic-pelagic linkage is a fundamental requirement for a holistic management; however, there is a lack of this understanding for most of the systems (Thrush and Dayton, 2010). For example, the strong top-down BPC around the coastal reef systems suggests that benthic-focused marine protection would benefit from the incorporation of pelagic protection (Grober-Dunsmore et al., 2008). Alternatively, fish activity could be a major bottom-up mechanism for BPC via sediment re-suspension with implications for carbon sequestration and benthic oxygen demand (Yahel et al., 2008).

In temperate systems, biotic and abiotic conditions change considerably in the course of the year with possible effects on food availability and food web interactions (Polis and Strong, 1996). Food quantity and quality, in turn, depend on multiple factors interacting along the water column, including nutrient availability, primary production, water stratification, mixed-layer depth, mid-water grazing rates, and bacterial degradation (Fenchel, 1988). The quantity and quality of food available for benthic communities determine their species richness, abundance, biomass and feeding patterns (Smith et al., 1997; Watts et al., 1992).

Assessment of feeding behavior in diverse species over multiple trophic levels is challenging. Conventional analyses based on gut contents involve large sample sizes, require the researchers to have a good taxonomic knowledge of organisms, and represent only a snapshot of the diet (Miller et al., 2010). Furthermore, biases related to what material is identifiable in the stomach may underestimate rapidly digested and evacuated prey (Hyslop, 1980).

Stable isotope analyses provide an alternative and complimentary approach for trophic analysis (Fry, 1988), and are predicated on the assumption that there are systematic and predictable changes in the isotopic signatures of a consumer relative to its prey or food resource (Deniro and Epstein, 1981; Minagawa and

Wada, 1984). These trophic changes, termed as trophic discrimination factor, vary among elements. The trophic discrimination factor of nitrogen ($\Delta^{15}N$) is high, with an average value of 3.4 ‰, when compared with other elements (Post, 2002). In contrast, the trophic discrimination factor of carbon ($\Delta^{13}C$) is lower, at around 1.0 ‰ (Deniro and Epstein, 1981; Peterson and Fry, 1987), and the carbon isotope signatures ($\delta^{13}C$) vary substantially among different production sources (Fry and Sherr, 1984). The $\delta^{15}N$ and $\delta^{13}C$ values act as proxies for the trophic level of species and indicate the production source or source mixtures sustaining a species (Post, 2002), respectively. Thus, the combined measurements of $\delta^{15}N$ and $\delta^{13}C$ can provide information on an organism's assimilated diet and facets of the associated food web (Peterson and Fry, 1987; Post, 2002).

Stable isotope analyses are particularly useful in elucidating the degree to which benthic consumers are coupled to pelagic primary production. For example, close coupling of consumers with pelagic primary productivity results in lower $\delta^{13}C$ in the consumer tissues, when compared with these links in the deposit feeders and detrital-based food webs (Hobson et al., 1995). Moreover, strong correlations between $\delta^{13}C$ and $\delta^{15}N$ are indicative of a single type of primary source material supporting the food web (Fanelli et al., 2011a; Polunin et al., 2001). The Balearic Islands separate two sub-basins in the western Mediterranean, the Balearic sub-basin (BsB) in the north and the Algerian sub-basin (AsB) in the south. Although they are connected, differences occur across the hydrographical transition represented by the Balearic Islands in this area: i) different abundance, biomass, and composition of demersal species have been detected (Guijarro et al., 2012; Moranta et al., 2008); ii) the BsB is more influenced by atmospheric forcing and Mediterranean waters, which are colder and more saline, whereas the AsB receives warmer and less saline Atlantic waters (Pinot et al., 2002); and iii) wider variability in suprabenthos biomass has been found in the BsB (Cartes et al., 2008). Therefore, the extent of BPC might be reflected in different trophic structures and the role of specific species.

The deep sea in the northwestern Mediterranean is influenced by the overlaying water column (Carrassón and Cartes, 2002; Cartes, 1998). The main inputs of pelagic material for benthic life are through the downward flux of detrital material (Miquel et al., 1994) as well as the trophic linkages between the benthic organisms and species that perform vertical migrations (Andersen and Sardou, 1992; Angel and Boxshall, 1990; Cartes et al., 1994; Olivar et al., 2012). In oligotrophic areas, such as the Balearic Islands, with little supply of terrigenous nutrients and without advective inputs of organic matter via submarine canyons, trophic webs are supported to a greater extent by planktonic biomass, rather than by benthic biomass (Cartes et al., 2001; Maynou and Cartes, 2000; Polunin et al., 2001). However, the extent to which the source materials supporting the dominant species vary at different depths and geographic areas is still unknown. For example, in the AsB, food chains are heavily dependent on a single production source delivered as marine snow (Cartes et al., 2008; Madurell et al., 2008; Polunin et al., 2001). In contrast, more dispersed patterns suggest production from multiple sources sustaining the community, as observed for macrozooplankton and micronekton in the Catalan Sea slope (Balearic sub-basin), in an area under the influence of river discharges (Fanelli et al., 2011a; Papiol et al., 2013).

Previous studies carried out in the western Mediterranean have described deep-water food webs and trophic balances using stable isotopes data, and have focused on specific taxa: fishes (Fanelli and Cartes, 2010), decapods (Fanelli and Cartes, 2008;) and cephalopods (Fanelli et al., 2012) or compartments such as hyperbenthos (Fanelli et al., 2009; Madurell et al., 2008), zooplankton (Fanelli et al., 2011a), epi- and endobenthos (Fanelli et al., 2009; Fanelli et al., 2011b), and deep-sea megafauna (Fanelli et al., 2013b ; Papiol et al., 2013; Polunin et al., 2001), elucidating their trophodynamics. To comprehensively explore the spatial effects governing the trophic structure of deep-sea assemblages in oligotrophic conditions, a more complete characterization of species along the water column (particularly, fishes) and the simultaneous inclusion of more trophic levels across all zones have been considered to be necessary (Cartes et al., 2008; Moranta et al., 2008).

Thus, the present study explored the trophodynamics of a wide array of species from near-surface to the bottom by using a stable isotope approach based on a broad, multidisciplinary, multi sampling gear project examining the trophic coupling of nektobenthic and benthic slope communities and the populations of the mesopelagic domain in the oligotrophic western Mediterranean. In particular, this study (1) describes the trophic structure along a bathymetric gradient (250–850 m), (2) identifies the food sources supporting the dominant species, and (3) investigates the degree of coupling between the vertical compartments at two locations and three depth zones.

3.2 Material and methods

Sample collection

The pooled size-based groups of zooplankton (50–200, 200–500, and >500 μm), together with 89 megafaunal species, were used for the analysis (48 species at SB, 51 species at US, and 54 species at MS). A total of 930 samples from the dominant species in terms of total biomass or numerical abundance (>70% of total catch), encompassing hyperbenthic (53 species) and pelagic (36 species) compartments, were used for isotopic analyses. Whenever possible, a minimum of three individuals of similar size were collected per species, location (S and C), season (autumn and summer), and depth (SB, US, and MS). Ideados survey design, sampling methodology and stable isotope analysis are explained in Chapter 2 (Section 2.1.1.2 and 2.2.2, respectively).

Food web structure

The trophic level (TrL) was derived from $\delta^{15}N$ as follows:

$$TrL_i = ((\delta^{15}N_i - \delta^{15}N_{ref})/\Delta^{15}N) + \lambda$$

where TrL_i is the trophic level of the species i, $\delta^{15}N_i$ is the mean species $\delta^{15}N$, $\delta^{15}N_{ref}$ is the mean $\delta^{15}N$ of the food web baseline, $\Delta^{15}N$ is the discrimination factor

per trophic level, and λ is the trophic level of the baseline. Two species were used to define the reference δ^{15}N: 1) *Cymbulia peroni* (mean δ^{15}N= 3.87‰ ± 0.45), a pelagic gastropod which performs extensive vertical migrations (Sardou et al., 1996), 2) *Calocaris macandreae* (mean δ^{15}N= 6.45‰ ± 0.83) a burrowing thalassiinid shrimp. *C. peroni* was used as a reference for pelagic species and was allocated λ=2, assuming that it is predominantly primary consumer. Whereas *C. macandreae,* a POM feeder, was used for hyperbenthic species and positioned at a λ=2.6 (Fanelli et al., 2009). The Δ^{15}N value was defined as 3.4‰ (Post, 2002). Although the use of a mean fractionation value for the whole food web is subjected to substantial errors and several studies have noted a relatively high variability in Δ^{15}N across trophic level and species (e.g. Caut et al., 2009; McCutchan et al., 2003; Post, 2002), there is a lack of more refined estimates for a majority of species, and considering all other sources of error, the variability of the trophic fractionation values has a minor effect on the computation of trophic positions (Post, 2002). Furthermore, a Δ^{15}N of 3.4‰ was considered to keep our results comparable with those from previous works analyzing similar trophic levels from the study area (Polunin et al., 2001).

As this study was primarily interested in spatial patterns with depth, the species data were pooled across seasons and replicated hauls per location (Layman et al., 2007). Such averaging is important for two reasons. First, small-sized species and individuals represent isotopic signatures over shorter temporal scales than large-sized individuals and species, giving rise to differences in the isotopic turnover rate. Second, species differ in their mobility; thus, individuals reflect spatial variation in isotopic signatures as a function of their mobility and isotopic turnover rate. Averaging isotopic signatures over space reduces such spatial variation and increases statistical power.

Bentho-pelagic coupling

In this study, the term BPC was used to indicate the connections through trophic relationships along the water column, from the near-surface layer to the near-bottom region. According to the vertical habitat, the marine fauna samples include: 1) pelagic species with a wide vertical distribution range in the water column (Hulley, 1981), which generally eat along the water column and 2) hyperbenthic species that spend part or all of their lives in association with the bottom, many of which interact within a framework of horizontal and vertical linkages (Mees and Jones, 1997; Giller and Gee, 1987). To determine the energy transfers between both the communities, the marine organisms were classified according to these categories as belonging to the pelagic compartment (PC) or hyperbenthic compartment (HC). Such classification was based on published literature and supported by the species presence in the different gears used during the surveys (Olivar et al., 2012; Quetglas et al., 2013b).

Statistical analyses

The food web structure was examined by applying hierarchical cluster analysis (average grouping methods) on the resemblance matrix (Euclidean distance) of mean δ^{15}N and δ^{13}C. As depth is the major factor determining the assemblage composition in both the demersal and mesopelagic communities in the study area (Moranta et al., 2008; Olivar et al., 2012), the clusters were performed separately for each of the three strata (250, 650, and 850 m) to reduce the effect of depth. The choice of the cut-off point that defines the clusters was somewhat arbitrary, but comparisons between the cluster results and the biological and ecological information available enabled a useful basis for the description of different trophic structures (Davenport and Bax, 2002). The differences among the clusters obtained at each stratum were tested by using a distance-based permutational analysis of variance based on Euclidean distances, and subsequently, pair-wise comparisons were carried out. The significance was set at p=0.05 and p values were obtained by using 9999 permutations of the raw data. When there were very few possible permutations, the Monte Carlo p value (p-MC) was preferred. For each stratum, δ^{15}N–δ^{13}C scatter plots were developed and the trophic groups were highlighted to obtain an overview of the trophic structure.

Shapiro-Wilk and Levène tests were used to assess the normality and homogeneity of variances, respectively. The correlations between δ^{15}N and δ^{13}C from each depth within location were determined to check the association strength between the trophic positions and food source. The species (co-occuring at both locations) isotopic values were compared by using pair t-test or Wilcoxon Signed-Rank test. The spatial differences in the POM were tested by using one-way ANOVA, and only contemporary POM data from both the locations were used for comparisons.

In addition, to reveal spatial differences and BPC, the differences in mean δ^{15}N and δ^{13}C values by species between locations (S and C), depth within location (C250, S250, C650, S650, C850, and S850), and vertical guild (PC and HC), together with their crossed effects, were tested by using a distance-based permutational analysis of variance based on Euclidean distances. All factors were treated as fixed, significance was set at p=0.05 and p values were obtained using 9999 permutations of the untransformed data. To elucidate the differences among the statistically significant levels for each factor, pair-wise tests were performed. *Gryphus vitreus* was excluded from this analysis because this species clustered apart, exhibiting highly enriched isotopic signature. Besides, the values of *Ophiura* spp. and *Anapagurus laevis* were not included owing to δ^{13}C analysis constraints after acidification. All the multivariate analyses were performed with PRIMER 6 + PERMANOVA software package from Plymouth Marine Laboratory, UK (Anderson et al., 2008).

Table 3.1. $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SD) of species sampled at Shelf-break, Upper slope and Middle slope, and the size ranges (in mm) of cephalopods, elasmobranches, fishes, and some crustaceans. VG: vertical guild; P: pelagic; HB: hyperbenthic; TrG: trophic group from cluster analysis (I–VII); n: number of replicates.

Species	Size	VG	Shelf-break				Upper slope				Middle slope			
			$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	TrG	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	TrG	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	TrG	n
POM		P									−22.46 ± 1.00	2.38 ± 1.00		36
Macrozooplankton[a]	>0.5	P	−20.21 ± 0.88	4.24 ± 0.63	I	9					−20.38 ± 0.72	4.52 ± 0.73	I	9
Mesozooplankton[a]	0.2–0.5	P	−20.28 ± 0.86	3.98 ± 0.38	I	9					−20.38 ± 0.94	3.87 ± 0.51	I	9
Microzooplankton[a]	0.05–0.2	P	−20.51 ± 1.00	3.87 ± 0.52	I	9					−20.62 ± 0.57	4.03 ± 0.23	I	9
Amphipoda														
Amphipoda unidentified		P	−20.22	3.92	I	1								
Phronima sedentaria		P			I						−19.54 ± 0.39	6.68 ± 0.5	II	2
Phrosina semilunata		P	−19.97 ± 0.49	5.68 ± 0.77	I	8					−19.33	5.94	II	1
Thaliacea														
Pyrosoma atlanticum		P	−21.50 ± 0.18	3.24 ± 0.35	I	5					−21.75 ± 0.46	4.01 ± 0.33	I	2
Salpa maxima		P	−21.45 ± 0.46	3.59 ± 1.05	I	4	−21.43	4.09	I	1				
Brachyopoda														
Gryphus vitreus		HB	−14.47 ± 1.36	8.61 ± 0.24		11	−15.46	9.17		1	−12.98 ± 1.4	10.20 ± 0.61		7
Echinodermata														
Ophiura spp[a].		HB		5.98		1		6.78		1				
Mysidacea														
Boreomysis arctica[a]		HB	−19.15 ± 1.59	6.59 ± 0.33	II	2	−19.89	6.64	II	1				
Eucopia unguiculata[a]		P									−20.45	6.87	II	1
Lophogaster typicus[a]		HB	−19.65	7.57	IV	1								
Euphausiacea														
Meganyctiphanes norvegica		P									−20.08 ± 0.48	6.57 ± 0.67	II	10
Decapoda														
Calocaris macandreae		HB					−19.20 ± 0.59	6.21 ± 0.83	II	3	−18.74	7.15	III	1
Alpheus glaber		HB	−18.10 ± 0.32	7.31 ± 0.71	III	6								
Aristeus antennatus	20–59	HB					−17.95 ± 0.36	10.06 ± 0.73	VI	9	−17.77 ± 0.24	10.32 ± 1.19	V	15
Chlorotoccus crassicornis	16	HB	−18.97	7.09	II	1								
Pandalina profunda		HB	−18.22 ± 0.61	7.98 ± 0.94	IV	4	−18.35	8.08	III	1				
Parapenaeus longirostris	11–27	HB	−18.25 ± 0.25	8.46 ± 0.49	IV	15								
Pasiphaea multidentata (A)	31–36	HB					−18.70 ± 0.06	7.65 ± 0.60	III	3	−18.82 ± 0.13	7.39 ± 0.41	III	3
Pasiphaea multidentata (J)	9–18	P					−19.19 ± 0.45	7.97 ± 0.73	II	5				
Pasiphaea sivado	18	P					−19.85	7.36	II	1				
Philocheras echinulatus		HB	−18.05 ± 0.39	9.049 ± 0.43	VI	25					−16.61	11.80	VI	1
Plesionika acanthonotus	7–15	HB									−17.99 ±0.76	7.78 ± 1.15	III	9
Plesionika antigai		HB	−18.91 ± 0.30	7.71 ± 0.45	IV	3								

Species	Size	VG	Shelf-break δ¹³C (‰)	δ¹⁵N (‰)	TrG	n	Upper slope δ¹³C (‰)	δ¹⁵N (‰)	TrG	n	Middle slope δ¹³C (‰)	δ¹⁵N (‰)	TrG	n
Plesionika heterocarpus	10–13	HB	−18.46 ± 0.25	7.96 ± 0.39	IV	10	−18.39	7.49	III	1				
Plesionika martia	15–17	HB					−18.47 ± 0.33	8.01 ± 0.37	III	7	−18.34	7.78	III	1
Processa canaliculata	6–12	HB					−18.90 ± 0.20	8.39 ± 0.44	III	7				
Richardina sp.		HB									−17.93 ± 0.18	11.29 ± 0.61	VI	4
Sergestes arcticus	34–40	HB									−20.75 ± 0.33	6.68 ± 0.41	II	4
Solenocera membranacea	8–14	HB	−18.60 ± 0.14	8.38 ± 0.56	IV	4								
Geryon longipes	37–55	HB					−18.09 ± 0.13	9.80 ± 0.36	VI	5	−17.72 ± 0.31	9.68 ± 0.54	V	6
Macropipus tuberculatus	13–35	HB	−18.80 ± 0.47	9.29 ± 0.95	V	12								
Monodaeus couchii		HB									−17.18	8.54	V	1
Anapagurus laevis		HB						6.10		1				
Munida tenuimana		HB									−18.25 ± 0.26	8.78 ± 0.15	V	4
Pagurus alatus		HB	−17.61 ± 1.12	7.10 ± 0.19	III	3								
Gastropoda														
Cymbulia peroni		P	−20.38 ± 0.15	3.73 ± 0.49	I	3	−20.56 ± 0.56	3.91 ± 0.54	I	3	−21.20	4.17	I	1
Cephalopoda														
Abralia veranyi	30–35	P	−19.82 ± 0.19	9.37 ± 0.42	V	2	−19.21	9.98	VI	1	−19.63	9.66	IV	1
Ancistrocheirus lesuerii	165	P									−20.18	9.62	IV	1
Ancistroteuthis lichtensteinii	13–155	P									−19.35 ± 0.22	10.07 ± 0.10	IV	3
Bathypolypus sponsalis	51–271	HB					−18.46	9.18	VI	1	−18.39 ± 0.14	9.61 ± 0.41	V	7
Heteroteuthis dispar	16–25	HB					−19.65	9.11	VI	1	−19.93 ± 0.18	9.02 ± 0.53	IV	10
Histioteuthis bonnellii	22–43	P					−20.44 ± 0.05	10.02 ± 0.14	V	2				
Histioteuthis reversa	21–145	P					−20.08 ± 0.38	9.84 ± 0.41	V	14	−20.17 ± 0.21	10.45 ± 0.73	IV	10
Illex coindetii	169–206	HB					−18.55 ± 0.17	9.15 ± 0.50	VI	8	−18.65	9.14	V	1
Loligo forbesii	150–475	HB	−19.34 ± 0.42	9.53 ± 0.65	V	25	−18.76	10.90	VII	1				
Neorossia caroli	51	HB					−18.36	9.00	VI	1				
Octopus salutii	45–90	HB	−18.77 ± 0.08	9.28 ± 0.17	V	2								
Opisthoteuthis calypso	30–40	HB									−18.34 ± 0.33	9.11 ± 0.15	V	2
Pteroctopus tetracirrhus	85–140	HB	−18.28 ± 0.31	8.36 ± 0.44	IV	5	−17.87 ± 0.20	9.24 ± 0.22	VI	2				
Rondeletiola minor	11–18	HB	−19.54 ± 0.14	8.31 ± 0.54	IV	18								
Rossia macrosoma	25–50	HB	−19.26 ± 0.22	8.27 ± 0.34	IV	19								
Scaeurgus unicirrhus	34–34	HB									−19.91 ± 0.01	9.24 ± 0.26	IV	2
Sepietta oweniana	13–28	HB	−19.73 ± 0.35	9.25 ± 0.47	V	10								
Galiteuthis armata	153	HB									−19.51	8.75	IV	1
Todarodes sagittatus	207–400	HB	−19.17 ± 0.57	8.76 ± 0.27	IV	3	−19.02 ± 0.30	9.58 ± 0.73	VI	8	−19.50 ± 0.55	9.04 ± 0.86	IV	3
Chondrichthyes														
Galeus melastomus	123–584	HB	−18.31 ± 0.24	8.51 ± 0.38	IV	3	−18.09 ± 0.34	9.93 ± 0.68	VI	22	−17.91 ± 0.24	10.01 ± 1.01	V	21
Raja clavata	200–815	HB	−17.84 ± 0.19	9.43 ± 0.92	VI	17								

| Species	Size	VG	Shelf-break δ¹³C (‰)	δ¹⁵N (‰)	TrG	n	Upper slope δ¹³C (‰)	δ¹⁵N (‰)	TrG	n	Middle slope δ¹³C (‰)	δ¹⁵N (‰)	TrG	n
Osteichthyes														
Alepocephalus rostratus	228–363	HB									−19.92 ± 0.91	10.02 ± 0.94	IV	10
Argyropelecus hemigymnus	13–39	P					−19.10 ± 0.07	8.97 ± 0.72	VI	3	−20.21 ± 0.42	6.42 ± 0.70	II	4
Benthosema glaciale	35–42	P					−20.80 ± 0.38	8.50 ± 0.59	IV	3	−21.56 ± 0.68	9.01 ± 0.53	IV	6
Cyclothone braueri[a]	22–25	P	−19.40	6.92	II	1	−19.63	7.11	II	1	−20.17 ± 0.51	6.85 ± 0.18	II	4
Capros aper	63–113	HB	−19.83 ± 0.41	8.94 ± 0.56	V	15	−19.93 ± 0.02	8.30 ± 0.57	II	3				
Ceratoscopelus maderensis	38–59	P	−19.74 ± 0.94	7.66 ± 0.38	IV	7	−21.06 ± 0.32	8.16 ± 0.73	IV	3	−19.79 ± 0.67	8.59 ± 0.77	IV	10
Diaphus holti	25–49	P					−21.24 ± 1.67	9.42 ± 0.88	IV	6				
Electrona risso	43–50	P					−19.54	8.18	II	1	−20.56 ± 0.20	8.37 ± 0.77	IV	3
Glossanodon leioglossus	126–136	HB	−19.49 ± 0.13	9.48 ± 0.40	V	3								
Helicolenus dactylopterus	140–209	HB	−18.88 ± 0.27	10.38 ± 0.74	V	12								
Hygophum benoiti	46–56	P	−19.35 ± 0.06	7.98 ± 0.58	IV	3	−19.27	7.53	II	1				
Hygophum hygomii	41–58	P	−18.94 ± 0.41	9.18 ± 0.78	V	2					−20.23 ± 0.38	9.08 ± 0.73	IV	3
Lampanyctus crocodilus (A)	108–181	HB					−19.09 ± 0.25	9.17 ± 0.87	VI	7	−18.73 ± 0.29	9.85 ± 0.73	V	15
Lampanyctus crocodilus (J)	57–92	P					−19.67 ± 0.11	7.89 ± 0.07	II	2	−20.45 ± 0.53	7.84 ± 0.60	IV	6
Lampanyctus pusillus	37–41	P					−20.06 ± 0.66	8.24 ± 0.03	II	2	−20.51 ± 0.84	9.15 ± 0.35	IV	2
Lepidion lepidion	79–239	HB					−20.37 ± 0.16	7.58 ± 0.23	II	3	−18.61 ± 0.46	11.32 ± 0.72	VI	17
Lepidorhombus boscii	74–269	HB	−18.76 ± 0.53	9.30 ± 0.83	V	21	−18.76	9.90	VI	1				
Lepidorhombus whiffiagonis	187–410	HB	−18.71 ± 0.38	9.41 ± 0.96	V	7								
Lobianchia dofleini	32–37	P									−20.23 ± 0.57	10.25 ± 0.45	IV	6
Lophius budegassa	86–446	HB	−18.40 ± 0.26	10.95 ± 0.64	VII	30								
Maurolicus muelleri	36–39	P	−20.16 ± 0.26	9.0 ± 0.03	V	3	−19.66 ± 0.15	7.79 ± 0.67	II	3				
Merluccius merluccius	85–551	HB	−19.07 ± 0.22	9.49 ± 0.69	V	37	−18.55 ± 0.58	10.92 ± 1.02	VII	8	−17.96 ± 0.42	11.89 ± 0.88	VI	2
Micromesistius poutassou	172–339	HB	−18.88 ± 0.14	9.91 ± 0.50	V	6	−18.94 ± 0.21	9.47 ± 0.46	VI	3	−18.28 ± 0.53	10.63 ± 0.84	V	6
Mora moro	80–436	HB					−19.62 ± 0.49	8.90 ± 0.89	VI	6	−18.25 ± 0.37	11.14 ± 0.56	VI	13
Myctophum punctatum	41–60	P	−18.85 ± 0.40	7.66 ± 0.48	IV	3	−20.27 ± 0.34	8.20 ± 1.32	II	3				
Nezumia aequalis	26–54	HB					−18.43 ± 0.41	11.56 ± 0.88	VII	7	−17.72 ± 0.31	12.95 ± 0.70	VI	12
Notolepis rissoi	132–193	P									−19.92 ± 1.41	7.94 ± 0.72	IV	5
Notoscopelus elongatus	39–95	P					−21.05 ± 0.68	9.05 ± 1.06	IV	5	−20.42 ± 1.16	8.92 ± 0.56	IV	9
Phycis blennoides	114–463	HB					−18.84 ± 0.52	11.12 ± 1.23	VII	12	−18.24 ± 0.67	11.57 ± 0.62	VI	21
Stomias boa	76–125	P	−17.97	9.11	VI	1	−19.17	9.23	VI	1	−19.51 ± 0.52	8.53 ± 0.32	IV	3
Symbolophorus veranyi	45–84	P					−20.02 ± 1.15	8.11 ± 0.98	II	7				
Trachurus mediterraneus	30–40	P									−19.11 ± 0.05	8.01 ± 0.54	III	2
Trachurus trachurus	37	P	−19.75	6.52	II	1								
Vinciguerria attenuata	34–37	P					−19.45 ± 0.32	8.59 ± 0.96	VI	6				

[a] number of isotopic values for a pool of individuals.

3.3 Results

Food web structure

The 89 species analyzed encompassed (excluding zooplankton) 33 teleosts, 2 elasmobranchs, 19 cephalopods, 23 decapod crustaceans, and many other invertebrates (Table 3.1). In the SB, δ^{15}N values ranged from 3.47‰ for *Pyrosoma atlanticum* to 10.96‰ for *Lophius budegassa*. δ^{15}N values in the US spanned from 3.92‰ for *C. peroni* to 11.57‰ for N*ezumia aequalis*. In the MS, δ^{15}N values ranged from 4.01‰ for *P. atlanticum* to 12.96‰ for *N. aequalis*. The overall range of δ^{15}N within the food webs analyzed increased with depth (Δ^{15}N $_{SB}$ = 7.5‰, Δ^{15}N $_{US}$ =7.7‰, Δ^{15}N $_{MS}$ = 9.0‰). The majority (~70%) of the species collected fell between the 3rd and 4th trophic level at both the pelagic and the hyperbenthic compartments (Fig. 3.1). In the SB, δ^{13}C values ranged from −21.51‰ for *P. atlanticum* to −17.61‰ for *Pagurus alatus*. δ^{13}C values in the US spanned from −21.43‰ for *Salpa maxima* to −17.87‰ for *Pteroctopus tetracirrhus*. In the MS, δ^{13}C values ranged from −21.75‰ for *P. atlanticum* to −16.62‰ for *Philocheras echinulatus*. The markedly enriched δ^{13}C values of the brachyopoda *G. vitreus* were excluded.

Figure 3.1. Trophic levels (TrL) (mean ± SD) for hyperbenthic (circles) and pelagic (squares) species sampled. The species are arranged in ascending order of their values.
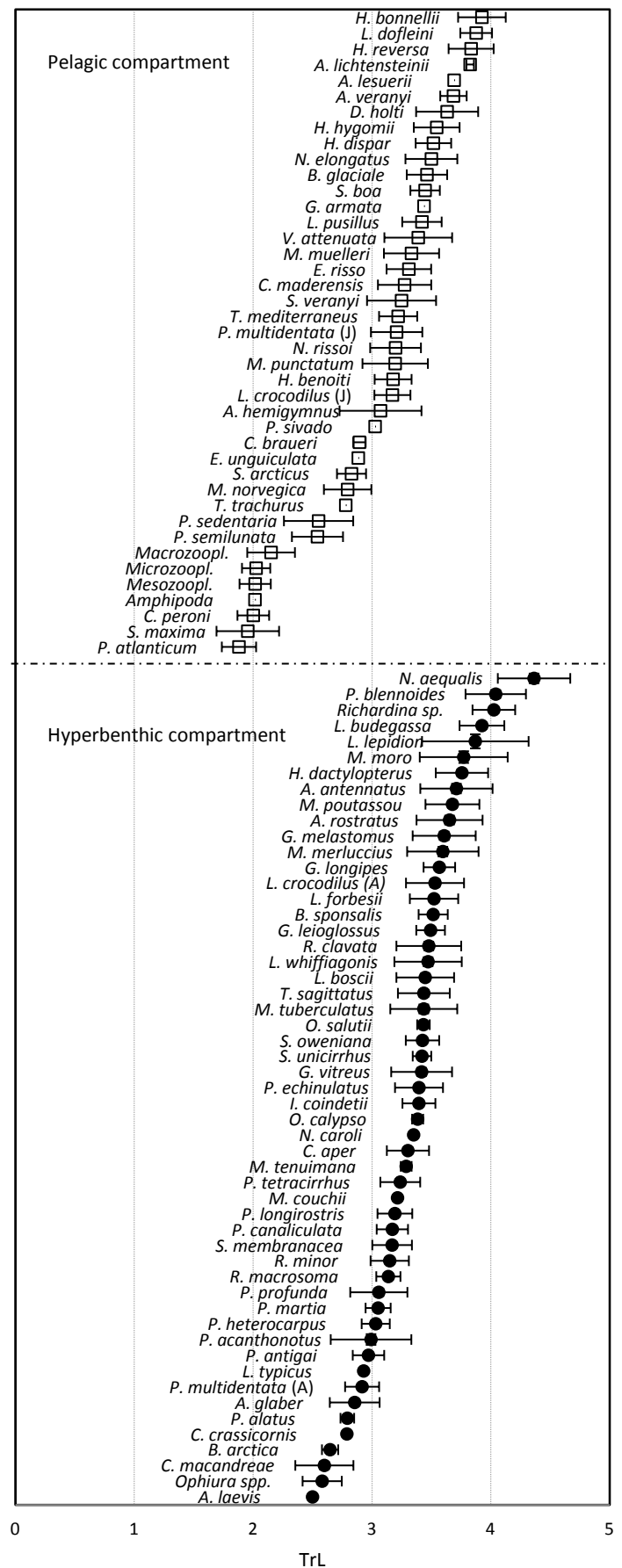
The analyses of POM for both the δ^{15}N (mean±SD; Sóller: δ^{15}N = 2.36‰±1.12 and Cabrera: δ^{15}N = 2.41‰±0.87) and δ^{13}C signatures (mean±SD; Sóller: δ^{13}C = –22.54‰±0.72 and Cabrera: δ^{13}C = –22.37‰±0.58), showed no significant differences between areas (p > 0.05).

Cluster analyses for each bathymetric stratum (SB, US and MS) revealed separation of species into 6–7 trophic groups in each of them (Fig. 3.2). These groups were labelled with roman numerals, from I to VII, in ascending order through the food web (see Table 3.1 for species included in each trophic group at each stratum).

With regard to the SB assemblage, group I comprised water column filter feeders with lowest mean δ^{15}N (3.80‰) and mean δ^{13}C (–20.66‰) values (e.g. *P. atlanticum* and *C. peroni*). Group II included typically zooplankton feeders (more closely dependent on mesoplankton) with mean δ^{15}N and δ^{13}C values of 6.56 and –19.45‰, respectively. Group III comprised benthic decapod crustaceans (*Alpheus glaber* and *P. alatus*) with most depleted mean δ^{13}C (–17.86‰) and mean δ^{15}N of 7.21‰, while group IV included hyperbenthic species (e.g. *Plesionika* spp.) characterized by mean δ^{15}N of 8.11‰ and mean δ^{13}C of –18.90‰. Furthermore, group V consisted of a mix of hyperbenthic (e.g. *Micromesistius poutassou* and *Merluccius merluccius*) and mesopelagic species (e.g. *Stomias boa* and *Maurolicus muelleri*) with mean δ^{15}N of 9.41‰ and mean δ^{13}C of –19.23‰. Group VI comprised hyperbenthic species such as *Raja clavata* with mean δ^{15}N of 9.20‰ and mean δ^{13}C of –17.96‰. Finally, the benthic fish *L. budegassa* was positioned in group VII, with the most enriched mean δ^{15}N (10.96‰) and mean δ^{13}C of –18.40‰.

A total of 20 species (excluding zooplankton) co-occurred in the SB of both the locations, and the mean δ^{13}C and δ^{15}N values were greater at Cabrera when compared with those at Sóller (paired-t=2.174, *p*<0.05 and paired-t=2.382, *p*<0.05, respectively) (Table 3.2).

In the US assemblage, the primary consumers (*C. peroni* and *S. maxima*) feeding on phytoplankton and other particles in the water column belonged to group I (mean δ^{15}N = 4.01‰, mean δ^{13}C = –20.99‰). Group II mainly comprised mesopelagic species (e.g. *Hygophum benoiti* and *Lampanyctus pusillus*) feeding on zooplankton with mean δ^{13}C value of –19.76‰ and mean δ^{15}N value of 7.61‰. Group III included the hyperbenthic affinity consumers (e.g. *Plesionika* spp. and *Processa canaliculata*), similar to that found at the SB, characterized by mean δ^{13}C of –18.56‰ and mean δ^{15}N of 7.93‰. Two new pelagic trophic δ^{15}N and δ^{13}C groups appeared: group IV, which clustered myctophids such as *Notoscopelus elongatus* and *Ceratoscopelus maderensis* with mean δ^{13}C and δ^{15}N values of –21.04‰ and 8.78 respectively, and group V, which comprised both pelagic squids of the genus *Histioteuthis* with higher δ^{13}C (–20.27‰) and δ^{15}N values (9.93‰). Group VI comprised a mixture of hyperbenthic organisms (e.g. *Mora moro* and *M. poutassou*) and, to a lesser extent, pelagic species (e.g. *S. boa*), characterized by mean δ^{13}C of –18.84‰ and mean δ^{15}N of 9.33‰. Finally, group VII included demersal fishes (e.g. *Neumia aequalis*, *Phycis blennoides*) and the squid *L. forbesii*, with mean δ^{13}C value of –18.65‰ and the most enriched mean δ^{15}N (11.13‰).

A total of 14 species co-occurred in the US of both the locations, whereas the mean $\delta^{13}C$ value did not differ between locations (paired-t=0.711, $p>0.05$), the $\delta^{15}N$ value was higher at Cabrera than at Sóller (paired-t=2.198, $p<0.05$) (Table 3.2).

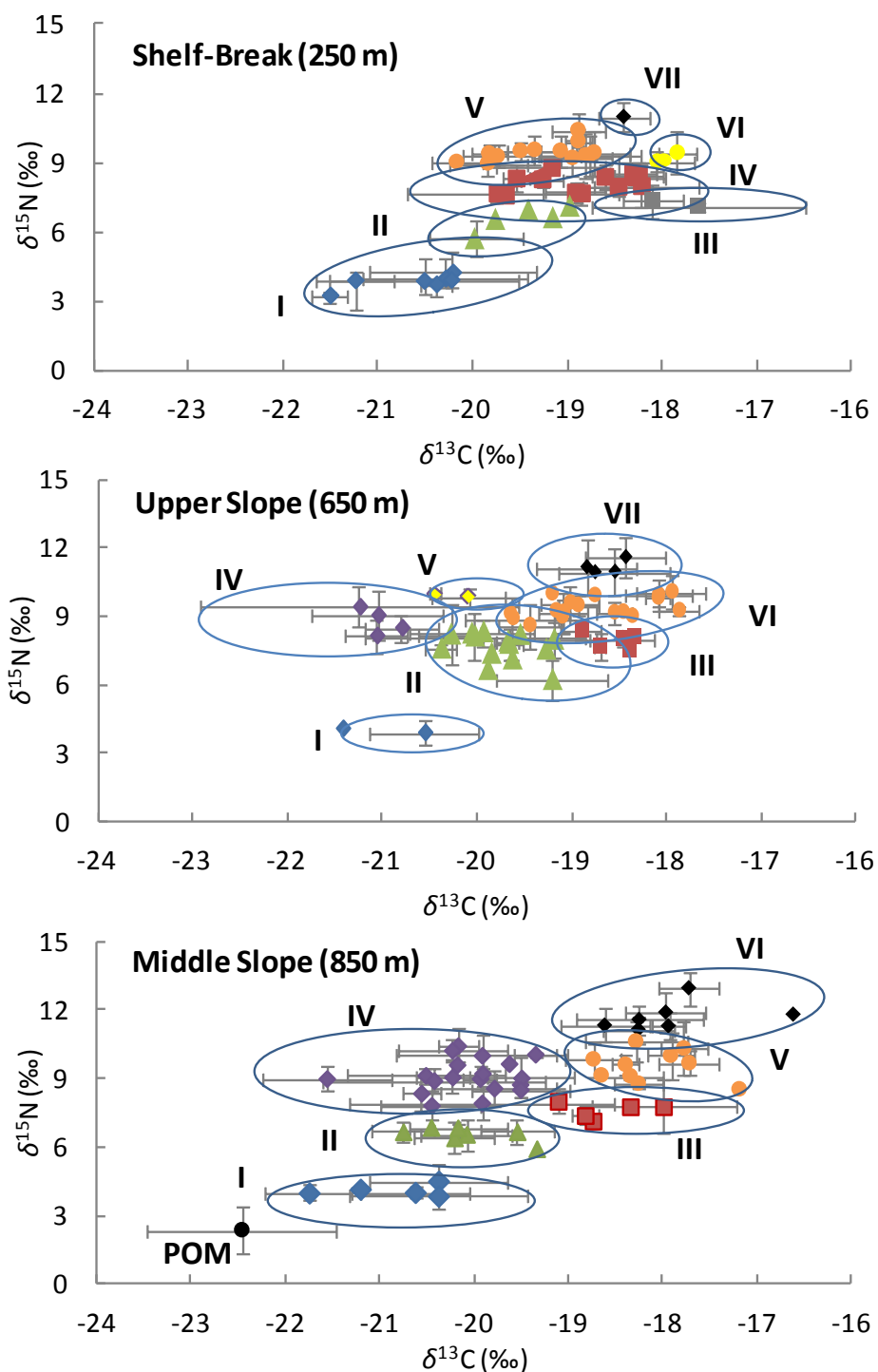Figure 3.2. Scatterplots of $\delta^{15}N$ and $\delta^{13}C$ values (mean ±SD) of each species per stratum. The symbols show the trophic groups (I–VII) from cluster analysis (see text). POM: Particulate organic matter.

Similarly to the US results, group I from the MS included pelagic basal species (e.g. *P. atlanticum* and *C. peroni*) (mean δ^{13}C = −20.87‰ and mean δ^{15}N = 4.12‰) and group II included mesopelagic species carnivorous on small zooplankton (e.g. *C. braueri* and *Sergestes arcticus*) with mean δ^{13}C and δ^{15}N values of −20.08 and 6.58‰, respectively. As for the SB and the US, group III clustered small natantian decapods among the others (e.g. *Plesionika* spp. and *Pasiphaea multidentata*) with mean δ^{13}C of −18.48‰ and mean δ^{15}N of 7.53‰, and group IV comprised many myctophids species and, to a lesser extent, pelagic cephalopods (e.g. *Galiteuthis armata* and *H. reversa*) (mean δ^{15}N = 9.06‰ and mean δ^{13}C = −19.89‰) relying on pelagic preys. Group V included mainly hyperbenthic species (e.g. *A. antennatus, G. melastomus*) with mean δ^{15}N value of 9.69‰ and high δ^{13}C values (mean δ^{13}C = −18.23‰) strongly affiliated with the benthic food chain. Finally, group VI, at the upper trophic level, was occupied by hyperbenthic fish species (e.g. *M. merluccius* and *M. moro*) and two crustacean decapods (*Richardina* spp. and *P. echinulatus*) with mean δ^{13}C value of −17.91‰ and mean δ^{15}N value of 11.72‰.

The 18 co-occurring species did not show differences in the mean δ^{13}C values between locations (paired-t=1.257, p=0.227), whereas the mean δ^{15}N values were higher at Cabrera, when compared with those at Sóller (paired-t=3.747, p<0.01) (Table 3.2). The average stable isotopic ratios differed significantly among the trophic groups identified by cluster analysis at all the strata (PERMANOVA, SB: Pseudo-F=46.263, US: Pseudo-F=48.318, MS: Pseudo-F=66.504, all p<0.001). The pair-wise comparisons showed significant differences among all the trophic groups (Table 3.3).

Food sources

There were significant relationships between δ^{15}N and δ^{13}C values across all the species at all the food webs, except for Cabrera in the US (Cabrera: R=0.09, p>0.05). The correlations were greater and highly significant in the MS (Cabrera: R=0.58, Sóller: R=0.50, both p<0.001), when compared with those in the SB (Cabrera: R=0.40, Sóller: R=0.36, both p<0.05) and the US (Sóller: R=0.43, p<0.01).

Benthopelagic coupling

Both pelagic compartment (PC) and hyperbenthic compartment (HC) covered a similar and narrow δ^{13}C range: from −21.58‰ for *P. atlanticum* to −19.11‰ for *Trachurus mediterraneus* (Δ ^{13}C= 2.47‰) in the PC, and from −19.92‰ for *Alepocephalus rostratus* to −17.19‰ for *M. couchii* (Δ ^{13}C = 2.73‰) in the HC. Significant differences in δ^{13}C were found between locations (Pseudo-F=4.81, p<0.05) and for depth within location per vertical guild (Pseudo-F=3.61, p<0.01). On an average, Cabrera showed enriched ^{13}C values, when compared with Sóller. Paired t-tests showed higher δ^{13}C values in the HC than those in the PC at all bathymetric strata of both the locations (p<0.001 for all pair wise comparisons). Besides, significant differences in the δ^{13}C values among the strata were only found

at Cabrera. On an average, over the PC, depleted ^{13}C values were observed in the MS when compared with those in the US ($p<0.05$), and over the HC, enriched ^{13}C values were found in the MS when compared with those in the SB ($p<0.01$).

The PC δ^{15}N values range from 3.67‰ for *P. atlanticum* to 10.42‰ for *Histiotheutis bonnellii (Δ ^{15}N = 6.8‰)*. The values in the HC spanned from 6.11‰ for *Anapagurus laevis* to 12.45‰ for *N. aequalis (Δ ^{15}N = 6.3‰)*. PERMANOVA test showed that δ^{15}N was influenced by depth within location (Pseudo-F= 5.5971, $p<0.001$) and vertical guild (Pseudo-F= 64.467, $p<0.001$). At both the locations, the δ^{15}N values in the SB were significantly lower than those in the MS ($p<0.01$) and US (CA: $p<0.05$; SO: $p<0.001$). Regarding the vertical guilds, HC had enriched ^{15}N values than PC.
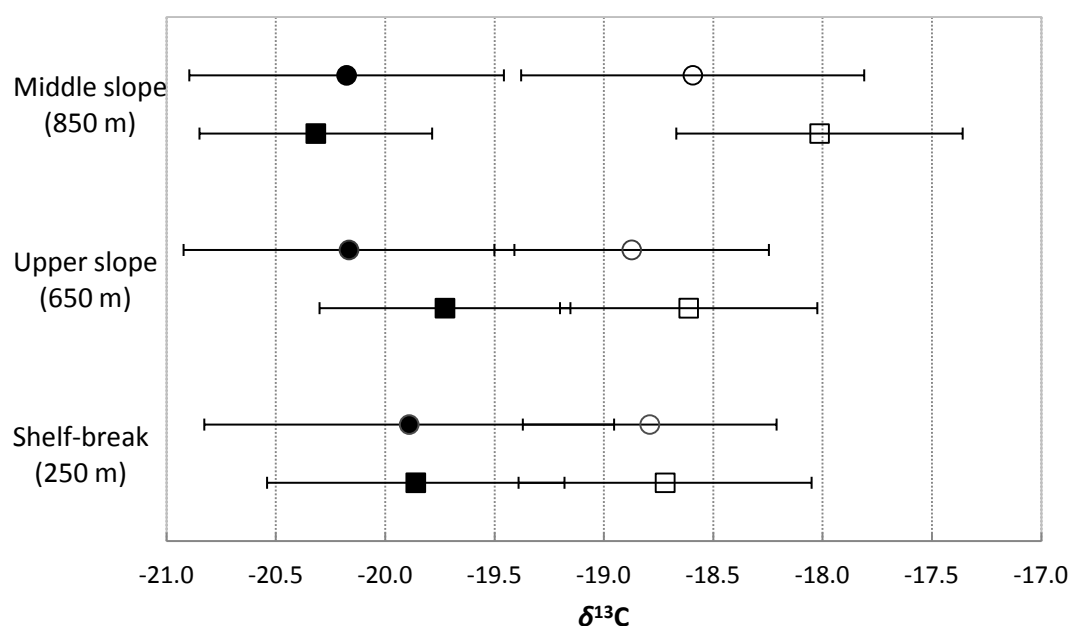
Figure 3.3. δ^{13}C values (mean ± SD) per vertical guild (filled symbols: Pelagic compartment; empty symbols: Hyperbenthic compartment) at each depth (250, 650, and 850 m) within location (squares represent Cabrera; circles represent Sóller).

3.4 Discussion

This study explored the trophic structure at different bathymetric strata (SB, US, and MS) in two oligotrophic locations from the western Mediterranean with contrasting oceanographic conditions. To cope with such issues, most of the available studies had analyzed faunistic associations and/or feeding strategies (Bergmann et al., 2009; Reid et al., 2012; Sahling et al., 2003). However, in the present study, we analyzed isotopic signatures of a broad range of taxa (89 species), covering the most important species in terms of biomass and abundance from different depth-related ecological compartments (pelagic *vs* hyperbenthic).

The comparison of isotopic signatures of species with very different generation times is complicated by their corresponding contrasting isotope turnovers: from days in plankton to months or years in large predators (O'Reilly et al., 2002; Post, 2002). Pooling data across seasons, as carried out in the present study, reduces such temporal variations. Considering all other sources of error, the variability of trophic discrimination has a minor effect on the computation of trophic positions (Post, 2002).

Food web structure

The most enriched ^{15}N values for hyperbenthic and pelagic species was exhibited by the macrourid *N. aequalis* and the squid *H. bonnellii*, respectively. We assumed an increase in δ^{15}N of 3.4‰ per trophic level and we considered as reference material a deposit feeder (*C. macandreae*) and a filter feeder (*C. peroni*) for the benthic and the pelagic food web respectively. This way, both the pelagic and hyperbenthic food webs spanned approximately four trophic levels. However, there was a lack of some high trophic level predators, including large pelagic fishes such as tuna, many elasmobranchs, or marine mammals that inhabit the study area, but are numerically uncommon or poorly selected using our sampling gears. Thus, it is possible that the overall food webs in these regions extend to approximately five trophic levels. Indeed, it has been reported that aquatic food webs rarely exceed four or five trophic levels (Hall and Raffaelli, 1993).

The existing isotope ecology analyses of the study area (Balearic Islands) have focused on specific ecosystem components, including zooplankton and suprabenthic species or benthopelagic megafauna, and thus were restricted to the lower two or three trophic levels (Fanelli et al., 2009; Madurell et al., 2008). However, Polunin et al. (2001) reported food chain lengths comparable to ours analyzing zooplankton, fishes and decapod crustaceans; in that work, the highest trophic levels were observed in the shark *Centroscymnus coelolepis* and, as in our case, in *N. aequalis*. The high δ^{15}N values in this small macrourid are thought to be related to the contribution of carnivorous polychaetes in its diet (Fanelli et al., 2013b; Papiol et al., 2013; Polunin et al., 2001).

Table 3.2. δ^{13}C and δ^{15}N values (mean ± SD where appropriate) for species co-occuring at both the locations (Cabrera and Sóller) at each bathymetric stratum (shelf-break, upper and middle slope). Size of specimens in mm (±SD), n: number of individual analysed. A: adults, J: juveniles.

Species	Cabrera				Sóller			
	Size	n	δ^{13}C (‰)	δ^{15}N (‰)	Size	n	δ^{13}C (‰)	δ^{15}N (‰)
Shelf-break								
Raja clavata	433.6 ± 258.4	8	−17.87 ± 0.22	9.46 ± 1.23	361.0 ± 141.3	9	−17.81 ± 0.18	9.41 ± 0.63
Capros aper	91.8 ± 5.1	6	−19.86 ± 0.22	8.92 ± 0.53	91.2 ± 15.7	9	−19.83 ± 0.53	8.96 ± 0.62
Helicolenus dactylopterus	171.5 ± 20.6	6	−18.79 ± 0.26	10.81 ± 0.59	171.8 ± 30.4	6	−18.98 ± 0.27	9.96 ± 0.66
Lepidorhombus boscii	190.0 ± 83.4	9	−18.61 ± 0.50	9.48 ± 0.71	169.7 ± 56.1	12	−18.87 ± 0.55	9.16 ± 0.93
Lepidorhombus whiffiagonis	386.7 ± 21.4	3	−18.41 ± 0.18	9.67 ± 1.29	226.8 ± 69.6	4	−18.95 ± 0.33	9.22 ± 0.80
Lophius budegassa	241.1 ± 96.0	18	−18.48 ± 0.27	10.92 ± 0.66	313.1 ± 78.5	12	−18.28 ± 0.22	11.01 ± 0.63
Merluccius merluccius	195.5 ± 73.6	19	−19.05 ± 0.21	9.53 ± 0.80	223.1 ± 74.0	18	−19.09 ± 0.24	9.45 ± 0.58
Micromesistius poutassou	174.0 ± 1.4	3	−18.79 ± 0.10	9.96 ± 0.69	182.0 ± 8.9	3	−18.98 ± 0.11	9.86 ± 0.38
Loligo forbesii	185.6 ± 23.2	9	−19.13 ± 0.21	9.80 ± 0.33	228.6 ± 63.2	16	−19.46 ± 0.48	9.38 ± 0.74
Pteroctopus tetracirrhus	95.0 ± 7.1	2	−18.28 ± 0.21	8.75 ± 0.17	90.0 ± 5.0	3	−18.29 ± 0.42	8.11 ± 0.37
Rossia macrosoma	34.7 ± 8.2	17	−19.24 ± 0.22	8.30 ± 0.36	29.5 ± 3.5	2	−19.48 ± 0.12	8.12 ± 0.07
Sepietta oweniana	19.0 ± 7.9	3	−19.29 ± 0.22	9.66 ± 0.72	14.9 ± 2.3	7	−19.93 ± 0.18	9.08 ± 0.23
Todarodes sagittatus	271.0	1	−19.78	9.08	207.5 ± 0.7	2	−18.87 ± 0.32	8.61 ± 0.06
Alpheus glaber		3	−17.88 ± 0.21	7.90 ± 0.32		3	−18.32 ± 0.26	6.74 ± 0.40
Pandalina profunda		3	−18.16 ± 0.74	8.14 ± 1.09		1	−18.39	7.51
Parapenaeus longirostris	17.1 ± 1.3	9	−18.25 ± 0.24	8.60 ± 0.49	19.5 ± 6.0	6	−18.26 ± 0.30	8.26 ± 0.47
Philocheras echinulatus	30.8 ± 1.8	13	−17.98 ± 0.16	9.22 ± 0.21	32.7 ± 1.5	12	−18.15 ± 0.54	8.86 ± 0.54
Plesionika heterocarpus	12.0 ± 1.0	3	−18.13 ± 0.06	8.33 ± 0.28	10.7 ± 0.5	7	−18.61 ± 0.13	7.80 ± 0.33
Macropipus tuberculatus		9	−18.66 ± 0.45	9.35 ± 0.84		3	−19.26 ± 0.03	9.13 ± 1.47
Pagurus alatus		1	−17.26	7.26		2	−17.79 ± 1.53	7.03 ± 0.20
Upper slope								
Galeus melastomus	367.2 ± 165.9	13	−18.10 ± 0.34	10.01 ± 0.65	378.7 ± 196.6	9	−18.08 ± 0.38	9.82 ± 0.76
Lampanyctus crocodilus (A)	186.0 ± 7.8	3	−18.85 ± 0.19	10.08 ± 0.14	142.5 ± 13.2	4	−19.27 ± 0.10	8.49 ± 0.24
Merluccius merluccius	282.3 ± 75.7	3	−19.00 ± 0.06	10.37 ± 0.56	462.6 ± 69.6	5	−18.28 ± 0.59	11.27 ± 1.15
Nezumia aequalis	30.3 ± 4.5	3	−18.42 ± 0.11	11.36 ± 0.30	42.8 ± 10.0	4	−18.45 ± 0.58	11.73 ± 1.20
Notoscopelus elongatus	79.5 ± 4.9	2	−20.31 ± 0.07	10.19 ± 0.22	89.3 ± 5.5	3	−21.55 ± 0.14	8.30 ± 0.34
Phycis blennoides	258.5 ± 111.6	6	−18.62 ± 0.21	11.45 ± 1.27	234.2 ± 116.2	6	−19.08 ± 0.67	10.51 ± 0.81
Symbolophorus veranyi	130.0	1	−20.30	10.10	61.2 ± 17.5	6	−19.98 ± 1.26	7.79 ± 0.50
Histioteuthis reversa	66.0	1	−20.15	9.27	50.3 ± 24.7	12	−20.08 ± 0.40	9.89 ± 0.40
Illex coindettii	189.3 ± 18.2	3	−18.74 ± 0.13	9.22 ± 0.16	187.6 ± 15.3	5	−18.45 ± 0.08	9.12 ± 0.65
Todarodes sagittatus	320.0 ± 69.3	2	−19.46 ± 0.25	9.31 ± 0.36	285.0 ± 66.6	6	−18.88 ± 0.13	9.68 ± 0.83
Cymbulia peroni		1	−19.93	4.43		2	−20.88 ± 0.21	3.66 ± 0.44
Aristeus antennatus	38.5 ± 7.5	6	−17.84 ± 0.36	10.01 ± 0.80	44.0 ± 1.7	3	−18.16 ± 0.32	10.17 ± 0.74
Pasiphaea multidentata (J)	17.3 ± 0.6	3	−19.02 ± 0.38	8.39 ± 0.66	13.5 ± 6.4	2	−19.45 ± 0.56	7.35 ± 0.01
Plesionika martia	15.7 ± 0.6	3	−18.43 ± 0.42	8.30 ± 0.30	15.8 ± 1.0	4	−18.51 ± 0.31	7.80 ± 0.29
Middle slope								
Galeus melastomus	328.3 ± 177.2	12	−17.88 ± 0.23	10.01 ± 1.14	409.9 ± 181.1	9	−17.96 ± 0.26	10.02 ± 0.88
Alepocephalus rostratus	322.7 ± 28.8	6	−19.34 ± 0.50	10.50 ± 0.89	237.8 ± 9.3	4	−20.79 ± 0.62	9.33 ± 0.53
Argyropelecus hemigymnus	13.0	1	−19.63	6.04	27.0 ± 1.7	3	−20.40 ± 0.22	6.55 ± 0.81
Benthosema glaciale	37.7 ± 2.3	3	−21.10 ± 0.64	9.30 ± 0.54	41.0 ± 1.0	3	−22.03 ± 0.36	8.73 ± 0.43
Ceratoscopelus maderensis	49.0 ± 3.7	6	−19.86 ± 0.80	8.90 ± 0.88	48.8 ± 4.3	4	−19.69 ± 0.50	8.14 ± 0.29
Lampanyctus crocodilus (A)	187.7 ± 11.0	6	−18.71 ± 0.32	10.05 ± 0.86	183.4 ± 16.3	9	−18.75 ± 0.30	9.73 ± 0.68
Lampanyctus crocodilus (J)	61.5 ± 9.2	3	−20.38 ± 0.79	7.83 ± 0.89	62.7 ± 6.0	3	−20.53 ± 0.28	7.86 ± 0.36
Lepidion lepidion	197.1 ± 51.0	8	−18.31 ± 0.28	11.85 ± 0.61	185.1 ± 24.5	9	−18.89 ± 0.43	10.86 ± 0.45
Lobianchia dofleini	35.0 ± 1.7	3	−20.70 ± 0.38	10.09 ± 0.66	32.0 ± 0	3	−19.77 ± 0.23	10.42 ± 0.06
Mora moro	377.3 ± 42.6	6	−18.09 ± 0.25	11.49 ± 0.38	340.0 ± 29.3	7	−18.40 ± 0.42	10.85 ± 0.55
Nezumia aequalis	48.5 ± 4.2	6	−17.52 ± 0.18	13.40 ± 0.56	46.8 ± 4.8	6	−17.92 ± 0.30	12.52 ± 0.66
Notoscopelus elongatus	56.3 ± 8.3	4	−21.11 ± 1.51	8.76 ± 0.32	52.4 ± 14.3	5	−19.88 ± 0.42	9.05 ± 0.71
Phycis blennoides	270.2 ± 99.3	10	−18.30 ± 0.30	11.97 ± 0.46	281.4 ± 105.7	11	−18.21 ± 0.91	11.25 ± 0.56
Histioteuthis reversa	74.8 ± 39.4	5	−20.19 ± 0.19	10.48 ± 0.86	59.8 ± 17.2	5	−20.16 ± 0.27	10.42 ± 0.70
Aristeus antennatus	44.3 ± 2.0	6	−17.75 ± 0.20	10.73 ± 1.39	39.2 ± 15.2	9	−17.79 ± 0.28	10.06 ± 1.03
Plesionika acanthonotus	11.3 ± 1.5	3	−17.78 ± 0.23	8.24 ± 0.77	11.3 ± 4.0	6	−18.11 ± 0.94	7.55 ± 1.31
Geryon longipes	53.0 ± 2.0	3	−17.46 ± 0.22	10.08 ± 0.27	53.3 ± 1.5	3	−17.98 ± 0.08	9.29 ± 0.44
Meganyctiphanes norvegica	7.2 ± 0.5	7	−19.90 ± 0.48	6.77 ± 0.73	7.3 ± 0.6	3	−20.50 ± 0.15	6.12 ± 0.21

In the present study, a wider range of $\delta^{15}N$ than $\delta^{13}C$ values were found at both the pelagic and the hyperbenthic compartments, which suggests the partitioning of a similar source of primary production among a variety of trophic levels. This pattern might be attributed to the oligotrophic conditions, which are thought to stimulate omnivorism to avoid competition for food (Pomeroy, 2001). The wide ranges of isotopic values were consistent with a high diversity of trophic strategies among species to maintain resource partitioning, although most of the species collected occupied intermediate trophic levels. The maintenance of these intermediate trophic levels suggested expansion of trophic niches (Gage and Tyler, 1991; Le Loc'h et al., 2008; Sokolova, 1997), or feeding across multiple trophic levels (Miller et al., 2010). Indeed, the trophic structure of the mesopelagic fishes in the study area pointed to a trophic segregation related to different energetic requirements. Non-surface-migratory species (e.g. *C. braueri*, *A. hemigymnus*) displayed lower $\delta^{15}N$ values than migrant and more energetically demanding species (e.g. *L. dofleini*, *H. hygomii*) feeding at higher trophic positions (Pakhomov, et al., 1996; Valls et al.,2014b). A wide spectrum of feeding guilds and food resource partitioning has been suggested among suprabenthos, bottom zooplankton feeders, and megafauna species in previous studies carried out in the western Mediterranean (Fanelli et al., 2009, 2013; Madurell et al., 2008; Polunin et al., 2001).

The isotope data can also be used to explore the relative position of species in the δ-space to follow changes of the trophic structure over spatial or temporal gradients (Layman et al., 2007; Post et al., 2000; Vander Zanden et al., 1997). The $\delta^{15}N–\delta^{13}C$ scatter plots showed that the volume of the occupied δ-space increased with increasing depth. On one hand, the presumable segregation between trophic groups at greater depths could be attributed to a greater partitioning of food sources due to increased food scarcity (Fanelli et al., 2013b; Pomeroy 2001). On the other hand, mesopelagic fishes diversity was found to increase from SB to MS in the study area (Olivar et al., 2012). The presence of mesopelagic fishes (e.g. stomiiforms and myctophiforms) and cephalopods (e.g. *Histioteuthis* spp.) in the slope assemblages, which formed new clusters in the food webs with comparatively depleted $^{13}\delta C$ values, increased the trophic diversity. Mesopelagic fishes are thought to constitute an important component of the food web, linking upper trophic levels with the pelagic chain (Cherel et al., 2008). The increasing $\delta^{15}N$ values below the SB could possibly be due to the introduction of these new pelagic resources on the slope grounds. Benthic fishes that rely on suprabenthos for feeding can change its trophic guild in order to take advantage of pelagic prey when they are available (Papiol et al., 2013). Nevertheless, it was noted that the apex predators (mainly demersal fishes) at those food webs became increasingly segregated from pelagic clusters, but remained closely affiliated with benthic groups. Thus, the high $\delta^{15}N$ values of these demersal fishes may be related to a great dominance of benthic prey in their diets.

Food sources

The $\delta^{13}C$ data and $\delta^{13}C-\delta^{15}N$ relationship can provide useful insight into the potential energy sources sustaining a species assemblage and the relative importance of their uptake. According to previous studies, a strong linear $\delta^{13}C-\delta^{15}N$ relationship is expected if the sampled assemblage represents a food chain derived from a single carbon source (Polunin et al., 2001). The correlations become increasingly weak as the utilization of further $\delta^{13}C$ distinct food sources increases (Fanelli et al., 2011a) or owing to high isotopic variability of a single food source (Fanelli et al., 2013b).

The presence of significant linear $\delta^{13}C-\delta^{15}N$ relationships and moderately high correlation coefficients were suggestive of a limited range of basal $\delta^{13}C$. This was supported by the $\delta^{13}C$ values of the constituent species, which were consistent with the POM and/or plankton. In our study site, the analysis of POM collected on the MS confirms that the main source of organic matter for the nektobenthic communities is produced by phytoplankton photosynthetic processes (in form of Chl-a produced at surface waters and quickly degraded) (Pasqual et al. 2012). Indeed, the $\delta^{13}C$ of POM fell within the range of most common marine phytoplankton data (Darnaude et al., 2004; France, 1995). These data suggested that other sources such as macrophytes are negligible, as found in other isotope studies of bathyal (Fanelli et al., 2009, 2013; Polunin et al., 2001) and abyssal (Iken et al., 2001) environments.

Nevertheless, exceptions to this generalization were observed. Particularly, a weak correlation was found in the US of Cabrera, while the brachiopod *G. vitreus*, exhibited highly enriched $\delta^{13}C$ in all stations where it was present. With regard to the former case, it is possible that a potential C source remains still unidentified or that phytoplankton and/or POM are more temporally or spatially heterogeneous in the isotope signature. On the other hand, *G. vitreus* is an epifaunal suspension feeder (Emig, 1989) that might exploit bacteria directly or indirectly as a food source (Deming et al., 1981; Eardly et al., 2001).

Benthic-pelagic coupling

The $\delta^{13}C$ is commonly used to quantify energy flows in food webs (Post, 2002; Vander Zanden and Rasmussen, 2001), constituting a valuable tool to infer BPC in marine ecosystems based on the fact that deposit and detritus consumers exhibit higher $\delta^{13}C$ values when compared with predators feeding on pelagic food sources (Hobson et al., 1995). The comparison of spatial (depth and location) patterns carried out in the present study should be treated with some caution. The species composition varied between shelf and slope grounds, and the number of pelagic species was found to be unbalanced between locations in the SB and US.

Table 3.3. Results of PERMANOVA pair-wise tests comparing the isotopic ratios of the postulated trophic groups (I–VII) obtained from cluster analysis at each bathymetric stratum (SB, US, and MS). Significance is indicated by asterisks: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

	t		
Groups	SB	US	MS
I, II	3.11**	7.00**	7.19***
I, III	3.74*	11.66*	10.59**
I, IV	9.44***	9.21***	9.66***
I, V	12.36***	10.81**	16.19***
I, VI	6.55**	12.27**	17.41***
I, VII	4.69***	19.61***	
II, III	3.70**	3.47***	5.33**
II, IV	4.81**	4.24***	5.62***
II, V	8.58***	7.49***	10.98***
II, VI	8.07*	4.33**	13.87***
II, VII	6.50**	9.45***	
III, IV	2.80*	7.76**	3.63***
III, V	5.75**	4.25***	5.57**
III, VI	7.56**	7.80*	8.67**
III, VII	8.24*	11.95**	
IV, V	5.53***	3.01**	4.41***
IV, VI	3.59**	5.94***	7.16***
IV, VII	4.16***	9.69*	
V, VI	3.52**	2.99**	5.20***
V, VII	2.75***	6.77***	
VI, VII	6.70**	4.68***	

Spatial differences in mean $\delta^{13}C$ values were obtained in this study. On one hand, $\delta^{13}C$ values from the HC were always higher than at the PC at both the locations, suggesting isotopic variability in the carbon source. Planktonic macrofauna are mainly influenced by organic matter from surface production that is depleted in ^{13}C in comparison to degraded or remineralized sedimentary organic matter (Fanelli et al., 2011a, b). On the other hand, the small $\delta^{13}C$ differences between mean values from the PC and the HC (Fig. 3.3) were indicative of close coupling between both compartments. Dominant benthopelagic species from the slope ecosystem of the study area, such as *M. merluccius* or *G. melastomus,* are known to consume mesopelagic prey (Cartes et al., 2009; Valls et al., 2011), thus favoring the transfer of organic matter from the upper layers to the bottom.

The generally higher $\delta^{13}C$ values found in Cabrera when compared with those at Sóller, is consistent of higher degree of reworking of organic matter in the former. Differences in the environmental variables favoring the settling of fresh organic matter and re-suspension of material were noted in both the locations during the study period. López-Jurado et al. (2008) showed a higher occurrence of the oceanographic frontal systems and eddy formation in the BsB (Sóller) and

similarly, Amores and Monserrat (2014) found that the BsB is hydrodynamically more active all year round when compared with the AsB (Cabrera). These variables exerted significant influence on the quality and quantity of the fluxes of POM (Pasqual et al, 2014), altering isotopic composition (Macko and Estep, 1984; Lehmann et al., 2002). These processes lead to spatial variability of the available organic matter that supplies the benthic communities.

The results from the sediment trap showed that while Cabrera depends on recycled POM (isotopically enriched), Sóller relies on fresh POM (isotopically depleted) (Pasqual et al., 2012). Mean δ^{13}C differences along the bathymetric gradient were observed at Cabrera, where the pelagic and the hyperbenthic fauna from the MS exhibited depleted and enriched ^{13}C values respectively. According to the literature, benthic macrofauna exhibits higher δ^{13}C at the bathyal depth compared with zooplankton (Fanelli et al., 2013b; Polunin et al., 2001), probably because of the consumption of sediment microorganisms, meiofauna, and refractory organic matter (Gooday and Turley, 1990; Iken et al., 2001).

Together with δ^{13}C, the δ^{15}N values showed differences between the locations. When comparing co-occurring species, Cabrera always exhibited consistently higher δ^{15}N values than Sóller, presumably attributable to basal δ^{15}N as it was present through the food web. The δ^{15}N values obtained from the POM settled in the sediment traps did not explain these higher δ^{15}N values, although there was heterogeneity in the composition of POM (Pasqual et al. 2012). The consumers of the suprabenthos and zooplankton from Cabrera might have on average more trophic steps between them and the basal materials than those from Sóller, which consequently would cause the greater δ^{15}N values in Cabrera. The spatial differences detected in species δ^{15}N values, either between areas and depths, could also be a consequence of trophic plasticity, which allows individual species to adapt to environmental heterogeneity and site-specific variability in the availability of prey resources (Fanelli et al., 2013b; Jennings et al., 1997; Pinnegar and Polunin, 2000). Future stomach content studies, together with prey availability, could help to elucidate this point.

Chapter 4. Feeding ecology of demersal elasmobranchs from the shelf and slope off the Balearic Sea (western Mediterranean)

Chapter 4. Feeding ecology of demersal elasmobranchs from the shelf and slope off the Balearic Sea (western Mediterranean)

4.1 Introduction

Trophic relationships are fundamental to understand biological interactions in animal communities and how they respond to human exploitation. Elasmobranchs are top predators playing an important role in the marine ecosystems with a top-down control on the size and dynamics of many species (Wetherbee and Cortes, 2004). Globally, there is an increasing evidence that elasmobranchs are more affected than teleosts by fishing exploitation (Stevens *et al.*, 2000). Insular areas from the western and central Mediterranean sustain elasmobranch assemblages with higher diversity and abundance than adjacent mainland areas (Massuti and Moranta, 2003).

Despite the importance of feeding relationships to understand the food structure and dynamics of marine ecosystems, little is known about the feeding ecology of most elasmobranchs (Heithaus, 2004; Wetherbee and Cortes, 2004). This is especially true for batoids, which have received considerably less attention than sharks at a worldwide level (Ishihara, 1990, Motta, 2004). Research on trophic relationships among elasmobranch sympatric species is also scarce in the western Mediterranean, where selachian feeding habits have been studied exclusively considering both single species and groups of two or three species with similar ecological roles or habitat distribution (Macpherson, 1980, Carrason *et al.*, 1992, Saidi *et al.*, 2009). Furthermore, few studies have focussed on the feeding ecology of batoids individual species in the Mediterranean Sea (Jardas *et al.*, 2004, Romanelli *et al.*, 2007, Saglam and Bascinar, 2008). In the present paper we analyzed the diet, feeding habits and trophic interactions for three selachians (*Etmopterus spinax*, *Scyliorhinus canicula* and *Galeus melastomus*) and five batoids (*Raja polystigma, Myliobatis aquila, Leucoraja naevus, Raja miraletus* and *Raja clavata*) from waters off the Balearic Islands (western Mediterranean).

4.2 Materials and methods

The elasmobranch species were taken from the fishery-independent MEDITS bottom trawl surveys conducted around the Balearic Islands (Fig.2.3) during early summer from 2007 to 2009. A total of 141 hauls were performed during daylight hours between 45 and 755 m depth. The diet of each elasmobranch species was quantified using the following indices: 1) Frequency of occurrence (%F), 2) Numerical (%N) and volumetric (%V) composition, 3) Index of Relative Importance (IRI=%F(%N+%V) which was standardized following %IRI=(IRI/∑IRI)×100 (Cortes, 1997), 4) vacuity index (v), 5) diet breadth, which was calculated using the Levin's standardized index: $B_i = \dfrac{1}{n-1}\left(\dfrac{1}{\sum_j p_{ij}^2} - 1\right)$ where p_{ij} is the proportion of diet of predator i that is made up of prey j and n is the number of

prey categories. This index ranges from 0 to 1, low values indicating diets dominated by a few prey items (specialist predators) and higher values indicating generalist diets (Krebs, 1999); and 6) Species diversity both in prey number (H'$_n$) and prey volume (H'$_v$) calculated using the Shannon-Wiener index: H'= - $\sum$p$_{ij}$ ln(p$_{ij}$)

Medits survey design and sampling methodology are thoroughly explained in Chapter 2 (section 2.1.1.1 and 2.2.2, respectively).

To standardize data and to facilitate diet comparisons and analyses, the following twelve major prey categories were established: Polychaeta, Sipuncula, Gastropoda, Bivalvia, Cephalopoda, Euphausiacea, Amphipoda, Isopoda, Mysidacea, Crustacea Reptantia, Crustacea Natantia and Teleostea. Prey categories with frequency of occurrence lower than 3% and the unidentifiable remains were excluded from these analyses. Diet overlap between species was calculated using the Schoener index (Hurlbert, 1978), which ranges from 0 (no overlap) to 1 (complete overlap), with values >0.6 representing a significant overlap (Sala and Ballesteros, 1997, Wallace, 1981). In order to compare exclusively coexisting species, all indexes characterising the diet were analysed separately for the following four bathymetric strata described in the study area (Ordines et al., 2011): 1) continental shelf (CS: 45-180 m); 2) shelf-break (SB: 180-330 m); 3) upper slope (US: 330-495 m); and 4) middle slope (MS: 495-750 m). Diet overlap, niche breadth and diet diversities were calculated using Ecological Methodology software version 7.0 (Krebs, 1999).

Intra-specific trends in the diet related to predator size (total length in cm), and depth (m) of the most abundant species (*S. canicula*, *G. melastomus* and *R. clavata*) were studied applying partial Canonical Correspondence analysis (pCCA) using CANOCO (ter Braak and Smilauer, 1998). In the pCCA procedure, one explanatory variable (size or depth in this case) was set as a covariable, which allowed testing of the effect of the other one, after the variation explained by the covariable had been factored out. The significance of the explanatory variables was assessed by means of the Monte Carlo permutation-based test. Before the design of the model, the interactions between explanatory variables were analysed and found to be non-significant.

4.3 Results

General information for the eight species studied (e.g. sample size, size and weight range and depth range), the diet composition considering the twelve main major prey categories and the dietary indexes used (e.g. vacuity, diversity, niche breadth) are in Table 4.1. Total sample sizes were rather unbalanced, ranging from the 15 individuals of *R. polystigma* to about 900 of *S. canicula*. The number of different prey items ranged from 11 in *R. clavata* from the US to 79 in *S. canicula* from the CS. Considering depth strata, sample sizes ranged from 6 individuals of *R. clavata* caught on the US to 766 *S. canicula* individuals from the MS. With the exception of the batoids *M. aquila* and *R. clavata*, all other species had maximum sizes smaller than 65 cm length. Whereas *S. canicula* and *R. clavata* inhabited all depth strata,

the other batoid and shark species were exclusively caught on the shelf and slope, respectively.

The percentage of empty stomachs was clearly higher in selachian (18-46%) than in batoid species (0-11%). Globally, the highest dietary diversity was found on the CS, where *S. canicula* and *R. clavata* showed the highest values in terms of numbers (4.10) and volume (4.55) respectively (Table 4.1). The lowest diversity in numbers was found on the US (~2.1 for both *G. melastomus* and *S. canicula*) but the lowest in weight occurred on the CS (2.3 for *L. naevus*). The most generalist and specialist diets were found in *S. canicula* from the CS (B_i=0.67) and *G. melastomus* from the US (B_i=0.17). The rest of species had values ranging from 0.26 to 0.49 indicating moderate levels of feeding specialization.

The diet composition of each species is summarized in this paragraph taking into account both the main prey groups (Table 4.1) and the lowest identified taxonomic levels (Table 4.4). Beginning with the batoids, natantian crustaceans (68%IRI) followed far by teleosts (13%IRI), isopods (7%IRI) and mysids (7%IRI) were the most important preys of *R. polystigma*. The diet of *M. aquila* was based on anomuran crustaceans (63%IRI) such as *Dardanus arrosor* (18%IRI) and unidentified Paguridae species (11%IRI), molluscs (26%IRI) and polychaetes (8%IRI). The diet of *L. naevus* was almost exclusively based on natanian crustaceans (51%IRI), mainly *Solenocera membranacea* (15%IRI), and teleosts (41%IRI). Brachyuran (55%IRI) and natantian (35%IRI) crustaceans, followed far by teleosts (6%IRI), were the most important preys of *R. miraletus*. The skate *R. clavata* preyed on teleosts (31%IRI), natantian (27%IRI) and reptantian (29%IRI) crustaceans on the CS, but on teleosts (42%IRI) and natantians (32%IRI) on the SB; teleosts (46%IRI) and reptantians (41%IRI) were the main preys on the US, although these values must be taken with care owing to the small sample size (N=6).

Concerning the sharks, *S. canicula* preyed on reptantians (35%IRI), polychaetes (22%IRI) and teleosts (18%IRI) on the CS; euphausiids were the most important prey on both the SB (72%IRI) and US (78%IRI) followed by polychaetes (12%IRI) and teleosts (17%IRI) respectively. The catshark *G. melastomus* preyed almost exclusively on euphausiids on the US (93% IRI), but on a mixture of euphausiids (43%IRI), teleosts (26%IRI), cephalopods (16%IRI) and natantians (14%IRI) on the MS. Finally, the diet of *E. spinax* consisted primarily on cephalopods (64%IRI) and teleosts (25%IRI) followed far by natantian crustaceans (9%IRI).

Diet overlap were biologically significant (>0.6) in 9 out of the 19 coexisting species (Table 4.2). In other four cases, however, the overlap remained close to the cut-off value of significance (0.51-0.59). Diet overlap was significant among different pairs of skate species and between the shark *S. canicula* and *R. clavata* on the CS. Overlap also existed between the sharks *S. canicula* and *G. melastomus* on the US and between *E. spinax* and *G. melastomus* on the MS.

Table 4.1. General information and diet composition (standardized Index of Relative Importance, %IRI) of eight elasmobranch species caught at different bathymetric strata (CS: continental shelf; SB: shelf-break; US: upper slope; MS: middle slope) in the Balearic Sea (western Mediterranean). N: sample size; TL: total length; WR: weight range; DR: depth range; v: vacuity index; H'_n, H'_v: Shannon-Wiener diversity in number and volume respectively; B_i: Levin's niche breadth. Values into brackets are the number of taxa in the twelve major taxonomic groups of prey (see Annex 1). *R. pol., R. polystigma; M. Aqu., M. aquila; L. nae., L. naevus; R. mir., R. miraletus.*

	R. pol. CS	*M. aqu.* CS	*L. nae.* CS	*R. mir.* CS	*R. clavata* CS	*R. clavata* SB	*R. clavata* US	*S. canicula* CS	*S. canicula* SB	*S. canicula* US	*G. melastomus* US	*G. melastomus* MS	*E. spinax* MS
N	15	23	27	31	266	48	6	766	66	60	170	167	46
TL (cm)	28-45	51-116	22-52	24-43	18-88	18-77	64-91	11-53	14-46	15-49	14-35	10-63	11-47
WR (g)	91-546	514-4201	59-864	66-421	25-4005	27-2612	1445-4458	11-470	20-336	23-390	7-106	2-628	5-514
DR (m)	63-172	51-63	107-174	58-83	52-174	249-256	355-691	53-174	249-256	355-444	355-450	593-755	597-755
Polychaeta	1.17 (1)	8.07 (1)	0.29 (1)	0.05 (1)	0.08 (1)			22.10 (2)	12.53 (1)	0.5 (1)			0.26 (1)
Sipuncula		1.63 (2)						2.07 (2)					
Mollusca	0.5 (1)	26.3 (3)		0.08 (1)	0.59 (8)	0.15 (1)		2.38 (6)					
Bivalvia		6.28 (1)			<0.01 (1)			<0.01 (1)					
Cephalopoda	0.5 (1)	0.13 (1)		0.08 (1)	0.59 (6)	0.15 (1)		2.36 (4)	0.60 (1)	0.23 (1)	0.01 (1)	15.93 (8)	64.26 (4)
Euphausiacea					5.13 (2)	1.19 (1)		2.34 (2)	72.38 (2)	77.64 (2)	92.59 (2)	42.97 (2)	0.90 (3)
Amphipoda	1.10 (1)	0.09 (1)	2.78 (1)	3.87 (1)	0.04 (1)	0.24 (1)		0.81 (2)		0.52 (1)	0.32 (2)	0.15 (2)	
Isopoda	7.22 (1)		1.08 (1)	0.04 (1)	3.83 (1)	1.19 (1)		0.51 (1)	1.93 (1)	0.95 (1)		0.01 (1)	0.37 (1)
Mysidacea	6.63 (2)		2.66 (1)	0.36 (1)	2.55 (2)	10.49 (2)		7.40 (2)	0.21 (1)	0.21 (2)	<0.01 (1)	0.01 (2)	
Reptantia	3.35 (3)	81.94 (5)	1.32 (2)	54.88 (2)	29.39 (18)	12.96 (10)	40.61 (3)	34.71 (19)	0.15 (2)	0.53 (5)	0.02 (2)	0.08 (3)	0.08 (1)
Natantia	67.97 (4)	1.10 (2)	50.88 (5)	34.78 (5)	27.19 (8)	31.84 (5)	12.87 (3)	8.62 (11)	5.74 (4)	2.03 (7)	2.60 (7)	14.19 (10)	8.54 (4)
Pisces	13.51 (2)		40.80 (2)	5.88 (3)	31.08 (18)	41.82 (9)	45.87 (3)	17.85 (16)	5.46 (3)	16.68 (3)	4.42 (5)	25.61 (9)	25.25 (10)
Prey items	15	15	13	15	63	31	11	79	19	25	21	45	16
%v	0	4.3	11.1	0	9	10.4	0	20	33.3	18.3	27.7	19.8	45.7
H' (prey number)	3.22	2.18	3.05	2.83	3.24	3.95	3.27	4.10	2.16	2.09	2.07	3.50	3.44
H' (prey volume)	3.23	2.53	2.26	2.69	4.55	3.64	2.86	4.30	3.39	3.12	2.83	3.93	2.97
B_i	0.49	0.26	0.36	0.31	0.47	0.49	0.23	0.67	0.49	0.47	0.17	0.43	0.28

Table 4.2. Diet overlap (Schoener index, SI) for coexisting elasmobranch species on the continental shelf, shelf break, upper and middle slope from the western Mediterranean. Biologically significant diet overlaps (SI>0.6) are in bold.

Species	SI
Continental shelf	
R. clavata vs M. aquila	0.28
R. miraletus vs R. clavata	**0.73**
R. miraletus vs M. aquila	0.47
L. naevus vs M. aquila	0.16
L. naevus vs R. miraletus	**0.65**
L. naevus vs R. clavata	**0.74**
R. polystigma vs R. clavata	**0.76**
R. polystigma vs R. miraletus	**0.65**
R. polystigma vs L. naevus	**0.83**
S. canicula vs M. aquila	0.57
S. canicula vs R. clavata	**0.71**
S. canicula vs R. miraletus	0.58
S. canicula vs L. naevus	0.51
S. canicula vs R. ploystigma	0.59
Shelf break	
S. canicula vs R. clavata	0.42
Upper slope	
S. canicula vs G. melastomus	**0.73**
S. canicula vs R. clavata	0.37
G. melastomus vs R. clavata	0.31
Middle slope	
E. spinax vs G. melastomus	**0.67**

Considering intra-specific trends for the three most abundant species, size was found to significantly affect the diet of all of them, whereas depth affected exclusively the shark *S. canicula* (Table 4.3). There existed a gradient of increasing prey size with increasing predator size in all three species, ranging from small-sized preys such as amphipods, euphausiids or mysids to large-sized preys such as cephalopods or teleosts (Fig. 4.1). In the case of the bathymetric effect on *S. canicula*, the separation along the depth axis seems to be related to preferences for prey such as polychaetes and reptantian crustaceans in the shallow populations and euphausiids in the deeper populations.

Table 4.3. Results of the partial canonical correspondence analysis (pCCA) testing the effects of predator size (TL, cm) and depth (m) on the volumetric contribution of the diets of the sharks *Scyliorhinus canicula* and *Galeus melastomus* and the skate *Raja clavata*. The percentage of variance explained (V.E.), the F-ratio, and the p-value are shown (n.s.: non significant effect).

Predator species	Size			Depth		
	V.E.	F-ratio	p	V.E.	F-ratio	p
S. canicula	4.12	4.25	<0.01	7.42	7.65	<0.01
G. melastomus	9.63	8.37	<0.01	2.13	1.88	n.s.
R. clavata	11.54	15.64	<0.01	1.40	1.85	n.s.

4.4 Discussion

To our knowledge, this is the first study in the Mediterranean dealing with the trophic ecology (e.g. diet composition, diet diversity, niche breadth, diet overlap) of such a large number of elasmobranchs, since we have analysed data of the most abundant demersal species from our study area. In total, three sharks (*Etmopterus spinax*, *Scyliorhinus canicula* and *Galeus melastomus*), and five batoids (*Raja polystigma, Myliobatis aquila, Leucoraja naevus, Raja miraletus* and *Raja clavata*) species inhabiting the continental shelf (CS: coastal shelf and SB: shelf break) and the slope (US: upper slope and MS: middle slope) were analysed. Other studies in the Mediterranean analysed at most four elasmobranch species (Macpherson, 1981).

The vacuity index was clearly higher in sharks (18-46%) than in bathoids (0-10%), which could be related to the well-known diminution of trophic resources with increasing depth (Carrasson *et al*., 1992, Olaso *et al*., 2005). Fittingly, the shark *E. spinax,* which only inhabits the deepest stratum (MS), had near half of their stomachs empty. The fact that *E. spinax* feeds preferentially on cephalopods, which in general have high nutritional values and low non-edible remains (Boyle and Rodhouse, 2005) may also influence such a high vacuity index. With the only exception of *S. canicula* from the CS, which showed the most generalist diet (0.67), and *G. melastomus* from the US which displayed the most specialist behaviour (0.17), all other species had niche breadth values between 0.26 and 0.49 that might be associated with moderate levels of specialization. Such moderate levels could be related to the high species richness and biomasses of shelf benthic ecosystems from the Balearic Islands (Massuti and Reñones, 2005, Ordines and Massuti, 2009). Relatively lower niche breadths on the slope than on the shelf might also be related with the already mentioned diminution of trophic resources with depth. As pointed out by Carrasson *et al*. (1992) the decrease of the number of prey per stomach and the trophic diversity with depth are indicative of the increasing resource scarceness along the depth gradient in the western Mediterranean.

On the CS, diet overlap was biologically significant among all the batoid species, except *M. aquila*, and between the skate *R. clavata* and the shark *S. canicula.* The lack of significant dietary differences among comparably-sized shelf skates suggests that interspecific resource competition was not intense (Bizzarro *et al.*, 2007). High values of overlap do not imply necessarily competition, except when resources are in short supply (Macpherson 1979; Cartes, 1998). The coexistence between species with similar trophic habits and a narrow niche breadth might be possible by the abundance of food resources (Collwell and Futuyma, 1971). As aforementioned, this would be the case on the rich shelf bottoms from our study area. Otherwise, species that are spatially segregated are not driven to differentiate their diets and may easily converge in the use of resources overlapping areas (Ross, 1977). Elasmobranch species from the Balearic Islands showed different optimum depths (Ordines *et al.*, 2011), which could indicate a sort of fine-tuned bathymetric segregation in spite of coexisting on shelf and slope bottoms.
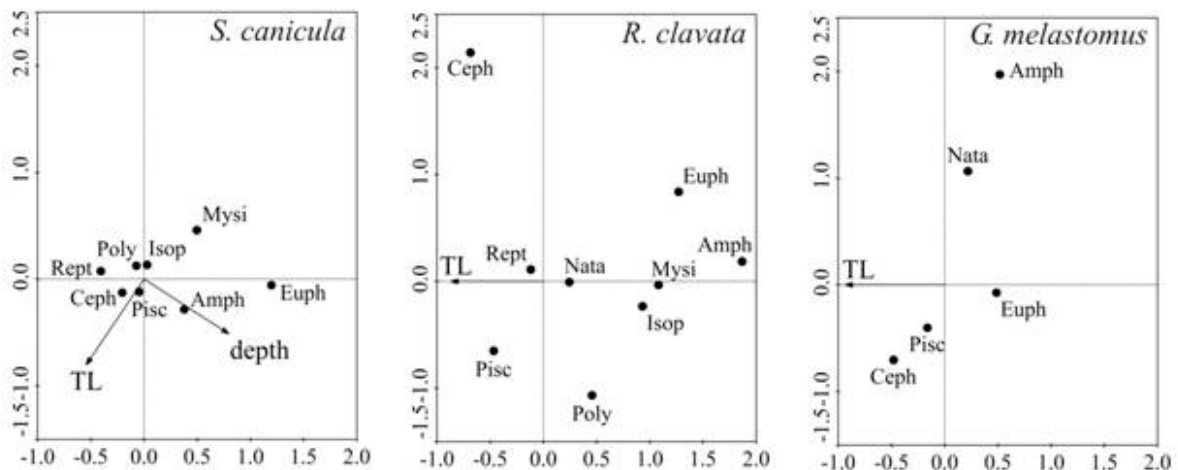
Figure 4.1. Partial canonical correspondence analysis (pCCA) biplots of the explanatory variables predator size (total length, cm) and depth of capture (in m) and nine different prey categories for the sharks *Scyliorhinus canicula* and *Galeus melastomus* and the skate *Raja clavata*. Euph: euphausiids; Amph: amphipods; Mysi: mysids; Isop: isopods; Poly: polychaetes; Nata: natantia; Rept: reptantia; Ceph: cephalopods; Pisc: pisces.

In accordance with previous studies carried out both in the Mediterranean (Romanelli *et al.*, 2007, Saglam and Bascinar, 2008) and the Atlantic (Ellis *et al.*, 1996, Gomes *et al.*, 1998, Farias *et al.*, 2006), the dominant prey taxa in all the batoids species, except *M. aquila*, were natantian crustaceans and teleosts. However, the diet of *M. aquila* was highly specialized on anomuran crustaceans and non-cephalopod molluscs (mainly bivalvians), two prey groups that were, with the exception of anomurans in *S. canicula*, barely present in the rest of species; it was also characterized by being the only species that did not prey on teleosts and

by the moderate importance of polychaetes. Such important differences would explain the lack of diet overlap between *M. aquila* and the rest of elasmobranch species. In general, other authors mentioned the same food preferences for *M. aquila* in the Mediterranean (Azouz and Capapé, 1971, Capapé and Quignard, 1974, Capapé, 1976, Jardas *et al.*, 2004) but differed in a such specialized diet based in anomuran crustaceans. The diet of *R. miraletus* differed slightly from the others skates, as it preyed mainly on a mixture of brachyuran crabs and natantian crustaceans besides displaying a narrow niche breadth. In accordance with previous studies (Farias *et al.*, 2006, Saglam and Bascinar, 2008), the skate *R. clavata* based its diet on teleosts and both reptantian and natantian crustaceans without showing any trend with depth.

The shark *S. canicula* inhabiting the CS showed the highest diversity of all species-strata, having a diet composed of a mixture dominated by reptantian crustaceans, polychaetes, teleosts and natantians. Prey diversity, however, decreased with depth, mainly because the shark changed to a diet based on euphausiids on deeper waters. Secondary preys were polychaetes and teleosts on the SB and US respectively. To our knowledge, the importance of polychaetes in the diet of *S. canicula* has not been reported previously. Polychaetes were also found in all the batoids analysed, but they only constituted accessory preys. In the Cantabrian Sea, Serrano *et al.* (2003) found that polychaetes preyed by *S. canicula* belonged to surface and subsurface mobile families. As pointed out Olaso *et al.* (1998) referring to the presence of subsurface species in the diet of *S. canicula*, high consumption rates of polychaetes might indicate the availability of endobenthic preys that are exposed to predation by the physical effects of trawling.

The diets of the catsharks *S. canicula* and *G. melastomus* overlapped on the US, where both species preyed mainly on euphausiids. The shark *G. melastomus* displays a marked bathymetric segregation of populations in the study area, with recruits and adults inhabiting the US and MS respectively (Massuti and Moranta, 2003) (Fig. 4.2). Consequently, competition for resources on the US, namely euphausiids, occurs between adults of *S. canicula* and recruits of *G. melastomus*. However, it is also possible that competition is lessened by a sort of size selection of preys owing to the marked size differences between the small-sized *G. melastomus* and the adults of *S. canicula*. The occurrence of euphausiids in the diet of a large number of species inhabiting the SB and US may be due to the high abundance of these organisms on those strata (Cartes *et al.*, 2009).
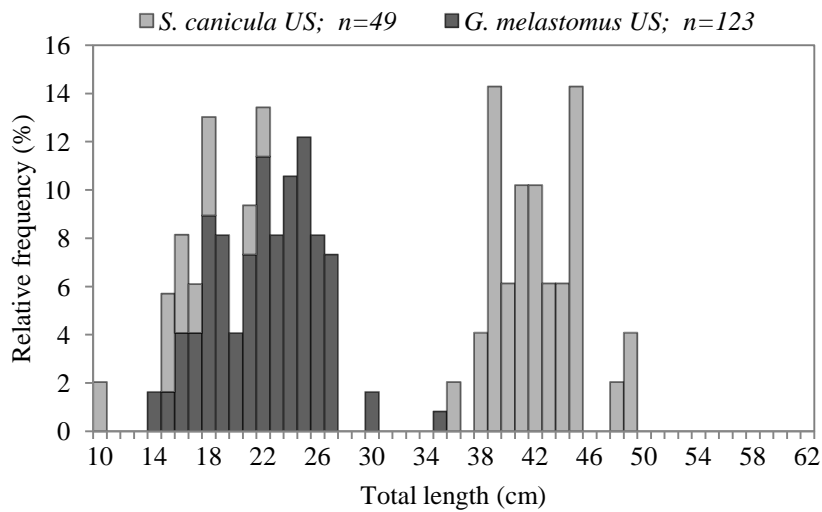
Figure 4.2. Length frequency distribution (in percentage) of *G. melastomus* and *S. canicula* sampled at upper slope (US) of the Balearic Islands.

The diets of the sharks *E. spinax* and *G. melastomus,* which were the only species coexisting on the MS, showed both similarities and notable differences. Concerning the similarities, both species had comparatively low to moderate values of natantian crustaceans (8-14%IRI) and high values of teleosts (≈25%IRI). Differences appeared in the relative importance of euphausiids and cephalopods, (Fig. 4.3) which showed an inverse pattern in both species: while cephalopods were the most important prey (64%IRI) and euphausiids only vestigial (1%IRI) for *E. spinax*, values in *G. melastomus* were inverted (16 and 43%IRI, respectively). Given that both species showed significant diet overlap, such inverse pattern would be a mechanism to lessen the competition on the MS. In agreement with this, Macpherson (1980) found that the diet overlap between these two species were significant for all size classes and seasons in the western Mediterranean. Both sharks consumed mesopelagic preys typical inhabitants of the Benthic Boundary Layer (BBL) (Angel and Boxshall, 1990) such as myctophids, euphausiids (e.g. *Meganycthyphanes norvegica*) and cephalopods (e.g. *Histioteuthis* spp.). These mesopelagic preys would be caught when the BBL remains close to the bottom, indicating the high dependence of slope demersal elasmobranchs on the pelagic ecosystem (Bizzarro *et al.*, 2007, Rinewalt *et al.*, 2007). A high dietary overlap between these two species was also reported in the Cantabrian Sea (Preciado *et al.*, 2009) but, in contrast with the preference of *E. spinax* for cephalopods in our samples, that population preyed mainly on euphausiids. On the contrary, high similarities were found in the diet composition of *E. spinax* analysed in the Ligurian sea (Wurtz and Vacchi 1978). Cephalopods (e.g. *Heteroteuthis* sp., Histioteuthidae) and myctophids were abundant prey in their stomach contents. Macpherson (1980) also described an increase in the consumption of cephalopods as *E. spinax* grows, although fishes and euphausiids were still the most important prey.

The three most abundant elasmobranch species (*R. clavata*, *G. melastomus* and *S. canicula*) showed ontogenetic variations in diet. The trend was the same in the

three species, with small individuals preying mainly on crustaceans but changing to a diet based on fishes in large-sized individuals. Such ontogenetic shifts have already been reported in other areas, both for the two catsharks (Macpherson, 1980, Olaso *et al.*, 2005) and the skate (Holden and Tucker, 1974, Ellis *et al.*, 1996, Saglam and Bascinar, 2008). However, some studies focused on *R. clavata* do not tally with this trend, such as the shift from benthic shrimps to pelagic crabs reported by Farias *et al.* (2006) or the lack of differences with size found by Morato *et al.* (2003). Ontogenetic shifts in diet are generally related to higher metabolic requirements of larger individuals (Carlson *et al.*, 2004) or to resource partitioning (Werner and Gilliam, 1984). The metabolic explanation would apply to *S. canicula*, because juvenile and adult populations live on different bathymetric strata in the Mediterranean (D'Onghia *et al.*, 1995, Massuti and Moranta, 2003). Given that there is not a bathymetric segregation of size classes neither in *R. clavata* nor in the populations of *G. melastomus* living on the MS in our study area (Massuti and Moranta, 2003), the observed ontogenetic shift might be a way of avoiding intraspecific competition.

Figure 4.3. Some of the main preys found in the stomachs of the elasmobranch species studied. **A**: *Glossanodon leioglossus*. **B**: *Meganyctiphanes norvegica*. **C**: *Alpheus glaber*. **D**: *Todarodes sagittatus*. **E**: *Solenocera membranacea*. **F**: *Liocarcinus sp*.

To conclude, the present work constitutes a comprehensive study on the feeding ecology of the most abundant demersal elasmobranchs, including both shark and batoid species, from an insular area geographically separated from the mainland in the western Mediterranean. Our results represent an important step forward in the knowledge of the trophic interactions among these elasmobranchs, but further studies dealing with aspects not treated here such as seasonal variations in diet, prey availability or diet of the coexisting teleost community are needed to improve the assessment of the role played by these species on the marine food webs from this area.

Table 4.4. Diet composition to the lowest possible taxon of eight elasmobranch species (five skates and three sharks) from the Balearic Islands (western Mediterranean). Frequency of occurrence (%F), the percentage of stomachs with a specific type of prey referred to the total number of stomachs containing food; volumetric composition (%V), expressed as the percentage contribution of each prey in volume to the whole content; standardized Index of Relative Importance (%IRI=(IRI/∑IRI)×100), where IRI=%F(%N+%V) and %N is the numeric composition or the percentage of each prey in number to the whole content.

Prey items	*R. polystigma*			*M. aquila*			*L. naevus*			*R. miraletus*			*R. clavata*			*S. canicula*			*G. melastomus*			*E. spinax*		
	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI
ALGAE																1.27	0.16	0.02	0.39	0.12	<0.01			
Phyllophora nervosa																0.14	0.01	<0.01						
Posidonia oceanica																0.14	0.00	<0.01						
Algae unid.																0.85	0.15	0.01	0.39	0.12	<0.01			
POLYCHAETA	13.33	4.18	1.17	36.36	4.87	8.07	4.17	0.17	0.29	3.23	0.09	0.05	4.81	0.35	0.09	31.90	12.38	22.45				4.00	0.16	0.26
Aphroditidae																1.56	0.49	0.04						
Polychaeta unid.	13.33	4.18	1.17	36.36	4.87	8.07	4.17	0.17	0.29	3.23	0.09	0.05	4.81	0.35	0.09	30.34	11.90	20.55				4.00	0.16	0.26
SIPUNCULA				13.64	8.02	1.63										7.65	6.30	2.02						
Sipunculidae				9.09	2.54	0.61										7.51	6.14	1.94						
Sipunculus nudus				4.55	5.47	0.24										0.14	0.15	<0.01						
NEMERTEA																0.28	0.25	<0.01						
MOLLUSCA	6.67	2.20	0.50	68.20	25.66	26.30				3.23	0.10	0.08	6.87	6.11	0.78	11.05	9.67	4.54	15.95	24.24	8.39	64.00	55.75	64.26
Bivalvia				31.82	14.93	6.28							0.69	0.18	<0.01	0.14	0.05	<0.01						
Gastropoda				13.64	4.41	0.86										0.14	0.01	<0.01						
Cephalopoda	6.67	2.20	0.50	4.55	0.45	0.13				3.23	0.10	0.08	6.19	5.93	0.66	8.22	8.95	2.93	16.34	24.24	8.60	64.00	55.75	64.26
Alloteuthis media													0.34	0.57	<0.01									
Eledone spp													0.34	1.74	0.01									
Heteroteuthis dispar																			0.39	0.70	0.01			
Histioteuthis bonnellii																						4.00	19.67	1.03
Histioteuthis spp																			0.39	0.05	<0.01			
Illex coindetii																			0.39	1.28	0.01			
Onychoteuthis banksii																			0.39	3.39	0.02			
Sepia spp				4.55	0.45	0.13																		
Sepiodea																0.28	0.25	<0.01						
Sepiola spp													0.34	0.07	<0.01									
Sepiolidae										3.23	0.10	0.08	0.69	0.15	<0.01	0.14	0.01	<0.01	0.78	0.65	0.01	4.00	2.95	0.21
Theuthoidea													1.72	2.78	0.08	0.57	3.36	0.06	0.39	1.54	0.01	4.00	2.75	0.20
Todarodes spp																			0.39	0.39	<0.01			
Cephalopoda unid.	6.67	2.20	0.50										2.75	0.62	0.05	7.22	5.33	1.71	13.23	16.24	4.88	52.00	30.38	33.49
Mollusca unid.				18.18	5.87	1.72							0.34	0.14	<0.01	2.55	0.66	0.12						
EUPHAUSIACEA													9.97	0.80	0.66	13.17	5.09	11.48	59.53	18.28	55.89	8.00	5.05	0.90
Meganyctiphanes norvegica													0.69	0.04	<0.01		3.82	3.76	1.69	15.48	14.93	8.00		5.05
Euphausiacea unid.													9.28	0.75	0.60	9.35	1.34	4.03	33.85	2.80	12.96			
AMPHIPODA	13.33	1.50	1.10	4.55	0.04	0.09	16.67	0.20	2.78	25.81	1.27	3.87	4.47	0.13	0.10	7.93	0.71	0.98	6.23	0.71	0.52			
Phronima sedentaria																0.71	0.40	0.01	4.67	0.60	0.32			
Amphipoda unid.	13.33	1.50	1.10	4.55	0.04	0.09	16.67	0.20	2.78	25.81	1.27	3.87	4.47	0.13	0.10	7.22	0.31	0.76	1.56	0.10	0.03			

Prey items	R. polystigma %F	%V	%IRI	M. aquila %F	%V	%IRI	L. naevus %F	%V	%IRI	R. miraletus %F	%V	%IRI	R. clavata %F	%V	%IRI	S. canicula %F	%V	%IRI	G. melastomus %F	%V	%IRI	E. spinax %F	%V	%IRI
ISOPODA	33.33	8.40	7.22				12.50	2.41	1.08	3.23	0.47	0.04	20.27	2.65	2.40	8.92	0.99	1.25	0.78	0.06	0.01	4.00	0.49	0.37
MYSIDACEA	26.67	1.99	6.63				20.83	1.38	2.66	6.45	0.19	0.36	18.90	1.03	2.33	9.07	1.24	1.58	1.56	0.11	0.03			
Lophogaster typicus	13.33	1.73	1.14				20.83	1.38	2.66				16.15	0.64	1.52	5.67	0.54	0.53	1.17	0.07	0.02			
Mysidacea unid.	20.00	0.26	3.26							6.45	0.19	0.31	4.12	0.39	0.12	4.82	0.69	0.39	0.39	0.04	<0.01			
REPTANTIA	20.00	5.74	3.35	100.00	60.96	81.94	12.50	0.72	1.32	80.65	36.81	54.88	48.45	26.74	33.93	33.00	19.58	32.65	2.72	0.89	0.11	4.00	0.16	0.08
Anomura	6.67	3.29	0.49	90.91	57.96	63.37							13.75	7.51	2.50	20.40	13.60	12.86	0.39	0.02	<0.01			
Dardanus arrosor				50.00	33.39	18.32							0.34	0.24	<0.01	0.99	0.80	0.03						
Galatheidae				4.55	0.05	0.12							2.06	0.08	0.02	1.70	0.08	0.05						
Munida intermedia																0.14	0.07	<0.01						
Munida rutllanti																0.14	0.10	<0.01						
Munida spp													1.03	0.24	0.02	0.57	0.39	0.01						
Paguridae				36.36	24.51	11.07							6.19	0.78	0.29	13.74	7.34	4.78	0.39	0.02	<0.01			
Pagurus prideauxi													3.09	5.38	0.27	2.12	3.36	0.24						
Pagurus spp	6.67	3.29	0.49										1.03	0.79	0.02	0.99	1.47	0.05						
Brachyura	13.33	2.44	1.26	22.73	3.01	2.78	12.50	0.72	1.32	80.65	36.81	54.88	45.70	18.99	22.57	15.72	5.73	5.12	1.17	0.23	0.01	4.00	0.16	0.08
Atelecyclus rotundatus													2.75	0.58	0.05	0.71	0.13	0.01						
Bathynectes spp													0.34	0.14	<0.01									
Calappa granulata													1.03	0.40	0.01									
Ethusa mascarone													1.37	0.07	0.01									
Goneplax rhomboides													1.03	0.84	0.02	0.14	0.10	<0.01						
Ilia nucleus																0.14	0.06	<0.01						
Liocarcinus depurator													0.69	0.51	0.01									
Liocarcinus spp	6.67	1.57	0.24	4.55	0.17	0.07	4.17	0.28	0.07	12.90	6.01	1.35	11.34	2.83	1.14	1.84	0.44	0.08						
Macropipus tuberculatus													6.87	5.67	0.72	0.57	0.24	0.01						
Monodaeus couchii													0.34	0.04	<0.01	0.14	0.16	<0.01						
Parthenope massena																0.14	0.01	<0.01						
Portunidae													0.34	0.11	<0.01	0.14	0.03	<0.01						
Xantidae													1.03	0.20	0.01	0.14	0.06	<0.01						
Brachyura unid.	6.67	0.87	0.39	18.18	2.84	1.96	8.33	0.44	0.73	67.74	30.79	39.02	18.56	7.61	3.68	11.76	4.50	3.05	1.17	0.23	0.01	4.00	0.16	0.08
Macrura													3.44	0.24	0.08	0.85	0.25	0.01	1.17	0.64	0.03			
Calocaris macandreae																			0.39	0.05	0.00			
Palinurus mauritanicus													3.09	0.23	0.06	0.42	0.04	<0.01						
Polycheles typhlops																			0.78	0.59	0.01			
Scyllaridae																0.14	0.02	<0.01						
Scyllarus spp													0.34	0.01	<0.01	0.28	0.19	<0.01						
NATANTIA	73.33	37.19	67.97	13.64	0.37	1.10	54.17	24.62	50.88	64.52	22.33	34.78	59.79	11.89	37.52	23.94	4.87	11.64	24.51	12.88	11.25	32.00	7.83	8.54
Alpheidae																0.28	0.07	<0.01						
Alpheus glaber													4.81	0.47	0.17	1.42	0.42	0.04	0.39	0.08	<0.01			
Alpheus spp													1.03	0.04	0.01	2.27	0.37	0.09						
Chlorotocus crassicornis													9.62	2.11	0.78	0.14	0.19	<0.01						
Crangonidae	13.33	6.00	2.06				4.17	0.11	0.18	6.45	0.20	0.26	2.41	0.18	0.03	0.71	0.06	0.01	0.39	0.15	<0.01			
Pandalina spp																0.14	0.00	<0.01						
Pasiphaea multidentata																			2.72	5.27	0.30			
Pasiphaea spp																0.57	0.20	0.01	4.67	2.30	0.35	12.00	2.39	1.17

Prey items	R. polystigma			M. aquila			L. naevus			R. miraletus			R. clavata			S. canicula			G. melastomus			E. spinax		
	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI
Pasiphaea sivado													0.28	0.13	<0.01				3.11	0.99	0.16			
Philocheras spp													0.14	0.02	<0.01									
Philocheras trispinosus													0.14	0.17	<0.01									
Plesionika spp													1.03	0.26	0.01	0.14	0.01	<0.01	0.78	0.09	<0.01			
Pontocaris spp																0.14	0.10	<0.01						
Procesa spp	33.33	11.79	11.29	9.09	0.23	0.43	29.17	2.94	5.92	22.58	2.97	2.72	24.74	2.44	4.05	10.20	1.15	1.67	0.39	0.05	<0.01			
Systellaspis debilis																			0.78	0.10	0.01			
Gennadas elegans																			0.39	0.03	<0.01			
Sergestes arcticus																			2.33	0.61	0.06			
Sergestes spp																			0.39	0.17	<0.01			
Sergia robustus																			2.72	1.87	0.15	4.00	1.97	0.16
Solenocera membranacea	13.33	14.30	3.01				33.33	18.16	14.80	22.58	14.30	4.71	13.75	3.27	1.58	1.27	0.60	0.04						
Solenocera spp													4.47	0.91	0.13	0.71	0.23	0.01						
Natantia unid.	26.67	5.11	5.53	4.55	0.14	0.15	16.67	3.41	4.17	25.81	4.87	4.38	21.99	2.21	3.82	8.50	1.15	1.32	7.78	1.16	0.75	16.00	3.47	2.05
Other crustacea																								
Rissoides desmaresti													1.03	0.29	0.01	0.71	0.51	0.02						
Crustacea unid.							4.17	2.27	0.14	3.23	0.77	0.06	5.50	0.27	0.16	6.80	1.35	0.82	3.89	1.33	0.22	4.00	0.83	0.22
TELEOSTEA	20.00	38.81	13.51				37.50	68.23	40.80	12.90	37.99	5.88	31.62	48.92	30.71	20.40	33.04	24.92	24.51	39.67	23.17	32.00	29.63	25.25
Anguilliformes													1.03	0.23	0.01	0.28	0.26	<0.01						
Aphia minuta													0.69	0.02	0.01									
Argentina sphyraena													0.34	0.06	<0.01									
Callionymus spp													0.34	0.12	<0.01	0.14	0.05	<0.01						
Capros aper																0.14	0.04	<0.01						
Cepola macrophthalma													0.34	0.07	<0.01	0.42	1.50	0.02						
Chauliodus sloani																			1.17	3.92	0.09			
Chelidonichthys cuculus													0.34	1.62	0.01									
Citharus linguatula													0.34	0.48	<0.01									
Diplodus spp																0.14	0.27	<0.01						
Gadiculus argenteus													0.69	1.26	0.01									
Glossanodon leioglossus													5.84	10.63	1.30	0.28	1.03	0.01						
Gobiidae													2.75	0.35	0.12	0.42	0.03	<0.01				4.00	0.15	0.21
Gymnammodites cicerelus	6.67	10.81	1.00							3.23	14.02	0.52												
Helicolenus dactylopterus													0.34	0.03	<0.01									
Lampanyctus crocodilus																			1.56	14.93	0.38			
Lepidorhombus spp																0.14	0.38	<0.01						
Maurolicus muelleri																			1.17	0.77	0.02			
Merluccius merluccius													1.03	0.37	0.01									
Micromesistius poutassou													1.03	1.11	0.02	0.14	0.01	<0.01						
Myctophidae																			3.11	1.25	0.18	12.00	4.90	1.95
Myctophum punctatum																			0.39	0.26	<0.01			
Nemichthys scolopaceus																			0.39	0.18	<0.01			
Nezumia aequalis																			0.39	1.16	0.01			

Prey items	R. polystigma			M. aquila			L. naevus			R. miraletus			R. clavata			S. canicula			G. melastomus			E. spinax		
	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI	%F	%V	%IRI
Notoscopelus spp																						4.00	17.21	1.04
Ophichthus rufus													0.34	0.02	<0.01	0.42	1.33	0.02						
Phycis blennoides													0.34	1.43	0.01									
Pleuronectidae													1.03	0.24	0.01	0.85	0.63	0.02	0.78	0.28	0.01			
Regalecus glesne							4.17	29.83	1.69															
Sardina pilchardus													1.03	1.70	0.03									
Serranus hepatus													1.03	3.12	0.05									
Spicara smaris										3.23	23.39	0.76	2.06	10.23	0.32	0.14	0.54	<0.01						
Stomias boa boa																0.14	0.19	<0.01	1.17	7.72	0.15			
Syngnathidae																0.14	0.16	<0.01						
Symphurus ligulatus																0.14	0.02	<0.01						
Symphurus nigrescens																0.14	0.22	<0.01						
Symphurus spp													0.34	0.07	<0.01									
Trachinus draco													0.69	1.62	0.01									
Trachurus spp																0.28	0.82	0.01						
Teleost unid.	13.33	28.00	7.00				33.33	38.40	22.75	6.45	0.58	0.38	16.84	14.16	4.97	16.86	25.56	16.01	17.51	9.21	6.03	24.00	7.37	7.05
THALIACEA																0.42	0.12	<0.01	2.33	0.70	0.10			
Doliolid																			0.39	0.08	<0.01			
Pyrosoma atlanticum																			0.78	0.38	0.01			
Salpidae																0.42	0.12	<0.01	1.17	0.24	0.03			
OTHERS				4.55	0.08	0.21							2.06	0.80	0.05	5.81	3.73	1.03	4.28	1.00	0.29	4.00	0.10	0.07
Cnidaria																0.57	0.19	0.01						
Echinoidea													1.03	0.55	0.01	0.28	0.28	<0.01						
Ostracoda																0.14	0.01	<0.01						
Eggs unid.																0.28	1.02	0.01	0.39	0.21	<0.01			
Ascidiacea																1.56	0.63	0.07	0.78	0.31	0.01			
Unid. remains				4.55	0.08	0.21							1.03	0.26	0.01	2.97	1.61	0.30	3.11	0.49	0.15	4.00	0.10	0.07
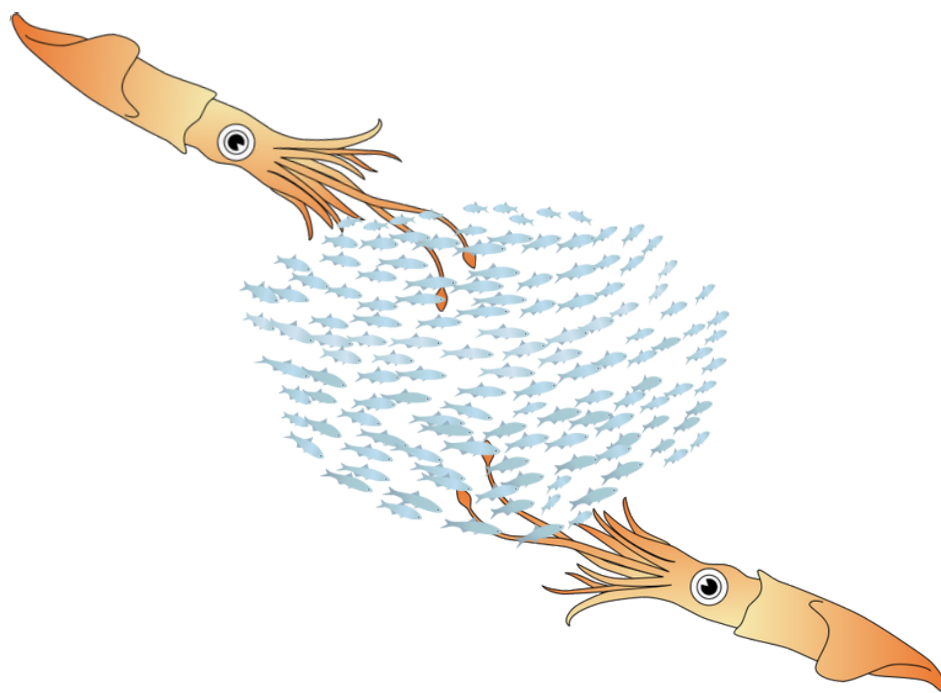
Chapter 5: Feeding ecology of two squid species from the western Mediterranean

Chapter 5: Feeding ecology of two squid species from the western Mediterranean

5.1 Introduction

The squid *Loligo vulgaris* Lamarck (1798) and *L. forbesii* Steenstrup (1857) are 2 cephalopod species with few external morphological differences and which inhabit the eastern Atlantic Ocean and Mediterranean Sea (Jereb & Roper 2010). They are important prey for a number of marine predators such as pelagic and demersal fish and marine mammals (e.g. Morte et al. 1997, Peristeraki et al. 2005, Bearzi et al. 2011). In turn, they prey on a broad spectrum of species, especially fish, but also crustaceans, cephalopods and polychaetes (Roper et al. 1984, Collins et al. 1994, Guerra & Rocha 1994, Pierce et al. 1994, Coelho et al. 1997, Wangvoralak et al. 2011). It is accepted that squid have a large trophic impact on other species in the food web and top-down control from squid to their prey can be high (Coll et al. 2013, Young et al. 2013).

They are also important fishery resources. Both species are taken as by-catch of the Mediterranean bottom trawl fishery (Guerra & Rocha 1994, Relini et al. 1999, Sifner & Vrgoc 2004, Massutí & Reñones 2005), while *L. vulgaris* also supports important artisanal and recreational fisheries (Guerra & Rocha 1994, Morales-Nin et al. 2005, Cabanellas-Reboredo et al. 2014a). Thus, the role of these squid, as predator and prey, and the interactions they have with other species, are key factors in the trophodynamics of marine ecosystems (Rocha et al. 1994, Navarro et al. 2013) and adequate knowledge about their role and interactions is important to allow appropriate resource managment (Moreno et al. 2013).

Diet composition and feeding ecology of these loliginids are well documented in Atlantic waters. They mainly feed on fish, with little frequency variation but different species composition depending on the region (Roper et al. 1984, Collins et al. 1994, Guerra & Rocha 1994, Pierce et al. 1994, Coelho et al. 1997, Wangvoralak et al. 2011). Seasonal and daily spatial migrations, related to reproduction and feeding, are known to occur in both species (Rocha & Guerra 1999, Cabanellas-Reboredo et al. 2012, 2014b). However, to date little is known about the diet of *L. vulgaris* and *L. forbesii* in the Mediterranean, although different aspects of their life cycle (e.g. growth and reproduction) are relatively well studied both in the western (Mangold-Wirz 1963, Worms 1979, Wurtz & Giuffra 1989, Sánchez & Demestre 2010) and central (Ragonese & Jereb 1986, Sifner & Vrgoc 2004) regions. In the Mediterranean Sea, both species show a clear bathymetric segregation, as *L. vulgaris* preferentially inhabits waters shallower than 200 m and *L. forbesii* is found at depths between 100 and 600 m, slightly overlapping on the deep continental shelf (Quetglas et al. 2000).

All available information on squid diet is based on stomach content analysis. In general, gut content analysis fails to provide information on long-term feeding habits (Jackson et al. 2007) and neglects some dietary materials. These analyses are further biased in cephalopods, which reduce the food to hardly recognizable pieces (Hyslop 1980, Boyle & Rodhouse 2005). Despite these shortcomings, stomach content analysis remains the main source of data for prey items and provides useful information on predator feeding habits and ecology (Clarke &

Kristensen 1980, Laptikhovsky et al. 2010, Miller et al. 2013). Although isotopic analysis performs better than dietary analysis in revealing assimilated food, it does not provide information on predator–prey interactions at the species level (Winemiller et al. 2007, Young et al. 2015).

We investigated the feeding habits of *L. vulgaris* and *L. forbesii* for the first time in the Mediterranean Sea by means of stomach content analysis. The diets were expected to reflect the bathymetric segregation of the 2 species in the area, yet some competition should exist at depths where the squid overlap. The main objective was to analyse the feeding habits of these 2 squid to determine differences and similarities in their feeding ecology. We also investigated whether these species display differences in diet related to sex, season or ontogenetic growth. Diet composition studies constitute a crucial first step in order to better understand trophic interactions, which in return allow building robust, meaningful marine food web models (Christensen & Walters 2004, Coll et al. 2006, 2008, Moreno et al. 2013).

5.2 Materials and methods

Species sampling

Individuals from both species were sampled monthly from commercial fishing boats off the Balearic Islands (western Mediterranean) between January 2009 and January 2010 (N = 984 *Loligo vulgaris* and 693 *L. forbesii*). Squid were obtained from bottom trawlers operating between 50 and 750 m and small-scale boats generally fishing down to 100 m. Species were identified on the basis of macroscopic external characters (e.g. tentacular club) after Roper et al. (1984). Whereas *L. vulgaris* were collected from trawl (76%) and artisanal fishery (24%), *L. forbesii* were all obtained from the trawl fishery. Additional samples were taken from the fishery-independent MEDITS bottom-trawl surveys (Bertrand et al. 2002) conducted in the study area during early summer from 2007 to 2010 (N = 468 *L. vulgaris* and 207 *L. forbesii*).

Stomachs sampling and diet indices

Prey items from gut contents were analysed under a binocular microscope and identified to the lowest possible taxon. The number of each prey category were recorded and the following indices calculated to analyse the diet and feeding intensity (Hyslop 1980): i) relative abundance (%N), ii) frequency of occurrence (%O) and iii) vacuity index (%v).
To determine the feeding strategy, niche breadth was calculated using the Levins'

Standardized index $B_i = \dfrac{1}{n-1}\left(\dfrac{1}{\sum_j p_{ij}^2} - 1\right)$ where p_{ij} is the proportion of diet of

predator *i* that is made up of prey *j* and *n* is the number of prey categories (Krebs 1999). The values of p_{ij} are determined from a matrix (e.g. Table 5.1) as described

by Colwell & Futuyma (1971). This index range from 0 to 1, low and high values indicating specialist and generalist diets respectively. Medits survey design, sampling methodology and stomach contents analysis are explained in Chapter 2 (Section 2.1.1.1 and 2.2.1, respectively)

Data analysis

The cumulative number of prey items was plotted against the cumulative number of non-empty stomachs in order to determine sample size sufficiency (Ferry & Cailliet 1996). PRIMER software was used to compute a prey species accumulation plot as an average of 999 curves based on different random orders of the stomachs. A curve approaching an asymptote with low variability indicates that the number of stomachs examined is sufficient to characterize the diet.

Different statistical analyses were performed to address the following goals: (1) a cluster analysis to detect ontogenetic shifts in diet; (2) a permutational multivariate analysis of variance (PERMANOVA) to test biological (sex and size) and environmental (season) factors affecting diet; and (3) a similarity percentage (SIMPER) analysis to identify the prey item contribution to diet dissimilarities.

Ontogenetic variation

Major prey categories were established from prey items (with N > 5) to eliminate biases associated with comparisons based on variable levels of prey identification (Cortés 1997). Fish prey were grouped to the family level (11 groups), and invertebrates were divided into 14 categories (see Table 5.1). The unidentifiable remains were excluded from these analyses.

For each species, individuals were grouped into 10 mm size classes. The first (<100 mm) and last (>250 mm) size group were based on the availability of a sufficient sample for the analyses. Specimens were then divided into small and large categories through hierarchical agglomerative and unweighted arithmetic average clustering (Clarke & Gorley 2006) by calculating Bray-Curtis similarity resemblance matrices. Analyses were done using abundance data. In order to reduce the weight of numerically dominant species, a prior square root transformation of the data was performed. The significant groups were determined using the SIMPROF test (p < 0.01) (Clarke & Gorley 2006).

Effects of sex, size and season

Data on diet based on major groups (mean abundance/sampling and size class) were analysed using multivariate analyses on all individuals with gut contents. First, the factors season (winter, W: January–March; spring, SP: April–June; summer, S: July–September; and autumn, A: October–December), size (small and large) and sex (males and females) were tested. Standardized prey group abundances were square-root transformed and used to develop a Bray-Curtis similarity matrix. PERMANOVA examined the effects of season, sex and size and

their crossed effects (9999 permutations under a reduced model) on the variation of the squid diet composition. Significant factors (p < 0.05) were further analyzed using a PERMANOVA pairwise comparison. Finally, SIMPER analysis was employed to evaluate the contribution of prey categories to variations among seasons (Clarke 1993). Homogeneity of multivariate variance was confirmed for all factors using PERMIDISP. All of these analyses were performed using the statistical software PRIMER6 & PERMANOVA (Clarke & Warwick 2001, Anderson et al. 2008).

5.3 Results

Diet composition

The diet composition of each squid species is summarized taking into account the lowest identified taxonomic levels (Table 5.1).

Loligo vulgaris

Of the 1452 stomachs analysed, 49.8% were from females (55–357 mm ML), 45.1% were from males (52–417 mm ML), and 5.1% could not be sexed (23–288 mm ML); the percentage of empty stomachs was 45%. Overall, 80 different prey items belonging to 12 taxa were identified (59 categories identified to genus/species level). The feeding spectrum included teleosts, cephalopods, stomatopods, polychaetes, cumaceans, decapod crustaceans, isopods, mysids, euphausiids, amphipods, salps and algae. The most important prey were fish (%O = 79.7, %N = 57.8), followed by crustaceans (%O = 21.6, %N = 19.8), cephalopods (%O = 19.5, %N = 6.8) and polychaetes (%O = 8.7, %N = 12.8). Among fish, 13 families and 26 species were identified, of which the sparids *Spicara* spp. (%O = 5.8, %N = 2.5) and benthic gobies *Lesueurigobius* spp. (%O = 3.9, %N = 3.4) were the most frequent and abundant prey.

L. forbesii

Of the 900 stomachs analyzed, 43.9% were from females (38–286 mm ML), 48.3% were from males (40–451 mm ML), and 7.8% could not be sexed (36–152 mm ML); the vacuity index was 45.2%. Overall, 65 different prey items belonging to 7 taxa were identified (50 categories to genus/species). The feeding spectrum included teleosts, elasmobranchs, molluscs, crustacean decapods, isopods, mysids and euphausiids. The main prey were fish (%O = 73.4, %N = 56.5), followed by crustaceans (%O = 39.0, %N = 41.5). Among fish, 12 families and 35 species were identified; the transparent goby *Aphia minuta* was by far the most important identified species (%N = 26.5, %O = 9.7), followed by the mesopelagic fish *Maurolicus muelleri* (%N = 3.1, %O = 6.5). Among crustaceans, euphausiids were the most common group (%N = 26.8, %O = 25).

Table 5.1. Relative abundance (%N) and frequency of occurrence (%O) of the prey items identified in *L. vulgaris* and *L. forbesii* stomach contents.

Prey	*Loligo vulgaris*		*Loligo forbesii*	
	%N	%O	%N	%O
PISCES	57.77	79.68	56.54	73.43
Argentinidae	0.04	0.12	1.47	5.27
Argentina sphyraena			0.09	0.41
Glossanodon leioglossus	0.04	0.12	1.38	4.87
Blenniidae	0.43	0.86		
Blennius ocellaris	0.11	0.25		
Parablennius tentacularis	0.22	0.25		
Blenniidae unid.	0.11	0.37		
Callionymidae			0.30	1.42
Callionymus maculatus			0.13	0.61
Callionymus sp.			0.04	0.20
Synchiropus phaeton			0.17	0.81
Carangidae	1.12	2.46	0.04	0.20
Trachurus mediterraneus	0.14	0.49		
Trachurus trachurus	0.04	0.12		
Trachurus sp.	0.94	1.85	0.04	0.20
Centriscidae			0.04	0.20
Macroramphosus scolopax			0.04	0.20
Cepolidae	0.04	0.12	0.09	0.41
Cepola macrophthalma	0.04	0.12	0.09	0.41
Clupeidae	0.61	2.09		
Sardina pilchardus	0.43	1.48		
Sardinella aurita	0.14	0.49		
Sprattus sprattus	0.04	0.12		
Epigonidae			0.04	0.20
Epigonus denticulatus			0.04	0.20
Gadiformes	0.25	0.86	1.51	6.69
Micromessistius poutassou			0.43	2.03
Molva dypterygia			0.04	0.20
Phycis blennoides			0.22	1.01
Gadiculus argenteus	0.04	0.12	0.56	2.64
Gaidropsarus biscayensis	0.22	0.74	0.26	1.22
Gobiidae	26.81	18.60	32.10	16.02
Aphia minuta	0.58	0.74	26.52	9.74
Pseudoaphya ferreri	0.18	0.25		
Crystallogobius linearis	1.84	1.35	3.89	2.23
Deltentosteus quadrimaculatus			0.04	0.20
Gobius niger	0.14	0.37		
Lesueurigobius friesii	1.30	1.85		
Lesueurigobius sanzi	0.18	0.49		
Lesueurigobius suerii	0.04	0.12		
Lesueurigobius sp.	1.84	1.48	0.13	0.61
Gobiidae unid.	20.7	14.53	1.51	4.46
Merlucciidae			0.13	0.61
Merluccius merluccius			0.13	0.61
Mullidae	0.04	0.12		
Mullus barbatus	0.04	0.12		
Myctophidae	0.04	0.12	2.68	8.52
Myctophum punctatum	0.04	0.12	0.04	0.20
Ceratoscopelus maderensis			0.95	3.85
Hygophum benoiti			0.04	0.20
Hygophum hygomii			0.09	0.41

Prey	Loligo vulgaris		Loligo forbesii	
	%N	%O	%N	%O
Lampanyctus crocodilus			0.17	0.81
Lampanyctus pusillus			0.52	1.62
Lampanyctus sp.			0.09	0.41
Lobianchia dofleini			0.04	0.20
Notoscopelus bolini			0.04	0.20
Notoscopelus elongatus			0.48	1.01
Notoscopelus sp.			0.09	0.41
Symbolophorus veranyi			0.13	0.61
Paralepididae			0.04	0.20
Lestidiops jayakari			0.04	0.20
Pleuronectiformes	0.11	0.25	0.21	1.01
Lepidorhombus boscii			0.13	0.61
Arnoglossus imperialis			0.04	0.20
Arnoglossus ruepellii			0.04	0.20
Citharus linguatula	0.11	0.25		
Sternoptychidae	0.33	0.12	3.67	7.10
Maurolicus muelleri	0.33	0.12	3.11	6.49
Argyropelecus hemigymnus			0.56	1.01
Sparidae	3.03	7.39		
Boops boops	0.11	0.37		
Centracanthus cirrus	0.47	1.35		
Spicara smaris	2.02	4.43		
Spicara sp.	0.43	1.35		
Stichaeidae	0.11	0.37		
Ophidium barbatum	0.11	0.37		
Pisces unid.	24.82	51.60	14.17	36.31
Elasmobranchii unid.			0.04	0.20
MOLLUSCA	6.79	19.46	1.56	6.69
Natica sp.			0.04	0.20
Nassarius sp.			0.04	0.20
Bivalvia unid.			0.22	0.60
Gastropoda unid.			0.08	0.80
Cephalopoda	6.79	19.46	1.26	5.89
Alloteuthis media	0.61	1.48		
Loligo vulgaris	0.18	0.49		
Loligo sp.	0.29	0.74		
Sepiida unid.	0.11	0.37		
Sepietta oweniana	0.36	0.49		
Theuthoidea unid.	1.81	5.17	0.09	0.41
Cephalopoda unid.	3.47	11.58	1.17	5.48
CRUSTACEA	19.80	21.55	41.47	38.95
Mysidacea	2.96	4.56	10.24	4.67
Gastrosaccus sanctus	0.33	0.62		
Gastrosaccus sp.	0.29	0.49	6.39	1.00
Mysidacea unid.	2.35	3.57	3.84	3.70
Amphipoda	0.22	0.62		
Phrosina semilunata	0.07	0.25		
Hiperiidea unid.	0.04	0.12		
Gammaridea unid.	0.07	0.12		
Amphipoda unid.	0.04	0.12		
Isopoda	7.44	8.87	0.22	0.81
Eurydice sp.	4.37	2.22	0.09	0.20
Idotea sp.	0.04	0.12		
Cymodoce sp.	0.07	0.12		
Paragnathia formica	0.18	0.25		
Natatolana sp.	1.08	1.60		
Gnathia sp. (Praniza)	0.07	0.12		

Prey	*Loligo vulgaris*		*Loligo forbesii*	
	%N	%O	%N	%O
Cirolanidae unid.	0.25	0.74		
Isopoda unid.	1.37	3.94	0.13	0.61
Euphausiacea	4.33	1.11	26.78	24.95
Meganyctiphanes norvegica	2.85	0.74	25.36	23.30
Eufausiacea unid.	1.48	0.37	1.43	1.80
Decapoda	3.32	6.16	3.41	8.52
Brachyura (Megalopa)	1.30	1.35		
Brachyura unid.	0.83	1.23		
Galatheidae	0.04	0.12		
Natantia				
Alpheus glaber	0.04	0.12		
Alpheus sp.	0.07	0.25		
Crangonidae	0.04	0.12		
Philocheras sculptus	0.04	0.12		
Plesionika heterocarpus			0.43	0.41
Plesionika sp.	0.14	0.37	0.91	0.41
Processa canaliculata			0.13	3.65
Processa sp.	0.18	0.62		
Parapenaeus longirostris			0.09	0.41
Pasiphaea sivado			0.09	1.01
Sergestes arachnipodus			0.95	1.22
Eusergestes arcticus			0.04	0.20
Solenocera membranacea			0.04	0.20
Decapoda unid.	0.65	20.9	0.73	1.62
Stomatopoda	0.51	1.23		
Rissoides desmaresti	0.07	0.25		
Squillidae unid.	0.07	0.25		
Stomatopoda unid.	0.36	0.74		
Cumacea unid.	0.43	0.49		
Crustacea unid.	1.01	3.20	0.82	3.04
POLYCHAETA	12.79	8.74		
Nereididae unid.	12.57	8.13		
Polychaeta unid.	0.22	0.74		
SALPIDAE	0.07	0.25		
ALGAE	1.81	6.16		
Unidentified	0.43	1.48	0.35	1.60

Table 5.2. Frequency of occurrence (%O) of the main prey groups found in stomach contents of *Loligo vulgaris* and *L. forbesii* by season (W: winter; SP: spring; S: summer; A: autumn) and size-class (small, large). The number of stomachs analysed (N), percentage of empty stomachs (%v) and Levin's standardized index (B_i) are also shown. Cut-off values defining small and large individuals are 210 and 140 mm dorsal mantle length for *L. vulgaris* and *L. forbesii*, respectively; unid.: unidentified

| | *L. vulgaris* (%O) | | | | | | | | *L. forbesii* (%O) | | | | | | | |
| | W | | SP | | S | | A | | W | | SP | | S | | A | |
	Small	Large	Small	Large	Small	Large	Small	Large	Small	Large	Small	Large	Small	Large	Small	Large
Pisces	73.5	78.9	85.1	87.8	79.8	87.2	79.4	76.2	33.8	76.2	88.7	83.2	62.5	74.3	78.6	79.8
Argentinidae									1.4		2.1	9.9				13.8
Blenniidae	1.0	2.6	0.3				2.8	2.4								
Callionymidae									1.4			3.0		1.4	7.1	1.1
Carangidae		5.3	1.7	2.0	6.7	2.6	0.9	4.8								
Clupeidae			3.1		5.9		0.9									
Sparidae	4.1	10.5	1.7	14.3	6.7	28.2	11.2	14.3								
Gadiformes		2.6	2.1								6.2	8.9	12.5	12.9		7.4
Gobiidae	10.2	2.6	29.4	6.1	25.2	2.6	18.7	2.4	4.2		46.4	17.8	31.3	5.7	7.1	3.2
Myctophydae			0.3				0.9					5.0		22.9		22.3
Pleuronectiformes										4.8		2.0				2.1
Sternoptychidae									1.4	4.8	4.1	17.8		1.4		10.6
Pisces unid.	60.2	55.3	52.6	67.3	41.2	61.5	53.3	57.1	26.8	66.7	34.0	32.7	18.8	41.4	64.3	41.5
Mollusca																
Cephalopoda	32.7	21.1	17.0	6.1	20.2	20.5	21.5	26.2		14.3	1.0	6.9	6.3	10.0	7.1	9.6
Bivalvia									1.4			1.0		1.4		
Crustacea	23.5	28.9	21.1	16.3	30.3	15.4	22.4	21.4	67.6	28.6	49.5	25.7	37.5	32.9	21.4	34.0
Brachyura	1.0		2.1		7.6		4.7									
Decapoda unid.			1.7				1.9									
Natantia		2.6	2.8	2.0	5.9		3.7	4.8			3.1	8.9		12.9	21.4	19.1
Cumacea			0.3		1.7		0.9									
Isopoda	14.3	26.3	7.3	6.1	8.4	15.4	4.7	7.1				1.0		1.4		2.1
Mysidacea	10.2		4.8		5.9		5.6				15.5		37.5		14.3	
Euphausiacea	2.0		1.7		1.7				63.4	28.6	33.0	13.9		15.7		16.0
Amphipoda	2.0		0.3				1.9									
Stomatopoda			0.3		2.5		2.8	7.1								
Crustacea unid.	1.0		4.2	8.2	1.7		5.6	4.8	4.2		5.2	2.0		2.9		3.2
Polychaeta	2.0	2.6	18.7	12.2	2.5	2.6	3.7									
Algae	8.2		1.0		8.4	15.4	9.3	26.2								
N	191	63	601	88	189	60	191	69	102	42	193	204	59	131	35	134
% v	46.6	36.5	49.6	42.0	35.4	31.7	41.9	39.1	49.2	50	49.2	48.5	72.9	45	57.1	29.1
B_i	0.13	0.12	0.17	0.08	0.25	0.12	0.20	0.16	0.05	0.06	0.15	0.27	0.11	0.19	0.08	0.24

Ontogenetic and seasonal effects

Cumulative prey curves (Fig. 5.1) revealed that our data provide a good description of the diet of *L. vulgaris* and *L. forbesii* by size group.
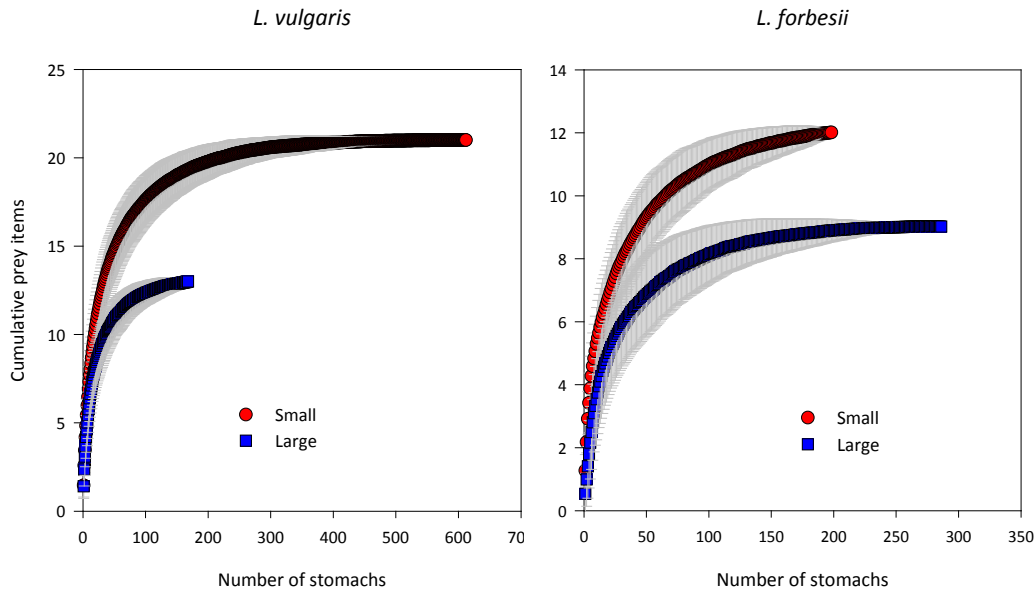
Figure 5.1. Cumulative prey curves as a function of sample size (full stomachs) analyzed by size class (small and large) for *Loligo vulgaris* and *L. forbesii*.
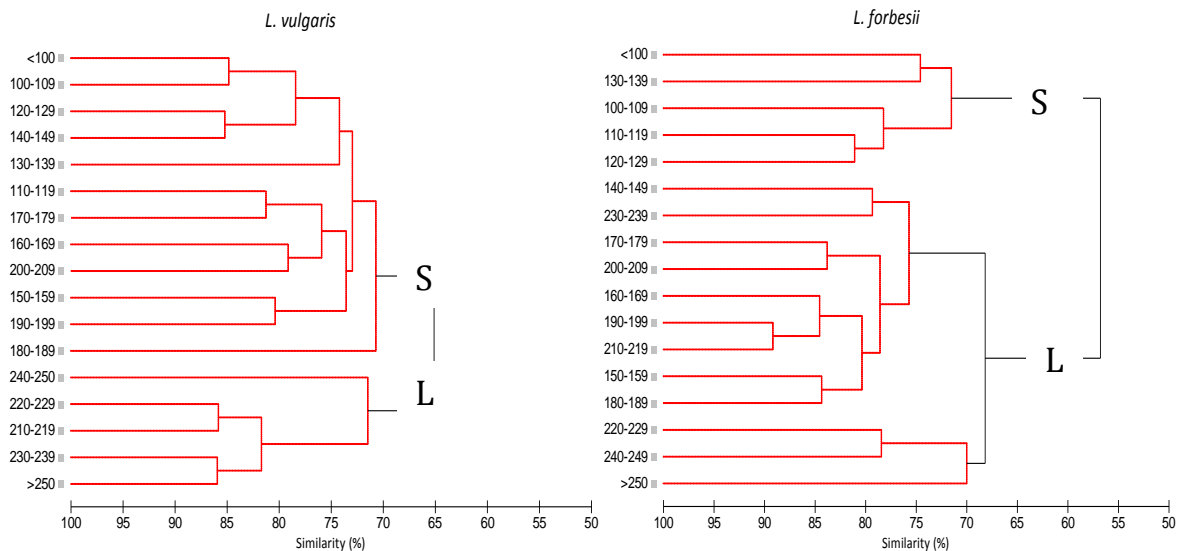

Figure 5.2. Cluster analysis applied on the Bray-Curtis similarity matrix for *Loligo vulgaris* and *L. forbesii* (based on prey occurrence). Red lines indicate size groups not separated (at p<0.01), black lines separate significantly different groups (S: small; L: large)

L. vulgaris

Four main prey groups were found in the diet: fish, crustaceans, molluscs and polychaetes. Clustering classification revealed diet differences between size-classes. This allowed the identification of 2 size groups (Fig. 5.2): small (<210 mm ML) and large (>210 mm ML); this grouping was validated by the SIMPROF test. Clupeids, myctophids, brachyurans, mysids, euphausiids, amphipods and cumaceans were only found in stomachs of small squid. The rest of prey was present in both size categories (Table 5.2). Fish were always the most frequent prey across all sizes (Fig. 5.3). Diet composition differed among seasons and size group (Table 5.3); spring diet differed from all other seasons (Table 5.4). A greater abundance of unidentified fish, gobiids and polychaetes, and lower abundance of isopods, sparids and cephalopods contributed to these differences (Table 5.5). Two fish families (Sparidae and Gobiidae), cephalopods and isopods were present in the diet of both size groups in every season. No differences were detected between sexes.

A narrow niche breadth ranging from 0.08 to 0.25 was observed across seasons and group size, indicating a specialized foraging strategy. Large individuals displayed a narrower niche breadth during spring, while the highest value was found in small individuals during summer (Table 5.2).

L. forbesii

Three main prey groups were found in the diet of *L. forbesii*: fish, crustaceans and molluscs. Clustering classification and SIMPROF test also differentiated 2 size groups (Fig. 5.3): small (<140 mm ML) and large (>140 mm ML). Myctophids, pleuronectiformes and isopods were only found in stomachs of large squid, while mysids were only present in small individuals. Fish were always the most frequent prey across all sizes (Fig. 5.4).

The interaction between size and season was found to significantly influence the diet of *L. forbesii* (Table 5.3). As in *L. vulgaris*, diet did not vary between sexes. Within small *L. forbesii*, winter diet differed from that in spring (Table 5.4), with the highest consumption of euphausiids in the winter, whereas unidentified fish and pelagic gobiids (mainly *A. minuta*) dominated the diet in the spring. Gobiids and mysids were most frequently found in summer compared to autumn (Table 5.6). For large individuals, the spring diet differed from that of summer and autumn. Unidentified fish, gobiids, natantian decapods, euphausiids and gadiforms (in descending order of abundance) were the main food categories in spring, in contrast to the greater abundance of unidentified fish, natantian decapods, gadiforms, cephalopods and myctophids in summer (Table 5.6). Diet variation between autumn and spring was due to the greater consumption of unidentified fish, euphausiids, natantian decapods, myctophids and cephalopods in autumn.

L. forbesii diet also varied seasonally between size classes in summer and autumn (Table 5.4). In summer, gobiids and mysids were the main contributors to the diets of small individuals, while unidentified fish, natantian decapods, cephalopods and

gadiforms were the main prey for large individuals. During autumn, euphausiids and myctophids were absent from the diet of small individuals (Table 5.7).

Niche breadth values were overall low (0.05 to 0.27) regardless of size or season, which indicates high specialization (Table 5.2). The lowest B_i values were found in winter for both size groups, whereas a wider niche breadth was observed for large individuals during spring and autumn.
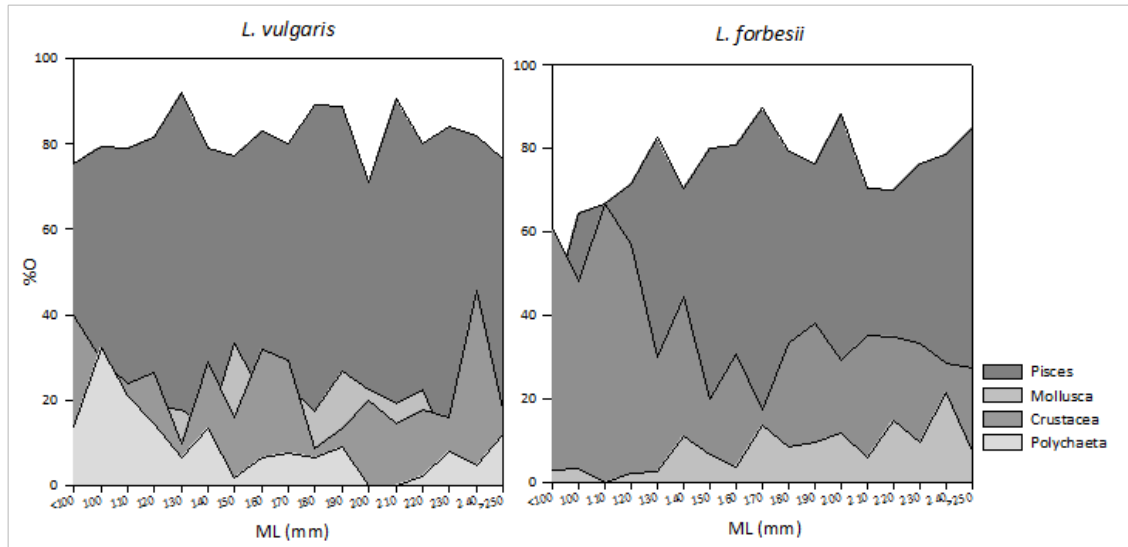
Figure 5.3. Variation with growth of the frequency of occurrence (%O) of the main prey groups (Pisces, Crustacea, Mollusca, Polychaeta) in the diet of *Loligo vulgaris* and *L. forbesii*. ML: dorsal mantle length.

5.4 Discussion

Although squid have been identified as a keystone group in Mediterranean ecosystems (Coll et al. 2006, Tsagarakis et al. 2010, Bǎnaru et al. 2013), further studies are needed to better understand their importance in the functioning of marine trophic webs in this area (Tsagarakis et al. 2010, Navarro et al. 2013), where the currently available information is very scarce (Table 5.8). In this study, we present for the first time a description of the diet of *Loligo vulgaris* and *L. forbesii* in the Mediterranean and analyse the trophic ecology of these loliginids.

Diet composition and spatial segregation

In agreement with previous work carried out in Atlantic waters, our study revealed that in the Mediterranean, both squid species display a mixed diet including pelagic, benthopelagic and benthic organisms with a clear preference for fish (e.g. Collins et al. 1994, Boyle & Pierce 1994, Pierce et al. 1994, Coelho et al. 1997, Wangvoralak et al. 2011). Although fish are known to be the most important prey for both squid, differences at the prey-specific level occur among regions probably due to their opportunistic behaviour (Jereb & Roper 2010, Wangvoralak et al.

2011). The families Ammodytidae, Gadidae, Carangidae or Gobiidae are among the most common prey along the geographic range of the squid (e.g. Collins et al. 1994, Rocha et al. 1994, Coelho et al. 1997).

Table 5.3. Results of PERMANOVA testing the effect of sex, size (small, large) and season (winter, spring, summer, autumn) for *Loligo vulgaris* and *L. forbesii*. Significant effects are shown in bold; res: residual, perm: permutation

Source	df	SS	MS	Pseudo-*F*	p(perm)	Unique perms
L. vulgaris						
Sex	1	1448.1	1448.1	0.6804	0.6628	9949
Size	1	17983	17983	8.4501	**0.0001**	9945
Season	3	17531	5843.7	2.7460	**0.0004**	9924
Sex×Size	1	2566.2	2566.2	1.2059	0.2896	9943
Sex×Season	3	3031.6	1010.5	0.4748	0.9615	9900
Season×Size	3	8403.9	2801.3	1.3163	0.1697	9920
Sex×Season×Size	3	4395.1	1465	0.6884	0.8125	9920
Res	137	2.91×10^5	2128.1			
Total	152	3.52×10^5				
L. forbesii						
Sex	1	1189.8	1189.8	0.4179	0.8440	9959
Size	1	10144	10144	3.5636	**0.0042**	9951
Season	3	22020	7340	2.5785	**0.0013**	9885
Sex×Size	1	2975.3	2975.3	1.0452	0.3855	9948
Sex×Season	3	4235.2	1411.7	0.4959	0.9446	9925
Season×Size	3	15684	5228.1	1.8366	**0.0299**	9922
Sex×Season×Size	3	3541.1	1180.4	0.4146	0.9739	9922
Res	78	2.22×10^5	2846.7			
Total	93	2.85×10^5				

In the present study, benthic gobiids and sparids were of particular importance for *L. vulgaris*, while *L. forbesii* preferred pelagic gobiids and mesopelagic species. Unfortunately, data on prey availability are absent in the area and we cannot attribute the higher frequency of these prey to their abundance in the environment. The stomach content analysis also revealed the bathymetric segregation of both species in the Mediterranean. Typical coastal species such as the sparids *Centracanthus cirrus* and *Spicara smaris* and the gobiids *Lesueurigobius* spp. were frequent prey only present in the diet of *L. vulgaris*. By contrast, shelf-break and upper slope species such as myctophids (e.g. *Ceratoscopelus maderensis*, *Lampanyctus* spp.), the argentinid *Glossanodon leioglossus* or the euphausiid *Meganyctiphanes norvegica* were the main prey of *L. forbesii* with hardly any presence in *L. vulgaris* diet. The bathymetric segregation is further evidenced by the scarce overlap of the most frequent prey.

Table 5.4. Pairwise tests for significant factors found in PERMANOVA (see Table 5.1 for abbreviations). Significant effects are shown in bold.

Species	Factors		t	p(perm)	Unique perms
L. vulgaris					
	Season				
		W, SP	1.7896	**0.0121**	9948
		W, S	1.3588	0.0895	9944
		W, A	1.2361	0.1818	9952
		SP, S	2.1402	**0.0014**	9945
		SP, A	1.8316	**0.0102**	9948
		S, A	0.6497	0.8704	9955
L. forbesii					
	Season				
	Small (<140 mm)				
		W, SP	1.5921	**0.0370**	9956
		W, S	1.7291	0.0648	3314
		W, A	1.6690	0.0719	8281
		SP, S	1.5311	0.0613	9948
		SP, A	0.9196	0.4981	9948
		S, A	1.6534	**0.0493**	3466
	Large (>140 mm)				
		W, SP	1.2983	0.1346	9944
		W, S	1.4407	0.1055	9941
		W, A	1.4650	0.1062	9941
		SP, S	1.5652	**0.0330**	9943
		SP, A	1.6702	**0.0146**	9952
		S, A	1.2079	0.2080	9950
	Size				
		W	0.7071	0.6251	8093
		SP	1.1094	0.2865	9957
		S	2.4063	**0.0005**	9894
		A	1.7135	**0.0398**	9947

These results indicate that these closely related squid segregate according to differences in horizontal habitat and food preferences. Further striking differences in the diet of both squid include cephalopods and isopods, which were more frequent in *L. vulgaris* than in *L. forbesii* (19.46% vs. 5.89% and 8.87% vs. 0.81%, respectively), euphausiids (24.95% in *L. forbesii* vs. 1.11% in *L. vulgaris*) and the importance of polychaetes (8.74%) in *L. vulgaris* that were absent in *L. forbesii* diet (see below). We also found noticeable quantities of algae in the stomach contents of small and large individuals of *L. vulgaris* that were completely absent from those of *L. forbesii.* In the Balearic Islands, the soft algae bottoms, characterized by a high abundance of free-living species and erect red algae, cover large areas of sea bottom at 40 to 90 m (Ballesteros 1994, Ordines & Massutí 2009). In previous work, algae present in *L. vulgaris* stomachs were categorized as non-food items and considered misleading owing to the carnivorous habits of this species (Macy 1982, Guerra & Rocha 1994, Pierce et al. 1994). If algae were accidentally eaten when foraging benthic prey, its appearance in squid diet should be higher in spring; however, we recorded the lowest values in that season.

Ontogenetic shift

The analysis of data at a high resolution level (e.g. family) evidenced ontogenetic changes in diet for both species that were not revealed when major groups were used, highlighting the importance of identifying prey at low taxonomic levels. Our work far outnumbered the list of prey reported in previous studies (e.g. Pierce et al. 1994, Rocha et al. 1994, Coelho et al. 1997, Wangvoralak et al. 2011), which allowed consistent statistical analysis of data by squid size. In contrast to our cluster analysis, ontogenetic changes were investigated separating individuals by maturity stages or pre-defined length classes in all previous studies (Collins et al. 1994, Pierce et al. 1994, Rocha et al. 1994, Coelho et al. 1997). From these studies, only *L. forbesii* specimens larger than 250–300 mm ML were found to vary from the small individuals (Rocha et al. 1994, Coelho et al. 1997). In studies using stable isotope analysis, *L. forbesii* did not show a clear ontogenetic pattern, whereas *L. vulgaris* showed an ontogenetic shift at 100 mm ML (Chouvelon et al. 2011).

Food preferences related to morphological and physiological changes as squid grow are expected. Accompanying these changes, species often undergo niche shifts involving diet, habitat use and interspecific interactions (Bergman & Greenberg 1994). Thus, ontogenetic variations in diet might be due to interactions between life-cycle traits (e.g. sex, maturity, size) and environmental conditions (e.g. season, climate and habitat). Given the impossibility of testing all putative drivers, 2 biotic (sex and size) and 1 abiotic (season) factors were tested in the current analysis.

Remarkably, although both species are so similar in morphology and size, *L. forbesii* underwent the shift in diet much earlier than *L. vulgaris* (140 mm *vs* 210 mm ML), suggesting that the factors triggering the ontogenetic shifts might be different. Whereas size and season statistically affected the diet of both squid, *L. forbesii* was also influenced by the interaction of those factors. This result indicates that neither of these factors on its own allows explanation for intraspecific differences in prey composition. The diet of small-sized individuals of *L. vulgaris* was more dependent on bottom-living organisms (e.g. suprabenthic/ benthic species such as mysids and gobiids) than large individuals that mainly prey on benthopelagic fish (e.g. sparids). On the other hand, prey composition in *L. forbesii* diet was only size-dependent during summer and autumn, which is probably due to intraspecific differences in depth distribution (Guerra & Rocha 1994, Stowasser 2004, Smith et al. 2013). It is likely that changes in both biotic and abiotic factors contribute to the ontogenetic diet shifts observed. Regardless of the mechanism, it is clear that size shifts in diet occur in both species, and this has important implications for both trophodynamics and marine food models (Garrison and Link 2000).

Seasonal variation

The diet of *L. vulgaris* in spring differed significantly from all other seasons. An increase in benthic prey such as polychaetes and gobiids (e.g. *Lesueurigobius* spp.) together with a decrease in highly motile prey such as sparids and cephalopods was observed in that season. Additionally, the highest values of diet specialization in large-sized individuals also occurred in spring. A shift to benthic feeding

coinciding with movement to the spawning grounds was also observed in *L. opalescens* (Karpov & Cailliet 1979). Moreover, a considerable increase in the consumption of polychaetes, mainly nereidids, was observed in both small and large *L. vulgaris* individuals. Polychaetes, and specially nereidids, are known to improve the reproductive fitness of cultured animals due to their high content of omega-3 fatty acids (García-Alonso et al. 2008); thus consumption of these prey might improve the reproductive condition of squid. Spring diet differences might also reflect variations in spatial or temporal food resource availability (Macy 1982, Collins et al. 1994).

The diet of large-sized individuals of *L. forbesii* was characterized by a high proportion of mesopelagic prey in summer and autumn during their reproduction period. Most prey were fish and crustacean species performing nycthemeral movements (e.g. *Hygophum* spp., *C. maderensis*, *Lampanyctus* spp., *Sergestes arachnipodus, Pasiphaaea sivado*) or were inhabitants of the deep scattering layer situated off-shore at 400–600 m depth (e.g. *A. hemigymnus*, *M. muelleri*) (Abelló et al. 2002, Olivar et al. 2012, Simao et al. 2014). This indicates that predator–prey interactions might occur during day–night vertical migrations of mesopelagic species or as a result of oblique offshore displacements displayed by large *L. forbesii*. Such oblique movements have been suggested for hake in our study area (Cartes et al. 2009) and the mesopelagic community around the Hawaiian Islands (Reid et al. 1991).

Vertical movements into the water column at night chasing mesopelagic prey such as lanternfish, euphausiids and mysids have also been reported in *L. pealei* (Vinogradov & Noskov 1979, Vovk 1985). Although euphausiids have been reported as important prey for other loliginids (Karpov & Cailliet 1979, Macy 1982, Vovk 1985), they have not previously been reported in *L. forbesii* (Pierce et al. 1994, Rocha et al. 1994, Wangvoralak et al. 2011), except for the smallest sizes (<60 mm) in Irish waters (Collins et al. 1994). In our results, euphausiids were the most important crustacean prey both in small and large *L. forbesii* individuals. The most frequent species was *M. norvegica*, a near-surface migrant, which is abundant down to 150 m depth in winter moving to waters below 700 m in summer (Sardou et al. 1996). Such differences agree with the aforementioned migration of large squid to deeper waters. Competition for *M. norvegica* in winter is unlikely owing to its high abundance (Sardou et al. 1996).

This euphausiid was consumed by large-sized squid year round, but only in winter and, to a lesser extent, in spring by small-sized. The transparent goby *Aphia minuta* was also a major prey for small-sized *L. forbesii* individuals in spring and summer. This gobiid supports an important small-scale fishery in the western Mediterranean which takes place in shallow epipelagic waters during winter (Iglesias et al. 1994). Driven by hydrographical conditions, the species migrates to deeper waters in spring (Iglesias & Morales-Nin 2001), where the squid would prey on them. The transparent goby was also an important resource for small *L. forbesii* in Irish waters (Collins et al. 1994).

To conclude, our study showed that, as expected, the diet composition of *L. vulgaris* and *L. forbesii* reflected the bathymetric segregation of these 2 squid species in the Mediterranean. Although both squid primarily preyed on fish, the most abundant were typical shallow shelf species in *L. vulgaris* and upper slope species in *L. forbesii*. In agreement with all previous studies, diet composition did not vary between males and females in either of the 2 squid species. Our results also revealed ontogenetic shifts of diet in both squid taking place at contrasting sizes (210 *vs* 140 mm ML, respectively), suggesting that the factors triggering these shifts might be different. During the reproduction period, *L. vulgaris* prioritizes benthic prey over nektonic prey, in particular the highly nutritive polychaetes which are known to improve the individual condition of cultured animals. Size-related differences in *L. forbesii* diet during the second half of the year indicate a deeper distribution of large individuals, which preferentially prey on mesopelagic species and are thus involved in benthic–pelagic coupling.

Table 5.5. SIMPER analysis identifying which prey contribute most (75% cut-off for low contribution) towards diet differences between size groups (Sm: small, L: large) and seasons (W: winter, SP: spring, S: summer, A: autumn) in *Loligo vulgaris* (75% cut-off for low contributions). Average abundance (Av.Ab.), average dissimilarity (Av.Diss.), standard deviation (SD), percentage contribution to the dissimilarity (Contrib%) and percentage contribution to the dissimilarity accumulated (Cum%) are shown; unid: unidentified.

	Prey items	Av.Ab.	Av.Ab.	Av.Diss	Diss/SD	Contrib%	Cum%
Size	Sm & L (Av.Diss. = 65.09%)	Group Sm	Group L				
	Pisces unid.	5.33	6.65	13.58	1.14	20.87	20.87
	Gobiidae	3.16	0.47	10.28	0.87	15.80	36.67
	Cephalopoda	2.11	1.73	8.66	0.92	13.31	49.97
	Isopoda	1.18	1.33	6.14	0.73	9.44	59.41
	Sparidae	0.71	1.25	5.17	0.60	7.95	67.36
	Polychaeta	0.90	0.53	4.07	0.48	6.25	73.61
	Algae	0.60	0.55	3.13	0.45	4.81	78.43
Season	SP & W (Av.Diss. = 61.48%)	Group SP	Group W				
	Pisces unid.	6.27	6.06	13.8	1.08	22.44	22.44
	Cephalopoda	1.45	2.77	10.11	0.93	16.45	38.89
	Gobiidae	2.58	1.09	9.77	0.78	15.89	54.78
	Isopoda	0.7	1.99	7.17	0.73	11.67	66.44
	Sparidae	0.3	1.05	4.22	0.47	6.87	73.31
	Polychaeta	1.23	0.16	4.08	0.48	6.63	79.94
	SP & S (Av.Diss. = 66.48%)	Group SP	Group S				
	Pisces unid	6.27	5.05	12.89	1.12	19.39	19.39
	Gobiidae	2.58	2.12	9.91	0.91	14.91	34.31
	Cephalopoda	1.45	1.96	7.35	0.91	11.06	45.37
	Sparidae	0.3	1.81	5.81	0.7	8.74	54.1
	Isopoda	0.7	1.7	5.49	0.87	8.25	62.36
	Algae	0.09	1.41	4.74	0.52	7.13	69.49
	Polychaeta	1.23	0.31	4.04	0.52	6.08	75.57
	SP & A (Av.Diss. = 64.80%)	Group SP	Group A				
	Pisces unid.	6.27	5.49	12.31	1.11	19	19
	Gobiidae	2.58	1.66	8.84	0.91	13.64	32.64
	Cephalopoda	1.45	2.3	7.88	0.94	12.16	44.79
	Sparidae	0.3	1.62	5.27	0.59	8.13	52.92
	Polychaeta	1.23	0.67	5.04	0.53	7.78	60.71
	Isopoda	0.7	1.25	4.95	0.56	7.64	68.35
	Algae	0.09	1.38	4.06	0.6	6.26	74.61
	Crustacea unid.	0.52	1.02	3.99	0.55	6.16	80.78

Table 5.6. SIMPER analysis identifying which prey contributed most (75% cut-off for low contribution) towards differences between seasons (W: winter, SP: spring, S: summer, A: autumn) for small and large *Loligo forbesii*. Average abundance (Av.Ab.), average dissimilarity (Av.Diss.), standard deviation (SD), percentage contribution to the dissimilarity (Contrib%) and percentage contribution to the dissimilarity accumulated (Cum%) are shown; unid: unidentified

Prey item	Av.Ab.	Av.Ab.	Av.Diss.	Diss/SD	Contrib%	Cum%
Small						
W & SP (Av.Diss. = 80.66%)	Group W	Group SP				
Euphausiacea	5.90	0.84	24.13	1.19	29.92	29.92
Pisces unid.	2.54	4.60	16.39	1.07	20.32	50.24
Gobiidae	1.73	3.20	13.97	0.87	17.32	67.56
Crustacea unid.	1.36	0.79	5.63	0.75	6.98	74.54
Sternoptychidae	0.81	0.81	5.23	0.48	6.48	81.03
S & A (Av.Diss. = 82.73%)	Group S	Group A				
Gobiidae	6.05	1.33	23.66	1.36	28.60	28.60
Pisces unid.	1.24	6.08	22.46	1.35	27.15	55.76
Mysidacea	4.75	1.15	18.27	1.03	22.09	77.84
Large						
SP & S (Av.Diss. = 71.80%)	Group SP	Group S				
Pisces unid.	3.61	5.45	13.00	1.14	18.11	18.11
Natantia	1.60	2.58	8.93	0.98	12.44	30.55
Gobiidae	2.58	0.27	8.27	0.73	11.52	42.06
Gadiformes	1.37	2.23	8.03	0.89	11.18	53.24
Euphausiacea	1.58	1.74	7.84	0.74	10.92	64.16
Cephalopoda	0.94	2.09	7.55	0.80	10.51	74.67
Myctophidae	0.69	1.46	4.91	0.79	6.84	81.52
SP & A (Av.Diss. = 71.31%)	Group SP	Group A				
Euphausiacea	1.58	3.83	10.10	1.18	14.16	14.16
Pisces unid.	3.61	4.16	9.75	1.14	13.68	27.84
Natantia	1.60	3.13	8.22	1.36	11.52	39.36
Gobiidae	2.58	1.13	7.56	0.82	10.61	49.97
Myctophidae	0.69	2.61	6.49	1.72	9.10	59.07
Cephalopoda	0.94	2.02	6.29	1.14	8.82	67.89
Sternoptychidae	0.96	1.34	4.80	0.71	6.73	74.62
Gadiformes	1.37	0.74	4.52	0.63	6.34	80.97

Table 5.7. SIMPER analysis identifying which prey contributed most (75% cut-off for low contribution) towards differences between small (Sm) and large (L) *Loligo forbesii* during summer and autumn. Average abundance (Av.Ab.), average dissimilarity (Av.Diss.), standard deviation (SD), percentage contribution to the dissimilarity (Contrib%) and percentage contribution to the dissimilarity accumulated (Cum%) are shown; unid: unidentified.

Prey item	Av.Ab.	Av.Ab.	Av.Diss.	Diss/SD	Contrib%	Cum%
Summer						
S & L (Av.Diss. = 84.06%)	Group S	Group L				
Gobiidae	5.50	0.35	16.58	1.18	19.72	19.72
Mysidacea	4.89	0.00	14.55	0.94	17.31	37.03
Pisces unid.	1.74	5.79	13.18	1.52	15.68	52.71
Natantia	0.00	2.89	8.74	1.06	10.39	63.11
Cephalopoda unid.	0.37	2.81	7.92	1.46	9.42	72.53
Gadiformes	0.53	2.28	6.09	1.29	7.24	79.77
Autumn						
S & L (Av.Diss. = 69.51%)	Group S	Group L				
Natantia	4.36	3.51	10.78	1.29	15.50	15.50
Euphausiacea	0.00	3.81	10.16	1.10	14.61	30.11
Pisces unid.	4.65	4.63	8.90	1.48	12.81	42.93
Myctophidae	0.00	3.23	8.47	3.02	12.19	55.12
Cephalopoda unid.	1.92	1.49	6.46	1.54	9.30	64.41
Gobiidae	1.45	1.06	4.38	0.99	6.30	70.71

Table 5.8. Prey composition (frequency of occurrence, %) of Atlantic *Loligo vulgaris* and *L. forbesii* and Mediterranean squid, according to the relevant literature (and N>75 stomachs). SP: sampling period; N: number of stomachs examined; Ceph: cephalopods, Crust: crustaceans, Poly: polychaetes.

Ocean	Species	Area	SP	N	Fish	Crust	Ceph	Poly	Reference
Atlantic Ocean	*Loligo vulgaris*	Lisbon	1990-1993	268	74	26	31	1	Pierce et al. 1994
		Algarve	1990-1993	137	67	16	18	17	Pierce et al. 1994
		Galicia	1991-1992	723	79	7	6	9	Rocha et al. 1994,
			1991-1992	662	87	3	6	2	Guerra and Rocha 1994
		Gulf of Cadiz	2008	77	70	9	20		Vila et al. 2010
	Loligo forbesii	Azores	1980-1981	622	82	12	13		Martins 1982
		Scotland	1990-1993	2122	85	20	8		Pierce et al. 1994
		Lisbon	1990-1993	267	54	55	7		Pierce et al. 1994
		Azores	1990-1993	224	94	22	19		Pierce et al. 1994
		Ireland	1991-1993	1293	73	26	8	<1	Collins et al. 1994
		Galicia	1991-1992	440	73	18	9		Rocha et al. 1994
			1991-1992	371	76	17	4		Guerra and Rocha 1994
		Algarve	1991-1994	964	73	7	19	2	Coelho et al. 1997
		Saharan Bank	1993-1994	848	51	20	26		Coelho et al. 1997
		Scottish Sea	2006-2007	360	96	29	11		Wangvoralak et al. 2011
Mediterranean Sea	*Loligo vulgaris*	Balearic Sea	2007-2010	1452	80	22	19	9	present study
	Loligo forbesii	Balearic Sea	2007-2010	900	73	39	6		present study
	Todarodes sagittatus	Balearic Sea	1995-1996	348	85	49	30		Quetglas et al. 1999
		Catalan Sea	2010-2012	94	54	53	18		Rosas-Luis et al. 2014
	Histioteuthis reversa	Balearic Sea	1996-2008	220	67	30	4		Quetglas et al. 2010
	Illex coindetii	Catalan Sea	1976-1979	802	65	30	5		Sánchez 1982
		Catalan Sea	2010-2012	121	60	72	11		Rosas-Luis et al. 2014
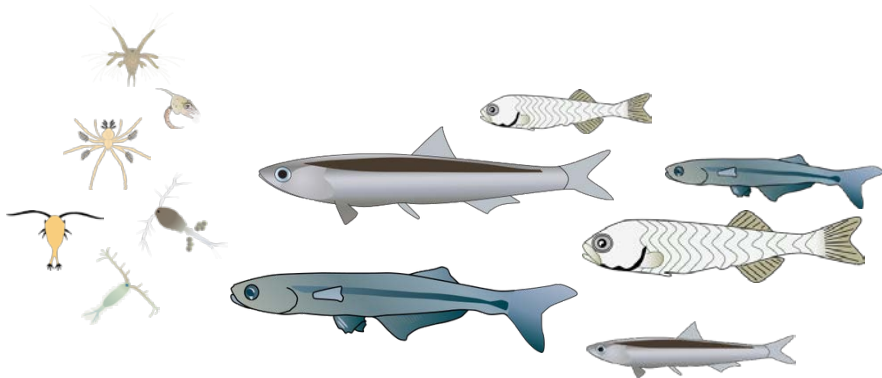
Chapter 6. Trophic structure of mesopelagic fishes in the western Mediterranean based on stable isotopes of carbon and nitrogen

Chapter 6. Trophic structure of mesopelagic fishes in the western Mediterranean based on stable isotopes of carbon and nitrogen

6.1 Introduction

Mesopelagic fishes nominally inhabit the water column between 200 to 1000 m (Gartner Jr. et al., 1997). For some species displacement is restricted to below the euphotic zone e.g. *Cyclothone* spp. (Badcock and Merrett, 1976) but many e.g. myctophids occur outside these depth bounds for short periods, even reaching surface layers during nyctimeral migrations (Hulley, 1986). Vertical migration follows prey movement and consequently mesopelagic fishes are important consumers of a wide variety of zooplankton (Pakhomov et al., 2006; Petursdottir et al., 2008) and in turn become significant prey for demersal, benthopelagic and other large pelagic fishes (Bulman et al., 2002; Cartes et al., 2009; Pakhomov et al., 2006), cephalopods (Phillips et al., 2001; Quetglas et al., 2010), seabirds (Hedd and Montevecchi, 2006; Navarro et al., 2009) and mammals (Cherel et al., 2008; Cherel et al., 2010).

Species that migrate to the near surface layers at night have very high caloric, lipidic and proteic contents relative to phylogenetically close species and stages resident at greater depths (Childress et al., 1990; Childress and Nygaard, 1973; Bailey and Robison, 1986) and thus represent particularly valuable prey items. In the highly oligotrophic western Mediterranean, mesopelagic fishes also form an important dietary contribution to the deep sea ecosystem (Cartes et al., 2009; Quetglas et al., 2010; Valls et al., 2011). Therefore, mesopelagic fish migration plays an important role in the transfer of matter synthesized in the euphotic zone to demersal and benthopelagic species, and consequently it is of foremost importance to ascertain the trophic position of these species.

Exploration of trophic structure in the higher mesopelagic food web, particularly in the Mediterranean have dealt with a few species, mainly to the oldest life stages of mesopelagic fishes collected at the benthic boundary layer (Fanelli et al., 2009; Papiol et al., 2012; Stefanescu and Cartes, 1992) and just a few include species in the water column (Bernal et al., 2013; Palma, 1990). This is the first attempt to analyse trophic structure of the pelagic assemblage of mesopelagic fishes in the Mediterranean.

Marked oligotrophic condition of the western Mediterranean makes mesopelagic vertical transport especially important to the benthos on the insular slope where it depend more directly on planktonic and nektonic prey along the water column (Cartes et al., 2008; Maynou and Cartes, 2000). However, compared to the nearby northeast Atlantic, Mediterranean mesopelagic fish assemblages are depauperate in species (Goodyear et al., 1972; Hulley, 1984; Olivar et al., 2012; Roe and Badcock, 1984). Although there are no estimates of the overall abundance of

mesopelagic species in the Mediterranean, lanternfishes (Myctophidae) and lightfishes (Gonostomatidae) usually dominate in both number of individuals and number of species (Goodyear et al., 1972; Olivar et al., 2012), and analysis of acoustic echograms also point to dominance in biomass (Olivar et al., 2012, Peña et al., 2014). Mesopelagic species therefore play important trophic functions in the Mediterranean marine system as a function of their abundance and in the interplay and transfer of energy between system components.

Mesopelagic fish may be grouped as vertically migratory to the epipelagic layers (mostly myctophids) and non-migratory (mostly the small size gonostomatid species) (Olivar et al., 2012). In addition to inter-species differences in vertical migration, intra-specific differences may also occur in relation to body size, with the largest individuals often remaining at depth (Olivar et al., 2012). Vertical migration has been advocated to follow the upper vertical migrating zooplankton, and the coincidence of large number of individuals performing the same behaviour and size based changes in behaviour imply competition for food and may involve intra and inter-species variations in feeding pattern and niche partitioning. Information on gut contents of mesopelagic fishes indicates that they may be micronektonivores, zooplanktivores and generalists (Gartner Jr. et al., 1997). While gut content approaches provide high dietary taxonomic resolution, the approach is restricted by short temporal representation, and includes substantial challenges in prey identification and biases from differential rates of digestibility (Hyslop, 1980). Such shortfalls may be mitigated by the use of alternative trophic techniques like stable isotope analyses (Miller et al., 2010).

Stable isotope analysis for food web studies are predicated on a stepwise change in the ratio of heavy and light atoms of carbon (^{12}C:^{13}C as $\delta^{13}C$) and nitrogen (^{14}N:^{15}N as $\delta^{15}N$) that generally occurs between consumer and dietary resource (Deniro and Epstein, 1981; Hobson et al., 1995; Minagawa and Wada, 1984; Petursdottir et al., 2008). $\delta^{13}C$ values are indicative of the food carbon source and habitat (Cherel et al., 2010), whereas $\delta^{15}N$ acts as an indicator or trophic level (Sweeting et al., 2007a).

Isotope based trophodynamic assessment of myctophiforms and or stomiiforms is limited. Existing data is dispersed globally and includes the sub-Antarctic (Cherel et al., 2010), Southern Ocean (Choy et al., 2012) and southern Tasman sea (Flynn and Kloser, 2012). Species have also been included in wider studies of food web structure (e.g., Cardona et al., 2012; Hedd and Montevecchi, 2006; Nilsen et al., 2008; Revill et al., 2009; Sugisaki and Tsuda, 1995) but were often sampled incidentally, for example in Mediterranean where mesopelagic species have been collected in association with bentho-pelagic and demersal food webs (Fanelli et al., 2009; Fanelli et al., 2011a; Navarro et al., 2011; Papiol et al., 2012; Tecchio et al., 2013). No work has systematically addressed the Mediterranean mesopelagic migrant community or explored trophodynamics at intra-population resolution.

The objectives of the present study are therefore to analyse trophic structure of the mesopelagic fishes at assemblage, interspecific and intra-population resolution in the Mediterranean and test generality in space and time. Specifically this study will use C and N stable isotope analyses of the 18 most abundant mesopelagic fishes and the associated likely preys, inhabiting the shelf-break and the slope of the western Mediterranean, and will examine inter species variation in i) trophic level and ii) potential food sources. This will be undertaken in iii) contrasting periods (late autumn mixing period and summer stratification season) at iv) two locations with expected isotopic changes in baseline due to contrasting environmental conditions, to establish generality and stability of trophic behaviour in the mesopelagic assemblage. A subset of the most numerous fish species will be assessed further using v) Bayesian mixing models to compare utilisation of potential food sources among closely related species to test for niche partitioning and vi) drivers of trophodynamics within species, particularly body size.

6.2 Material and methods

Source of the samples

Mesopelagic fishes and zooplankton were collected in two cruises off Mallorca Island (Balearic Islands, Western Mediterranean) in late autumn (December 2009) and summer (July 2010) at two locations on the southern (Cabrera) and northwestern (Sóller) above two depth strata (shelf and slope).

Mesopelagic fishes were collected at the near surface (40-80 m) or 400 m-Deep Scattering Layers (Olivar et al., 2012) with a pelagic midwater trawl. After on board identification specimens were frozen to -20°C until stable isotope analysis (SIA). Older life history stages of *L. crocodilus* were collected contemporaneously from bottom trawls.

Macro, mesozooplankton and microzooplankton samples were caught from vertical hauls by different small nets (IKMT, RMT, and MOCNESS) Samples were oven dried at 60°C on board and kept in a desiccator until preparation for SIA. Adult stages of the euphausiid *Meganycthiphanes norvegica* were collected from the pelagic midwater trawl with fishes, and kept frozen until analysis.

Particulate organic matter (POM) samples used in this study were collected from year-round moored time-series sediment. Only data taken a month before each survey (November and June) were considered to provide a better temporal match with macrofauna. The sampling methods can be found in Chapter 2 (section 2.1.1.2)

Stable isotopes analyses

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analyses were conducted on the 18 most abundant mesopelagic fish species (Table 6.1) and their most probable preys, i.e., the bulk zooplankton by size fraction (micro, meso and

macrozooplankton), the euphausiid *Meganycthiphanes norvegica* (adults) and POM. Zooplankton samples were left unacidified as i) previous analysis suggest only limited inorganic carbon bias (Bode et al., 2003; Bunn et al., 1995; Letessier et al., 2012) and ii) to maintain standardization among samples. Species sampling and stable isotope analysis are explained in Chapter 2 (Section 2.2.2)

Preliminary analyses of isotopic baselines were then conducted to inform any spatio-temporal isotope correction and to identify prey sources for later application to SIAR diet mixing models. Differences in zooplankton $\delta^{13}C$ and $\delta^{15}N$, among locations (Sóller and Cabrera), bathymetric strata (water column above shelf and slope), size (meso-, macrozoopalnkton and euphausiids) and season (late autumn and summer) were explored using ANOVA. POM variability by season could not be statistically tested due to small number of samples a function of logistical constraints.

Community metrics such total convex hull area (TA) and distance to the centroid (CD) in the $\delta^{13}C$-$\delta^{15}N$ isotopic space were calculated according to Layman et al., (2007). TA is the area of the smallest convex polygon containing all the species and can be used as a measure of trophic diversity. CD is the average Euclidean distance of each species to the $\delta^{13}C$-$\delta^{15}N$ centroid and provides a measure of the degree of trophic diversity. These metrics were calculated using the package 'siar' (Parnell and Jackson, 2013) for R v3.0.2 (R Development Core Team, 2013).

Trophic level (TrL) was calculated following the equation:

$$TrL_i = ((\delta^{15}N_i - \delta^{15}N_{ref}) / \Delta\delta^{15}N) + \lambda$$

where TrL_i is the trophic level of species i, $\delta^{15}N_i$ is the mean species $\delta^{15}N$, $\delta^{15}N_{ref}$ is the mean $\delta^{15}N$ of the food web baseline, $\Delta\delta^{15}N$ is the discrimination factor per trophic level and λ is the trophic level of the baseline. The value of $\delta^{15}N_{ref}$ was estimated from the 50-200 μm fraction of plankton (mean $\delta^{15}N=3.96‰±0.40$) as it was mostly composed of early stages of copepods, nauplii and copepodites (Fernández de Puelles et al., 2014) and other small zooplankters mostly filter feeders ($\lambda=2$). Mean isotopic discrimination factor of mesopelagic fishes was 3.15‰ obtained from the global mean of a fish specific data set (Sweeting et al., 2007b; Sweeting et al., 2007a).

Spatial and temporal variation in $\delta^{13}C$ and $\delta^{15}N$ differences in mesopelagic fauna (excluding *L. crocodilus* from the bottom), among locations, bathymetric strata and season were tested by ANOVA or permutational analysis of variance (PERMANOVA) as appropriate to the data structure followed by post hoc pair-wise tests. All factors were treated as fixed, significance was set at p=0.05 and *p* values were obtained using 9999 permutations of the untransformed data. As well as informing on systematic influences of species isotope signatures, these analysis dictated sample pooling for subsequent exploration of assemblage structure, resource mixing and exploration of intra-population trophodynamics.

Assemblage structure was explored using Cluster Analysis (average grouping methods) conducted on the resemblance matrix (Euclidean distance) of mean $\delta^{15}N$ and $\delta^{13}C$ species values. $\delta^{15}N$ showed no seasonality but $\delta^{13}C$ required standardisation ($\delta^{13}C_{sd}$). Standardisation was to a year average mid-point where $\delta^{13}C$ difference for each species between seasons was calculated (*i* differences from

i species), then the mean of that difference (*1-i*) was either subtracted or added to all individuals in either late autumn or summer season.

Inter-species analysis focused on temporal and species specific prey utilisation of common mesopelagic fishes using the Bayesian mixing model SIAR v4.1.3 (Stable Isotope Analysis in R) of (Parnell et al., 2010). Only those mesopelagic fishes with at least three specimens per season were included in the model.

SIAR analysis was conducted on 9 species of mesopelagic fish that contrasted in trophic level (*C. braueri* vs *L. dofleini*), represent different species of the same genus (*H. benoiti* vs *H. hygomii*, or *L. pusillus* vs *L. crocodilus*), inhabit different locations in the water column (mesopelagic *L. crocodilus* vs *L. crocodilus* from the bottom), or might have competition for food (the most common and abundant migratory myctophids, i.e., *C. maderensis* vs *N. elongatus,* and the most common and abundant non-migratory stomiiforms, i.e., *C. braueri* and *A. hemigymnus*).

Potential dietary endpoints applicable to all species included in SIAR analysis were derived from published data on stomach contents of mesopelagic fishes and authors observations. Models included 4 prey groups i) mesozooplankton ii) adult euphausiids (Bernal et al., 2013; Champalbert et al., 2008; Pakhomov et al., 2006; Sutton, 2005; Williams et al., 2001; Bernal, own observations), iii) other mesopelagic fishes (Podrazhanskaya, 1993; Roe and Badcock, 1984; Sutton, 2005) constructed from the isotopic mean of all mesopelagic fishes shorter than 40 mm SL and iv) POM; particulate material identified as marine snow or detrital material (Miller et al., 2012; Palma, 1990; Bernal, own observations). Due to seasonal differences in the potential preys, isotopic signatures mixing models were run separately for late autumn and summer. These four groups describe the potential sources covering all major diet sources, except for gelatinous plankton.

No trophic enrichment factors exists specifically for mesopelagic fishes, not an uncommon scenario (Galvan et al., 2012). This study therefore used literature derived means (and standard deviations) and explored the sensitivity of analysis by running five mixing models using different trophic enrichment factors that have been previously used in other studies dealing with fish muscle (Caut et al., 2009; Pinnegar and Polunin, 1999; Sweeting et al., 2007a, 2007b; Trueman et al., 2005; Vander Zanden and Rasmussen, 2001) (Table 6.2).

Intra-population variation in trophodynamics explained by body size is a frequent determinant of fish trophodynamics (Galvan et al., 2010). δ^{15}N and δ^{13}C relationships with size (as standard length, SL) were explored using least squares linear regression. In addition, to investigate variability in δ^{13}C associated with varying lipid content, C:N and non-lipid normalized δ^{13}C data were explored. Analysis was conducted for the most frequently sampled species that included *L. crocodilus, C. maderensis* and *N. elongatus* where samples size was acceptable and sampling included 61%, 30% and 47% of their length range respectively. This encompassed all the size range reported for these species in the water column in the western Mediterranean (Goodyear et al., 1972; Olivar et al., 2012).

Statistical analyses were conducted using STATISTICA 11. Shapiro-Wilk and Levène tests were used to assess the normality and homogeneity of variances respectively. All the multivariate analyses were performed with PRIMER 6 +

PERMANOVA software package from Plymouth Marine Laboratory, UK (Anderson et al., 2008).

6.3 Results

Baseline Identification

Mesozooplankton, macrozooplankton fractions and adults of euphausiids, exhibited lower $\delta^{13}C$ in summer (mean $\delta^{13}C= -20.69\pm0.40‰$) than late autumn (mean $\delta^{13}C = -19.45\pm0.42‰$) ($F_{1.45}=55.59$, p<0.001) but there was no influence of locations, bathymetric strata or size fraction (all p>0.05). $\delta^{15}N$ was independent of season, location or bathymetric strata (all p>0.05), but $\delta^{15}N$ differed among plankton size fractions ($F_{2.45}=66.25$, p<0.001), with higher values with the larger size (meso<macro<adults euphausiids) and statistically significant differences between adult euphausiids and the other smaller size fractions (p<0.001).

POM sample size was small, a consequence of logistic constraints on collection. POM data however, reflected that of zooplankton with a seasonal offset of 1.2‰ for $\delta^{13}C$ and $\delta^{15}N$ being similar among seasons within analytical precision (mean ±SD; $\delta^{13}C=-23.72\pm0.04‰$, $\delta^{15}N=2.34\pm0.22‰$ and $\delta^{13}C=-22.50\pm0.69‰$, $\delta^{15}N=2.55\pm1.08‰$ in summer and late autumn respectively) (Fig. 6.1).
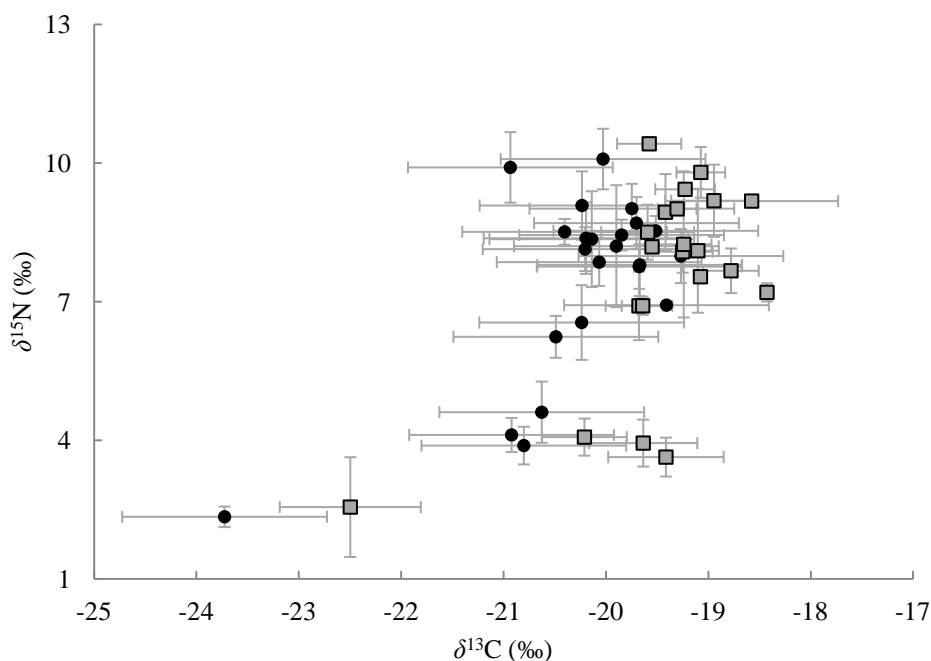
Figure 6.1. Scatterplot of the $\delta^{15}N$ and $\delta^{13}C$ values (mean ± s.d., ‰) of POM, zooplankton (micro-, meso-, macrozooplankton and adults of *M. norvegica*) and mesopelagic fishes from the Balearic Sea collected during July 2010 (black circles) and December 2009 (grey squares) surveys.

Table 6.1. Trophic level, size range (in mm), $\delta^{13}C$, $\delta^{15}N$ and C/N (‰ mean values, ±sd) of mesopelagic species and their potential preys sampled in 2009 and 2010. B: bottom; n: number of replicates.

Taxa/Family	Prey/Predator	TrL	December 2009					July 2010				
			n	Size (mm)	$\delta^{13}C'$ ‰	$\delta^{15}N$ ‰	C/N	n	Size (mm)	$\delta^{13}C'$ ‰	$\delta^{15}N$ ‰	C/N
POM	POM		7		-22.79 ± 0.41	2.33 ± 1.21		3		-23.72 ± 0.04	2.34 ± 0.22	
Zooplankton	Macrozooplancton	2.1 ± 0.2	6	>0.5	-19.64 ± 0.53	3.94 ± 0.51	4.16 ± 0.21	12	>0.5	-20.63 ± 0.69	4.61 ± 0.66	4.24 ± 0.37
Zooplankton	Mesozooplancton	2.0 ± 0.1	7	0.2-0.5	-19.41 ± 0.56	3.64 ± 0.42	3.91 ± 0.52	11	0.2-0.5	-20.92 ± 0.39	4.11 ± 0.37	4.28± 0.42
Zooplankton	Microzooplancton	2.0 ± 0.1	7	0.053-0.2	-20.21± 0.41	4.07 ± 0.40	4.78 ± 0.61	11	0.053-0.2	-20.80 ± 0.91	3.88 ± 0.41	5.66 ± 0.83
Euphausiacea	*Meganycthiphanes norvegica*	2.8 ±0.2	5		-19.68 ± 0.33	6.91 ± 0.74	3.37 ± 0.27	5		-20.49 ± 0.14	6.24 ± 0.45	3.65 ± 0.24
Aulopiformes	*Arctozenus rissoi*	3.2 ± 0.2	2	132-148	-18.43 ± 0.02	7.20 ± 0.20	3.48 ± 0.02	3	168-193	-19.85 ± 0.16	8.44 ± 0.33	4.44 ± 0.53
Myctophiformes	*Benthosema glaciale*	3.6 ± 0.2	3	37-41	-19.59 ± 0.45	8.50 ± 0.60	4.58 ± 0.16	6	35-42	-19.75 ± 0.38	9.01 ± 0.54	5.19 ± 1.03
Myctophiformes	*Ceratoscopelus maderensis*	3.3± 0.2	11	38-54	-19.25 ± 0.29	8.08 ± 0.46	3.59 ± 0.22	9	50-59	-20.14 ± 0.46	8.35 ± 1.04	3.80 ± 0.55
Myctophiformes	*Diaphus holti*	3.7 ± 0.3	3	25-48	-19.42 ± 0.05	8.94 ± 0.83	3.78 ± 0.57	3	43-49	-20.93 ± 0.26	9.91 ± 0.76	5.16 ± 0.17
Myctophiformes	*Electrona risso*	3.4 ± 0.2	1	50	-19.55	8.19	3.41	3	43-45	-20.19 ± 0.25	8.37 ± 0.77	3.73 ± 0.07
Myctophiformes	*Hygophum benoiti*	3.2 ± 0.2	1	56	-19.08	7.54	3.56	3	46-48	-19.27 ± 0.21	7.99 ± 0.59	3.45 ± 0.15
Myctophiformes	*Hygophum hygomii*	3.6 ± 0.2	2	56-58	-18.94 ± 0.42	9.18 ± 0.78	3.37 ± 0.01	3	41-47	-20.23 ± 0.38	9.08 ± 0.74	3.41 ± 0.05
Myctophiformes	*Lampanyctus crocodilus*	3.3 ± 0.2						8	55-69	-20.07 ± 0.37	7.86 ± 0.52	3.51 ± 0.29
Myctophiformes	*Lampanyctus crocodilus B*	3.8 ± 0.3	9	121-177	-18.87 ± 0.36	9.55 ± 0.98	3.10 ± 0.07	13	108-181	-18.83 ± 0.31	9.66 ± 0.76	3.09 ± 0.07
Myctophiformes	*Lampanyctus pusillus*	3.5 ± 0.2						4	37-41	-19.70 ± 0.38	8.70 ± 0.56	3.95 ± 0.39
Myctophiformes	*Lobianchia dofleini*	4.0 ± 0.1	3	32-32	-19.58 ± 0.31	10.42 ± 0.06	3.55 ± 0.19	3	34-37	-20.03 ± 0.40	10.09 ± 0.66	4.03 ± 0.03
Myctophiformes	*Myctophum punctatum*	3.3 ± 0.3	3	52-60	-18.78 ± 0.27	7.67 ± 0.48	3.41 ± 0.20	3	41-54	-19.90 ± 0.50	8.20 ± 1.32	3.74 ± 0.20
Myctophiformes	*Notoscopelus elongatus*	3.6 ± 0.2	5	64-83	-19.07 ± 0.24	9.80 ± 0.55	4.06 ± 0.49	9	39-95	-20.40 ± 0.46	8.51 ± 0.28	4.10 ± 0.55
Myctophiformes	*Symbolophorus veranyi*	3.3 ± 0.3	4	45-130	-19.10 ± 0.21	8.10 ± 1.34	3.59 ± 0.49	3	61-84	-20.20 ± 0.23	8.14 ± 0.48	4.10 ± 0.87
Stomiiformes	*Argyropelecus hemigymnus*	3.1 ± 0.5	4	13-39	-19.24 ± 0.27	8.24 ± 1.58	3.12 ± 0.12	3	26-29	-20.24 ± 0.08	6.55 ± 0.81	3.55 ± 0.27
Stomiiformes	*Maurolicus muelleri*	3.4 ± 0.3	3	37-39	-19.30 ± 0.19	9.01 ± 0.03	4.23 ± 0.35	3	36-39	-19.67± 0.15	7.80 ± 0.67	3.35 ± 0.05
Stomiiformes	*Stomias boa*	3.5 ± 0.1	2	105-125	-18.58 ± 0.84	9.18 ± 0.09	3.32 ± 0.08	3	76-122	-19.51 ± 0.53	8.53 ± 0.32	3.23± 0.10
Stomiiformes	*Vinciguerria attenuata*	3.5 ± 0.3	3	35-37	-19.23 ± 0.29	9.43 ± 0.14	3.26 ± 0.06	3	34-36	-19.67 ± 0.15	7.76 ± 0.48	3.45 ± 0.02
Stomiiformes	*Cyclothone braueri*	2.9 ± 0.1	5	30-30	-19.64 ± 0..20	6.91 ± 0.20	3.79 ± 0.30	1	30	-19.41	6.92	3.38

Trophic Structure - Assemblage

Mean $\delta^{13}C$ of the 18 mesopelagic fish species spanned a small range of $\delta^{13}C$, only 1.01 ‰ and 1.21‰ during summer and late autumn respectively. Species mean $\delta^{15}N$ spanned 3.54‰ in summer and 3.51‰ in late autumn, equivalent to ~1.1 trophic levels (assuming trophic discrimination of nitrogen of 3.15‰) (Fig. 6.1). $\delta^{13}C$ reflected basal patterns being lower in summer than late autumn (F=35.498 p<0.001). $\delta^{15}N$ and trophic level were similar between seasons, locations and strata (all paired t-test p>0.05), therefore data were pooled to obtain a mean trophic level value per species.

When microzooplankton represents the second trophic level, Trophic level of the mesopelagic fish fell between 2.9 for *C. braueri* to 4.0 for *L. dofleini* (Fig. 6.2). Trophic level among fish species were significantly different (ANOVA, $F_{18,132}=7.972$ p<0.001), with differences among species of lower levels like *C. braueri* and *A. hemigymnus* and those of the top e.g. *Diaphus holti*, *L. dofleini*, *N. elongatus* and the bottom dwelling stages of *L. crocodilus*.
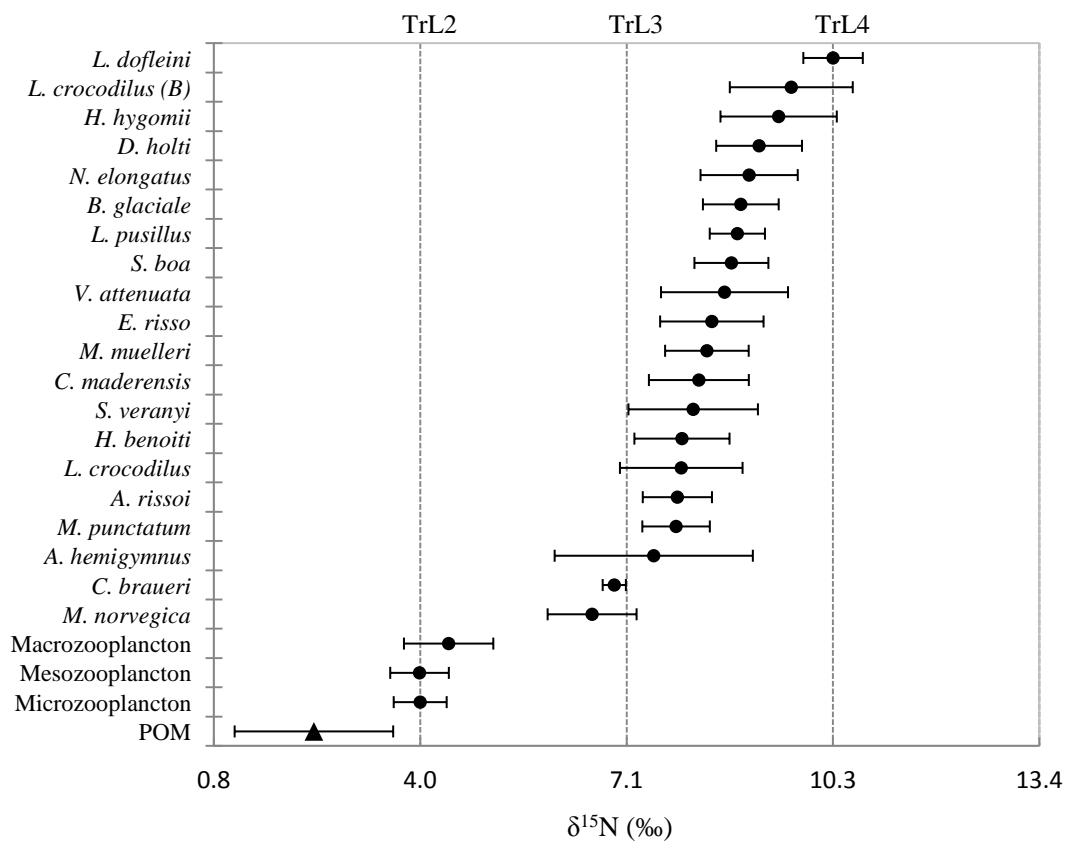
Figure 6.2. $\delta^{15}N$ (mean ± s.d., ‰) values and estimated Trophic level (TrL) of mesopelagic fishes and their potential preys.

Cluster analysis on the 18 mesopelagic fish species, zooplankton (the adult euphausiid *M. norvegica* and bulk of the three zooplankton fractions) and POM identified 5 trophic groups (TG) (Fig. 6.3). Comparisons between isotopic signatures and the biological characteristics within the assemblage, allowed a description of the different isotopic niches with clusters being strongly driven by differences in δ^{15}N.

The lowest trophic level values identified POM at the base of the food chain (TG 1) and primary consumers (the three size-groups of bulk zooplankton) (TG 2). Higher consumers were subdivided into three clusters: TG3 was occupied by the small stomiiform *C. braueri* and the adult euphausiid *M. norvegica*, which exhibited lower isotopic signature (mean δ^{15}N=6.74‰, mean δ^{13}C=-19.98‰) than TG4 and TG5. TG4 was the largest and middle group in the cluster containing 2 subgroups differentiated by their trophic level (4a: mean δ^{15}N=7.96‰, 4b: mean δ^{15}N=8.77‰) but with similar mean δ^{13}C values (-19.5‰). And finally, TG5 included *L. dofleini* and *D. holti* with the highest δ^{15}N signatures (mean δ^{15}N=9.42‰) which equates to trophic level of approximately 4.

Overall community metrics TA (6.78 and 7.20 for summer and autumn respectively) and CD (1.36 and 1.50 for summer and autumn respectively) were similar between cruises, although showed higher values for the autumn period.
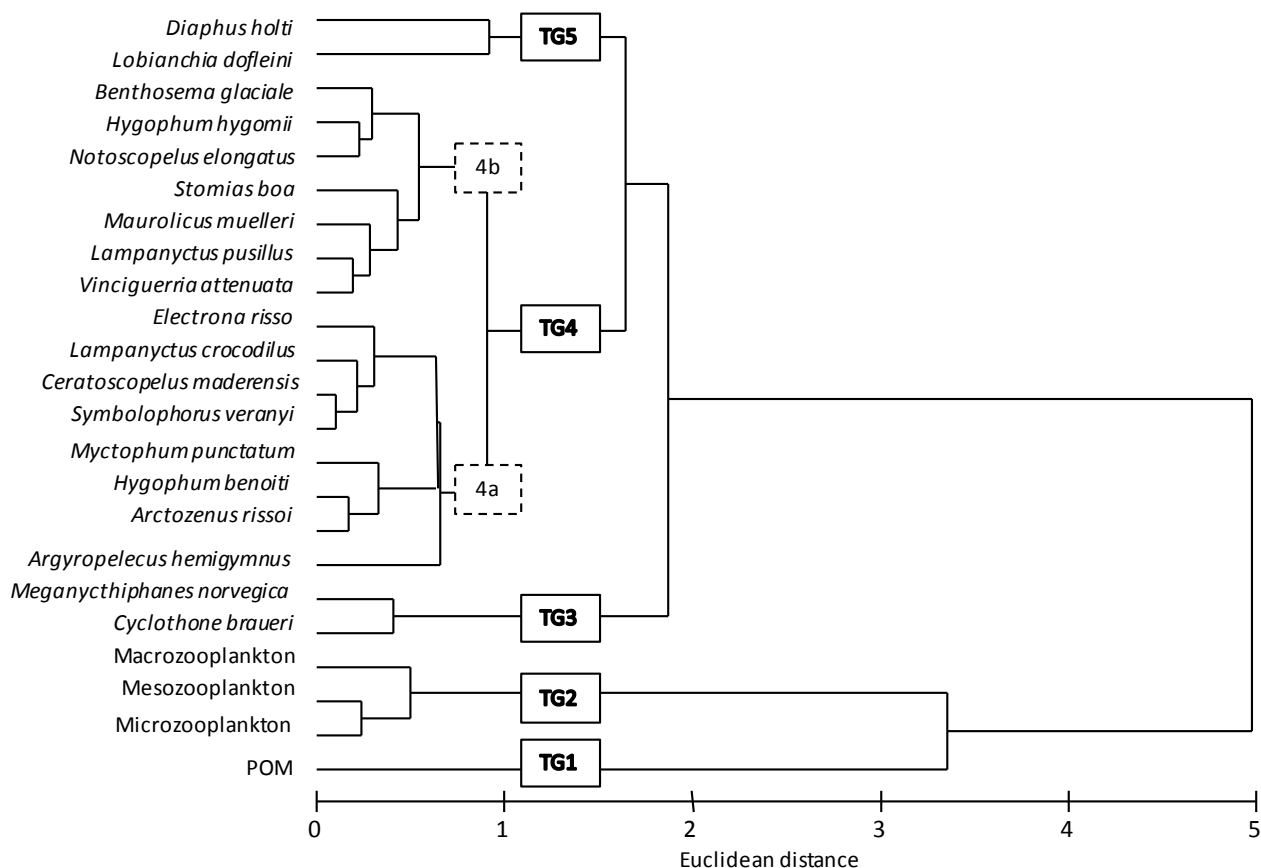
Figure 6. 3. Cluster analysis based on mean δ^{13}C and δ^{15}N values (see text) showing the mesopelagic trophic groups (TG1-TG5).

Table 6.2. Estimated contribution (mean ± sd) of the four potential preys to the diet of several mesopelagic species of the western Mediterranean during late autumn (December, 2009) and summer (July 2010) cruises. Trophic discrimination factors (TDF) coming from Sweeting et al. (2007a, b), Vander Zanden and Ramussen, (2001) and Caut et al., (2009) are included for comparisons.

	Sweeting et al. (2007a, b)				Vander Zanden and Ramussen (2001)				Caut et al. (2009)			
	POM	Zoopl	Euf	Fish	POM	Zoopl	Euf	Fish	POM	Zoopl	Euf	Fish
December												
A. hemigymnus	0.20 ± 0.09	0.24 ± 0.12	0.28 ± 0.11	0.28 ± 0.11	0.17 ± 0.10	0.29 ± 0.12	0.28 ± 0.13	0.27 ± 0.11	0.28 ± 0.08	0.22 ± 0.11	0.26 ± 0.13	0.25 ± 0.11
C. braueri	**0.37** ± 0.08	**0.35** ± 0.11	0.17 ± 0.10	0.11 ± 0.08	**0.35** ± 0.10	0.51 ± 0.13	0.08 ± 0.07	0.05 ± 0.05	**0.47** ± 0.08	**0.34** ± 0.11	0.12 ± 0.09	0.08 ± 0.06
C. maderensis	0.25 ± 0.05	**0.31** ± 0.07	0.25 ± 0.09	0.19 ± 0.08	0.17 ± 0.07	**0.49** ± 0.09	0.22 ± 0.10	0.12 ± 0.07	**0.35** ± 0.04	0.29 ± 0.07	0.23 ± 0.10	0.14 ± 0.07
L. crocodilus B	0.08 ± 0.06	0.18 ± 0.10	**0.34** ± 0.13	**0.40** ± 0.10	0.06 ± 0.05	0.20 ± 0.09	**0.36** ± 0.15	**0.39** ± 0.11	0.14 ± 0.06	0.16 ± 0.09	**0.34** ± 0.13	**0.37** ± 0.10
L. dofleini	0.20 ± 0.11	0.17 ± 0.11	0.29 ± 0.14	**0.34** ± 0.14	0.13 ± 0.09	0.16 ± 0.11	**0.33** ± 0.15	**0.38** ± 0.12	0.26 ± 0.12	0.15 ± 0.11	0.27 ± 0.14	**0.32** ± 0.14
N. elongatus	0.14 ± 0.07	0.18 ± 0.10	**0.32** ± 0.12	**0.36** ± 0.10	0.09 ± 0.06	0.24 ± 0.10	**0.32** ± 0.13	**0.35** ± 0.10	0.25 ± 0.06	0.13 ± 0.09	**0.30** ± 0.13	**0.33** ± 0.10
July												
A. hemigymnus	0.29 ± 0.11	0.29 ± 0.14	0.23 ± 0.13	0.19 ± 0.12	0.23 ± 0.12	0.29 ± 0.14	0.25 ± 0.14	0.23 ± 0.13	**0.35** ± 0.08	0.28 ± 0.14	0.21 ± 0.12	0.16 ± 0.11
C. maderensis	0.23 ± 0.07	0.27 ± 0.11	0.26 ± 0.12	0.25 ± 0.09	0.14 ± 0.07	**0.37** ± 0.12	0.28 ± 0.13	0.21 ± 0.10	**0.33** ± 0.06	0.24 ± 0.12	0.23 ± 0.13	0.20 ± 0.10
H. benoiti	0.18 ± 0.11	**0.31** ± 0.14	0.27 ± 0.14	0.24 ± 0.12	0.24 ± 0.13	**0.32** ± 0.16	0.24 ± 0.14	0.20 ± 0.12	0.18 ± 0.11	**0.33** ± 0.15	0.27 ± 0.14	0.22 ± 0.13
H. hygomii	0.21 ± 0.10	0.23 ± 0.12	0.27 ± 0.13	0.29 ± 0.11	0.18 ± 0.09	0.26 ± 0.13	0.27 ± 0.13	0.29 ± 0.11	**0.30** ± 0.09	0.20 ± 0.12	0.24 ± 0.13	0.26 ± 0.11
L. crocodilus	0.23 ± 0.07	**0.33** ± 0.10	0.26 ± 0.11	0.17 ± 0.08	0.17 ± 0.09	**0.53** ± 0.16	0.21 ± 0.12	0.10 ± 0.08	**0.30** ± 0.05	**0.35** ± 0.10	0.23 ± 0.11	0.11 ± 0.07
L. crocodilus B	0.03 ± 0.03	0.20 ± 0.10	**0.33** ± 0.13	**0.44** ± 0.09	0.04 ± 0.04	0.28 ± 0.11	0.28 ± 0.17	**0.40** ± 0.10	0.03 ± 0.02	0.28 ± 0.10	**0.32** ± 0.14	**0.38** ± 0.09
L. pusillus	0.16 ± 0.08	0.28 ± 0.12	0.29 ± 0.13	0.28 ± 0.10	0.15 ± 0.10	**0.32** ± 0.14	0.28 ± 0.14	0.25 ± 0.11	0.22 ± 0.07	0.27 ± 0.12	0.27 ± 0.13	0.24 ± 0.10
L. dofleini	0.16 ± 0.10	0.21 ± 0.13	0.28 ± 0.14	**0.35** ± 0.13	0.11 ± 0.08	0.20 ± 0.12	**0.30** ± 0.14	**0.39** ± 0.12	0.24 ± 0.10	0.20 ± 0.13	0.26 ± 0.14	**0.30** ± 0.13
N. elongatus	0.27 ± 0.06	0.22 ± 0.09	0.26 ± 0.10	0.25 ± 0.07	0.19 ± 0.07	**0.35** ± 0.11	**0.30** ± 0.11	0.16 ± 0.07	**0.38** ± 0.06	0.17 ± 0.10	0.24 ± 0.12	0.21 ± 0.08

TDF (mean ± sd): Sweeting (2007a, b): $\Delta\delta^{13}C$ = 0.97 ± 1.08, $\Delta\delta^{15}N$=3.15 ± 1.28; Vander Zanden and Ramussen, (2001): $\Delta\delta^{13}C$ = 0.47 ± 1.23, $\Delta\delta^{15}N$ = 3.46 ± 0.23; Caut et al., (2009): $\Delta\delta^{13}C$ = 1.40 ± 0.60, $\Delta\delta^{15}N$ = 3.52 ± 1.01. Potential prey: POM: particulate organic matter; Zoopl: zooplankton; Euf: *Meganyctiphanes norvegica* adults; Fish: fishes (see text). B: bottom. Values ≥ 0.30 are in bold.

SIAR dietary mixing - Population

Using the mean enrichment factors from Pinnegar and Polunin (1999) and Trueman et al. (2005) consumers were placed outside the prey polygon. Most mesopelagic consumers fit better into the polygon (including the standard deviations) when using Sweeting et al. (2007a; 2007b) and Vander Zanden and Rasmussen (2001), except for the larger *L. crocodilus* from the bottom, where Caut et al. (2009) appeared most appropriate, a consequence of the larger trophic discrimination of carbon (Fig. 6.4). Although the use of trophic discrimination factors led to differences in relative contributions of potential prey, the overall patterns of diet preferences for each species were consistent among models using Sweeting et al., (2007a; 2007b) and Vander Zanden and Rasmussen, (2001) (Table 6.2).

Species could be categorized as showing preference for i) mesozooplankton/POM ii) mesozooplankton/euphausiids or iii) euphausiids and fish prey. The lowest δ^{15}N was observed in small non-surface-migrating *C. braueri* for which SIAR derived a diet based heavily on mesozooplankton and POM. At the other extreme with the higher trophic level estimates, *L. dofleini* showed substantial contribution of larger prey, particularly fishes and euphausiids. In between, the two *Hygophum* species showed fairly similar contributions of the different potential preys, with only a slight tendency towards higher contribution of mesozooplankton for *H. benoiti* and a higher contribution of fishes for *H. hygomii*. *L. pusillus* diet included all dietary endpoints except POM.

For some species however, no discernible or consistent dominant prey type could be established. This latter group included *A. hemigymnus* but with some seasonal variation where late autumn individuals seem to utilise less POM than those collected in summer. High seasonal variation was also evident in *N. elongatus*, which showed a relatively higher contribution of fish in late autumn, compared to a higher contribution of zooplankton items in summer. *C. maderensis* and *N. elongatus* also exhibited seasonality with overlapping diet in summer but some separation in winter where *C. maderensis* was estimated to rely on smaller prey than *N. elongatus*.

Water column *L. crocodilus* showed a clear preference for mesozooplankton, with a small contribution of euphausiids and fishes. In contrast bottom non-migratory *L. crocodilus* exhibited high piscivory both in summer and late autumn, with only minor contributions from mesozooplankton.

Intra-population patterns

The pooled analyses of all individuals collected showed no effects of body size (SL) on δ^{13}C, δ^{15}N and Trophic level (δ^{13}C: $F_{1,70}$=0.123 R=0.043, p>0.05; δ^{15}N: $F_{1,70}$=0.021, R=0.018, p>0.05; TrL: $F_{1,70}$=0.081, R=0.035, p>0.05). Three species had body size ranges and samples sizes that were amenable to exploration of size influences on intra-population trophodynamics (Fig. 6.5).
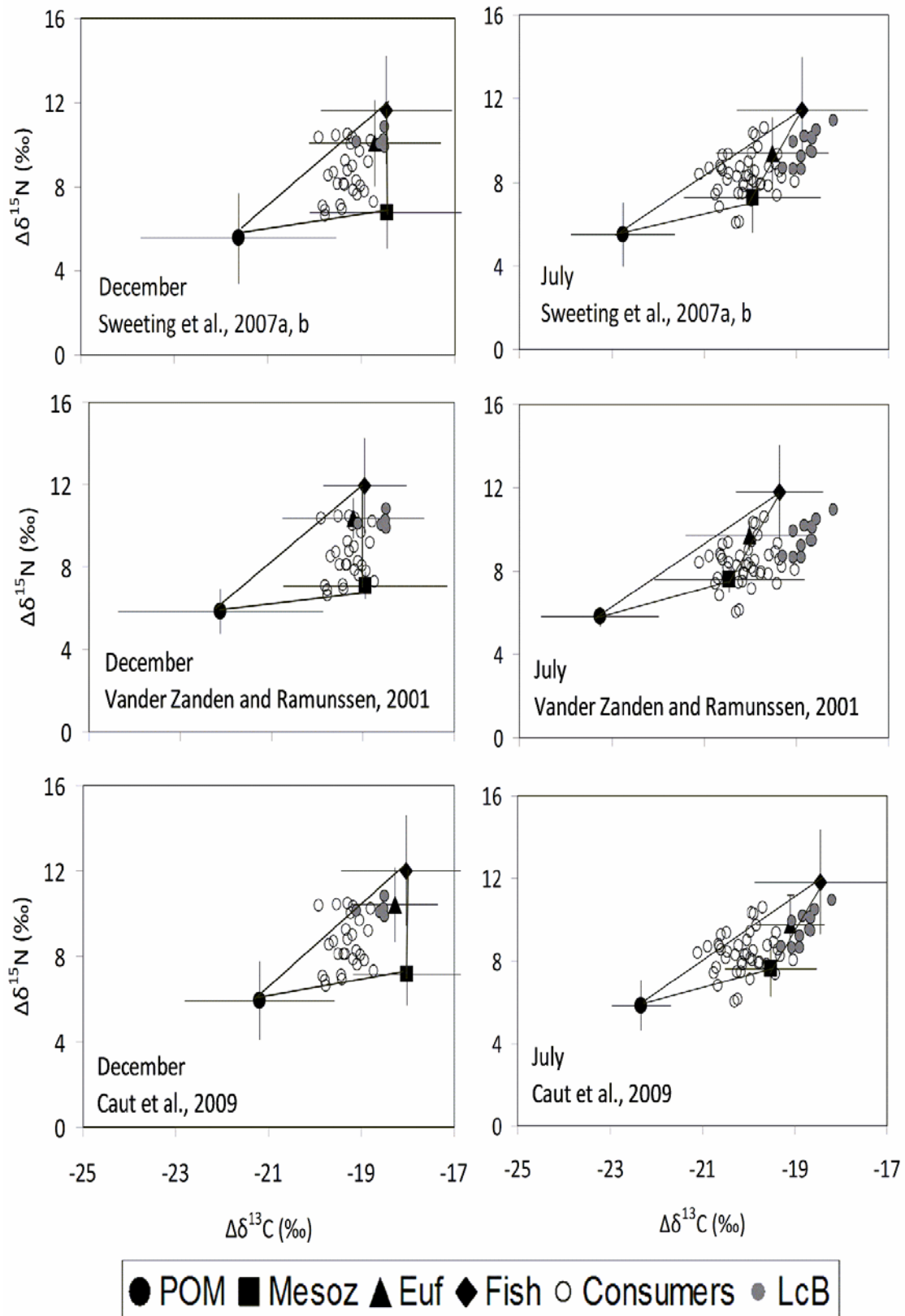
Figure 6.4. Stable isotope ratios of mesopelagic fishes (circles) and feasible contribution of potential prey (black symbols) to their diet according to SIAR. Bars denote standard deviations. *Lampanyctus crocodilus* specimens from bottom trawls identified in grey colour.
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L. crocodilus δ^{15}N and δ^{13}C both exhibited strong positive relationship with SL (R=0.863 and R=0.923 respectively, both p<0.001). In contrast, body size trends in δ^{15}N were absent for *N. elongatus* and *C. maderensis.* δ^{13}C decreased with increasing size in *C. maderensis* (R=0.559, p<0.01) but exhibited high variability and a non-significant positive trend in *N. elongatus.* Patterns in δ^{13}C are predominantly explained by lipid content as C:N and δ^{13}C were negatively correlated in all three species although only significantly so for *L. crocodilus* and *C. maderensis*, (R=0.892, p<0.001 and R=0.798, p<0.05 respectively). Further, C:N of both *L. crocodilus* and *C. maderensis* exhibited systematic but contrasting C:N trends with body size. C:N (lipid content) decreased with increasing size for *L. crocodilus*, while *C. maderensis* showed the opposite trend.

6.4 Discussion

Spatial and seasonal patterns

The present study reveals seasonal differences in δ^{13}C of both plankton and mesopelagic fishes but little spatial variation over the spatial scale sampled. By contrast δ^{15}N variation was minimal in both space and time. Approximately uniform δ^{13}C differences at both upper and lower trophic levels suggest δ^{13}C differences arise from the primary producers (Fry and Quiñones, 1994).

δ^{13}C differences are likely attributed to C-fixation dynamics in phytoplankton e.g. with temperature or could represent seasonal changes in the plankton community. The observed seasonality in δ^{13}C would be consistent with a higher fraction of diatoms (Fry and Wainright, 1991; Miller et al., 2008) in the late autumn mixing period, than during summer stratification seasons, where dinoflagelates dominated the phytoplankton community (Estrada et al., 1999) over large spatial scales. In the study region there are also differences in the copepod species present throughout the year and in their relative abundance (Fernández de Puelles et al., 2003, 2014), however, the most abundant genus, such as *Clausocalanus*, *Paracalanus* and *Oithona* are always there (Fernández de Puelles et al., 2014).

δ^{15}N was comparatively low in mesozooplankton and sinking POM but consistent with previously reported values in the NW Mediterranean (Fanelli et al., 2011b), and in oceanic waters of other oligotrophic regions (NW Atlantic, Fry and Quiñones, 1994; the eastern Mediterranean Sea, Koppelmann et al., 2009). Higher δ^{15}N values of larger sized zooplankton organisms, particularly the euphausiid *M. norvegica* (generally considered omnivorous) compared to mesozooplankton, has been observed elsewhere (Letessier et al., 2012) and considered as either increasing potential to switch to carnivory in the presence of available preys (Beyer, 1992) or the increasingly likelihood of a species in that size fraction being fully carnivorous (Fry and Quiñones, 1994).

Mean isotopic value of mesozooplankton coincide with those of copepods collected historically from the same region (Fanelli et al., 2009), reinforcing conclusions that copepods were the main constituents of the mesozooplankton (>70%), in both late autumn and summer across multiple years (Fernandez de Puelles et al., 2014).

Other main community metrics such as TA and CD (Layman et al., 2007) although similar between cruises, showed higher values for the autumn period, which points to a slightly higher trophic diversity for this period. Some caveats are associated to this approach but our data set allowed for robust results. That is, 1) number of sample species was balanced between seasons, 2) baseline δ^{15}N was independent of season, and 3) one single primary food source sustains the mesopelagic community in the study area (Valls et al., 2014a).

Trophic structure of mesopelagic fishes

Data suggest a tight trophic interactions between mesozooplankton and euphausiids and higher mesopelagic fishes representing an important link between primary production and the nektonic community (Letessier et al., 2012; Miller et al., 2010; Yoon et al., 2007). The mesopelagic species included in this study (5 Stomiiforms, 1 Aulopiform and 12 Myctophiforms), were the most abundant and frequent in the Mediterranean Sea (Goodyear et al., 1972; Olivar et al., 2012), and their distribution coincided with the main scattering layers marked by echosounders (Peña et al., 2014).

Although in a recent study on measures of trophic position Hussey et al. (2014) advocated the use of scaled discrimination factors in front of the conventional constant discrimination approach, as a matter of comparison with the other studies on mesopelagic fish (Cherel et al., 2010; Choy et al., 2012; Flynn and Kloser, 2012) here we also apply a constant enrichment between prey and fish. Trophic level estimations of Myctophidae in this study are similar to those elsewhere and indicate the myctophids act as secondary and tertiary consumers of the pelagic ecosystem (Cherel et al., 2010; Choy et al., 2012; Flynn and Kloser, 2012). Previous trophic level estimations for Stomiiforms include the larger dragonfishes (*Chauliodus sloani*, *Stomias boa* and *Idiacanthus* spp.) which were estimated at trophic level 3-3.4 (Choy et al., 2012). Here, the lowest trophic level positions among the whole mesopelagic fish populations were occupied by two small non migratory Stomiiforms; *C. braueri* (Gonostomatidae) and *A. hemigymnus* (Sternopthychidae). Other Stomiiforms such as the Phosichthydae *V. attenuata*, *M. muelleri* and the Stomiidae *S. boa* showed intermediate trophic level of 3.5, a good agreement with Choy et al. (2012), despite the smaller size of the specimens analysed here (80-125 mm SL in the Mediterranean *vs* 126-168 in the Mid-Atlantic Ridge (Choy et al., 2012).

This study showed that Mediterranean mesopelagic fish displayed a continuum δ^{15}N within a comparatively narrow δ^{13}C range, suggesting trophic segregation within the assemblage, but a shared ultimate production source, presumably phytoplankton. This narrow isotopic range in δ^{13}C appears a feature of mesopelagic fishes being absent in data from demersally caught benthic and benthopelagic species of the same region (Valls et al., 2014a).

The trophic continuum reflects behavioural factors among the mesopelagic species studied. The lower δ^{15}N / TrLs species including *C. braueri* and *A. hemigymnus*, occupy intermediate depths (mainly at the 400-600 m Deep Scatering Layer, DSL) and are non surface-migratory (Andersen and Sardou, 1992; Badcock and Merrett,

1976; Olivar et al., 2012; Ross et al., 2010). In contrast the upper Trophic levels were represented by migratory myctohpids such as *L. dofleini* or *H. hygomii.* This trophic segregation could be related to the more stable environment of the intermediate water column and adoption of less energetically demanding behaviour, compared to the changing environment experienced by the extensive migratory species.

The relatively high δ^{13}C and δ^{15}N of bottom living *L. crocodilus* indicated that they have an isotopic niche closer to other bottom dwelling species (Valls et al., 2014a) than to their younger migratory stages. Comparisons of δ^{15}N among a non-migrant and two migrant myctophids in the western Pacific showed lower values in those feeding in the upper water column than in those feeding in deeper layers (Sugisaki and Tsuda, 1995). Conversely, for the gonostomatid *C. braueri* the near bottom collected specimens (Fanelli et al., 2009) have isotope signatures similar to those obtained from the deep scattering layer here, pointing to similar dependence on pelagic organisms for feeding, independently of their location. Interpretation of dietary differences however, requires some caution due to potential confounding effects of depth on dietary isotope basal signatures.

Comparative trophic patterns and niche segregation

Trophic structuring of the assemblage is described as a single ultimate production source sustaining a mesopelagic assemblage of niches, separated across the trophic continuum. This idea is reinforced by results of SIAR mixing model and subsequent interspecific comparison of common genus.

The use of stable isotope mixing Bayesian models to ascertain diet is subject to a number of weaknesses as the actual food sources included in the analysis and those associated to the trophic discrimination factors used (TDFs) (Galvan et al., 2012). TDFs are not available for any of the species here, or for close relatives. This study used Vander Zanden and Rasmussen (2001), Sweeting et al. (2007a, 2007b) and Caut et al., (2009) values. Previous studies on mesopelagic and other fishes used similar values e.g. Δ^{15}N=3.1-3.4‰ for myctophids (Cherel et al., 2010; Flynn and Kloser, 2012) or Δ^{15}N=3.56‰ and Δ^{13}C=1.01‰ for the pelagic juvenile stages of sardine and anchovy in the northwestern Mediterranean (Costalago et al., 2012). These TDFs are similar to those of aquatic organisms reviewed elsewhere (Caut et al., 2009; Caut et al., 2010; Post, 2002; Sweeting et al., 2007a., 2007b; Vander Zanden and Rasmussen, 2001) and that have extensively been applied for the pelagic marine environment (Bode et al., 2007; Miller et al., 2010; Olson et al., 2010).

The widely utilised TDFs used appear suitable for the mesopelagic species of this study as often being close to maximising the number of individuals occurring in the prey polygon. Additionally the dietary endpoints included, in spite of euphausiids appearing within the prey polygon for one of the cruises, provided diet estimations consistent with pre-existing stomach contents of *C. braueri* (Palma, 1990), *L. pusillus* (Bernal et al., 2013) and *L. crocodilus* from bottom trawls (Stefanescu and Cartes, 1992; Valls, unpublished data). Results also revealed consistent patterns of partitioning between the four prey sources when using Sweeting et al. (2007a,

2007b) and Vander Zanden and Rasmussen (2001), suggesting a suitability to summarise feeding patterns of the mesopelagic assemblage more widely. Therefore, the model, as utilized here, is useful to give a comparative overview into the feeding patterns among species, although the reliability of the estimated contributions of each dietary endpoint is subject to great uncertainty. The accuracy of these types of models also depends on the accuracy of the potential prey used for the analysis, which is strongly dependent on the available information on stomach content analysis. In order to better differentiate the potential food sources, for future investigations it would be interesting to analyse separately a few groups among the mesoozooplankton, e.g. copepods.

Most mesopelagic fishes studied had feasible prey combinations that encompassed several trophic levels, consuming a mixed diet of mesozooplankton, euphausiids and, to a lesser extent either POM or other mesopelagic fishes. POM was particularly important for *C. braueri*, while in the opposite side, small mesopelagic fishes appeared particularly important in *L. dofleini* and the bottom living *L. crocodilus*. The diet of *C. maderensis*, the most common and abundant myctophid in the water column, seems mainly based on zooplankton, with scant contribution of fishes or POM, therefore sharing preys with most of the other mesopelagic fishes.

Meso- and macrozooplankton organisms, particularly copepods and euphausiids are usually the most abundant and common prey for mesopelagic fishes reported in the literature (Bernal et al., 2013; Gaskett et al., 2001; Hulley, 1990; Pakhomov and Perissinotto, 1996; Petursdottir et al., 2008; Pusch et al., 2004; Shreeve et al., 2009). Fishes are also frequently been cited as prey items, and although their contribution is not important in numerical terms, it is substantial expressed as carbon and nitrogen mass (Gaskett et al., 2001; Pusch et al., 2004). POM such as marine snow has been documented as part of the diet of different fish species and developmental stages and comprises detritus-like materials from all types of marine organisms (e.g. bacteria, phytoplankton, zooplankton) that aggregate together, such as appendicularians houses (Miller et al., 2012).

Despite similar migratory behaviour and overlapping vertical distributions, niche segregation was observed within genera e.g. for *H. benoiti* and *H. hygomii,* which differed in fish/mesozooplankton utilisation. Similarly, for the migrating stages of *L. crocodilus* and *L. pusillus*, *L. crocodilus* diet was more dependent on mesozooplankton and POM than *L. pusillus* that had a higher contribution of euphausiids and mesozooplankton. Stomach content for *L. pusillus* support this interpretation indicating that copepods and euphausiids were the main preys of adults stages, while fishes appeared in the diet, but in low abundance (Bernal et al., 2013)

Size Based Feeding

Size based feeding of marine fishes is common (Galvan et al., 2010) although, both pelagic and deep water species have been underrepresented in the literature to date (Tecchio et al., 2013). Indeed, with the exception of the large mesopelagic predators, pelagic species are largely absent. Of the 18 species included in this study just three could be assessed at intra-population level. Of them, only *L.*

crocodilus exhibited pronounced positive size based shifts that are commonly observed within the benthic and coastal systems (Galvan et al., 2010), although in this case size specific depth distribution suggests that trophic level changes may be confounded by basal changes in isotope signature with depth. Species inhabiting different environments and having distinct food sources can be distinguished, allowing for the identification of vagrant individuals (Bunn et al., 1989; Doucett et al., 1999), even at small scale (Maruyama et al., 2001; Takai and Sakamoto, 1999).
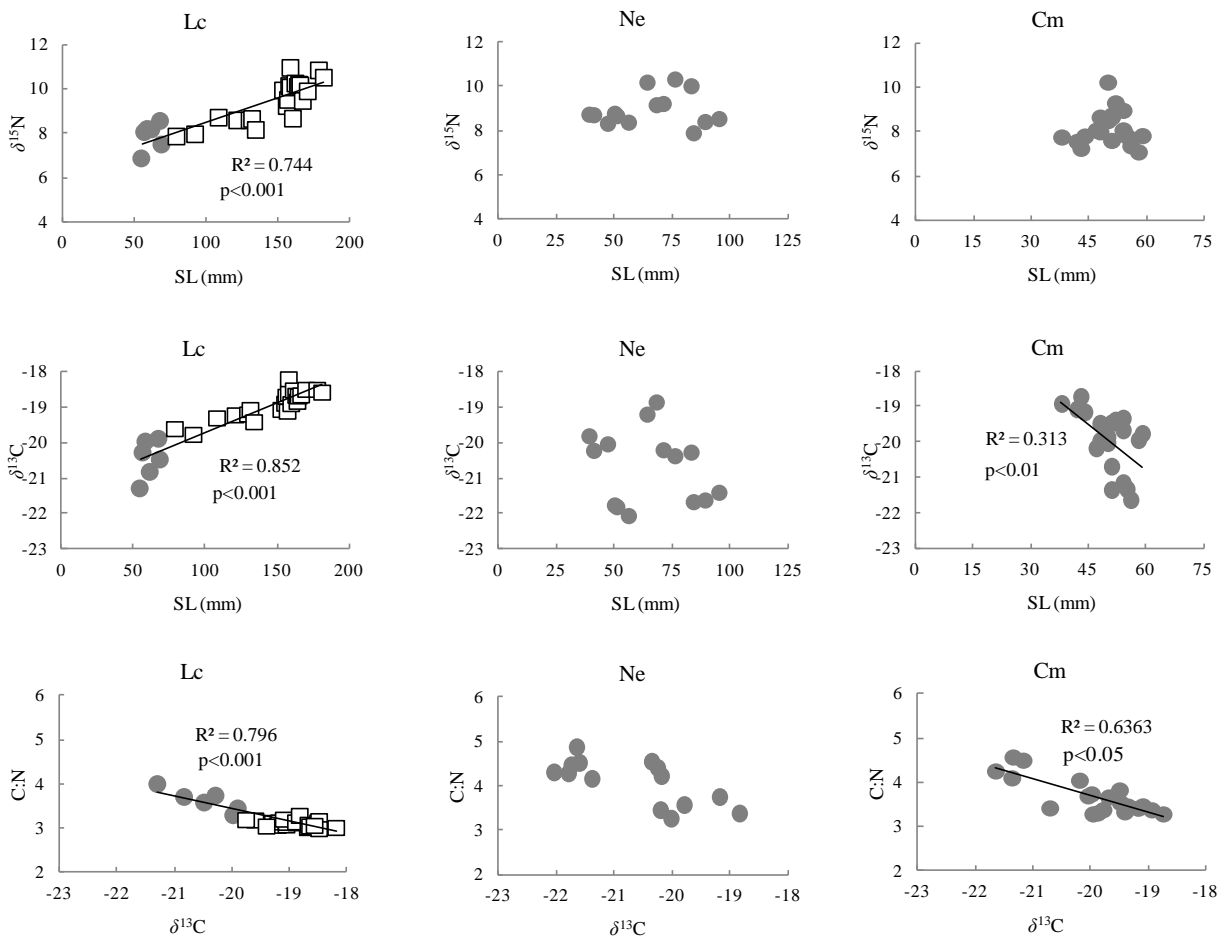
Figure 6.5. Relationship between the body size (SL, mm) and δ^{13}C (‰) and δ^{15}N (‰) and between the C.N ratio and δ^{13}C (‰) of *L. crocodilus* (Lc), *N. elongatus* (Ne) and *C. maderensis* (Cm). Squares represent non-migrating Lc.

Assuming potential confounding influences of depth are minimal, the isotope data suggest small *L. crocodilus* with lower δ^{13}C are more strongly dependent on the pelagic environment than the larger bottom dwelling individuals with higher δ^{13}C. Additionally, the higher δ^{15}N with increasing size also points to a higher dependence for preys, such fish or other preys, of higher trophic levels for the larger non migrating fish of this species. Concurrent stomach content analysis of adults collected in bottom trawls indicated that, after decapods (not included in the present pelagic sampling), euphausiids and fishes were their main preys

(Cartes pers. com.). Such patterns are also consistent with previously hypothesized migratory pattern of this species (Olivar et al., 2012).

However, here it is more likely that size trends in $\delta^{13}C$ are a function of size specific variation in lipid content. Small migrant *L. crocodilus* individuals exhibited higher lipid content (C:N) in muscle tissue than non-migrating larger individuals, suggesting differences in energy requirements and type of lipids they accumulate. C:N of the largest individuals was as low as 3.1 indicative of fish tissue with virtually no or very limited lipid content (Sweeting et al 2006). This contrasts with previous observations of relatively high lipid content in non-migrant *L. crocodilus* adults' (Suntsov and Brodeur, 2008).

No size trends were observed with *N. elongatus* $\delta^{13}C$ and $\delta^{15}N$ values but variability in $\delta^{13}C$ was high, suggesting a wider inter-individual contribution to total niche width (Bearhop 2004) than in the two other species. Variation could not be attributed to differences in lipid content (C:N). *C. maderensis* exhibited negative correlations between $\delta^{13}C$–size and $\delta^{13}C$–C:N owing to lower energy storage in the smallest individuals. Such variability could underline selectivity for rich-lipid prey with increasing size. Differences in general life strategies were thus observed between these vertical migrant species with overlapping distribution.

In summary, mesopelagic fishes showed strong dependence on the pelagic system with very limited spatial and temporal variation in trophic structure over the scale assessed. Trophic levels of mesopelagic fishes spanned the three trophic levels with the most noticeable niche segregation observed between the non-migratory stomiiforms (*C. braueri* or *A. hemigymnus*) and some of the extensive migratory myctophids (*L. dofleini* or *N. elongatus*). Niche segregation was also evident within genera for *Hygophum* spp. and *Lampanyctus* spp.. Bayesian SIAR mixing models identified interesting contrasting trophic patterns, e.g., i) *C. braueri,* the dominant species in the DSL, whose estimated diet was composed of POM and mesoozooplankton, ii) *C. maderensis,* the dominant upper migrating species, fed mostly on mesozooplankton, or iii) *L. dofleini* and *N. elongatus*, relatively abundant migrant species, which incorporate fishes to their diet. Finally, stage-habitat related differences in the diet were conspicuous for the older bottom dwelling stages of *L. crocodilus* and the migratory ones, with a lesser degree of piscivory for the pelagic stages.
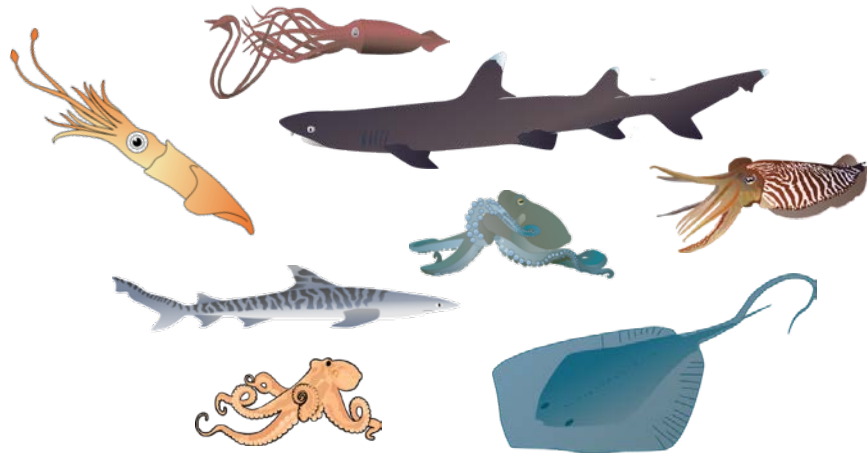
Chapter 7. Feeding strategies and resource partitioning among elasmobranchs and cephalopods in Mediterranean deep-sea ecosystems

Chapter 7. Feeding strategies and resource partitioning among elasmobranchs and cephalopods in Mediterranean deep-sea ecosystems

7.1 Introduction

Differences in locomotory costs, growth rates and reproductive strategies are important determinants of animal metabolism in any environment (Childress and Thuesen, 1995; Seibel, 2007; Seibel et al., 2004). Elasmobranchs and cephalopods display contrasting life history traits. The former are long-lived, and invest in few, well-developed young, whereas the latter have short life spans, high population growth rates and high fecundity. Cephalopods and elasmobranchs also show physiological divergences, both in the biochemical system (protein- and lipid-based systems) and biochemical composition (Pethybridge et al., 2010; Spitz et al., 2010). Cephalopods have a protein-based metabolism whereas elasmobranchs rely on lipids as an energy source (Gallagher et al., 2017; Pethybridge et al., 2010; Valls et al., 2016).

Given the differences in foraging dynamics and life history constraints, differences would be expected when comparing cephalopods and elasmobranchs feeding strategies and food consumption. Although elasmobranchs and cephalopods play a key role in food web dynamics, there is still limited knowledge on basic ecological aspects, particularly in terms of trophic ecology (Collins and Rodhouse, 2006; Young et al., 2013). As opportunistic predators with a wide trophic spectrum both taxonomic groups are naturally linked to a wide variety of prey (Boyle and Rodhouse, 2005; Valls et al., 2015, 2011; Wetherbee et al., 2012), potentially connecting ecologically distinct food webs.

Elasmobranchs and cephalopods also show differences in their long-term abundance trends. Profound declines in worldwide elasmobranch populations have become apparent owing to long-term disturbances such as intense fishing exploitation, pollution and habitat degradation (Dulvy et al., 2014; Stevens, 2000). By contrast, cephalopod populations have increased globally over the last six decades indicating their potential adaptation to changing environmental conditions (Doubleday et al., 2016). Cephalopods seem to be especially sensitive to short-term perturbations such as climate oscillations and prey availability (Pierce et al., 2008; Quetglas et al., 2016; Rodhouse et al., 2014).

The Mediterranean Sea constitutes one of the 25 biodiversity hotspots already recognised on a planetary scale (Myers et al., 2000a). However, habitat degradation and fishing exploitation have generated a strong impact on marine fauna in the last decades (Colloca et al., 2013; Vasilakopoulos et al., 2014). These threats, together with climate change, have been predicted to grow within the next decade, further affecting biodiversity in the Mediterranean (Coll et al., 2010). Elasmobranchs are the most endangered group of marine fishes in the Mediterranean, with 39 species assessed as critically endangered, endangered or vulnerable (Dulvy et al., 2016). In spite of being characterized by more oligotrophic conditions than the surrounding areas (Fanelli et al., 2009), the Balearic Islands (western Mediterranean) show one of the highest values of diversity and abundance of demersal elasmobranchs in the basin (Massutí and

Moranta, 2003; Ramírez-Amaro et al., 2015). Cephalopods diversity is also higher in waters around the Balearic Archipelago than in the adjacent areas (Quetglas et al., 2014). Higher habitat heterogeneity and a comparatively lower intensity of fishing exploitation seem to be the factors of such a high diversity (Ordines et al., 2011; Quetglas et al., 2012).

Given the aforementioned changes in many elasmobranch and cephalopod populations worldwide and the potential impacts on their prey and predators, detailed food web information will be needed to improve predictions of ecosystem responses to ongoing perturbations (Coll et al., 2013; Stergiou and Karpouzi, 2002; Young et al., 2015). Knowledge of trophic position, diet composition, and ontogenetic shifts in diet are essential for ecosystem modelling (e.g. Christensen and Pauly, 1992) and topological analysis (Navia et al., 2010), which will allow implementing a sound ecosystem-based management (Simpfendorfer et al., 2011). Despite well documented differences in feeding strategies, many existing ecosystem models still lump elasmobranchs and cephalopods together into very broad and generic categories (Angelini and Vaz-Velho, 2011; Tecchio et al., 2013; Tsagarakis et al., 2010). Aggregation within model groups has generally been due to the lack of information about feeding habits and life history of the different species. Such a lack of data has already been identified as a limiting factor in food web studies from the Mediterranean Sea (Bănaru et al., 2013; Coll et al., 2006; Navarro et al., 2013).

Trophic ecology studies have traditionally been done by means of stomach content analysis (SCA) (Cortes, 1999; Hyslop, 1980). Although this methodology allows high levels of taxonomic resolution, elasmobranchs and cephalopods show large percentages of empty stomachs and the identified prey are restricted in many cases to hard, difficult to digest species (Hyslop, 1980; Rodhouse and Nigmatullin, 1996). Stable isotope analysis (SIA), particularly of carbon (δ^{13}C) and nitrogen (δ^{15}N), provides time-integrated information on assimilated, rather than ingested, biomass. However, SIA cannot identify specific prey items, and multiple diet combinations can result in similar δ^{13}C and δ^{15}N values of a consumer (Hussey et al., 2014; Post, 2002). The isotope data can also be used to infer various structural aspects of a particular food web (Jackson et al., 2011), such as niche width of species or population (Layman et al., 2007). Integrating SIA and SCA results helps thus to have a better understanding of species trophic ecology in marine systems (Navarro et al., 2014; Stowasser et al., 2006).

Classification of trophic guilds provides a useful framework for simplifying dietary information and assessing the trophic structure across species assemblages (Fanelli et al., 2013b; Preciado et al., 2016). Dietary guild analysis can be used to identify groups of species that use similar resources within a community and thus play similar functional role in an ecosystem (Garrison and Link, 2000). Resource partitioning among community members is frequently attributed to competitive interactions (Root, 1967; Schoener, 1970) and the interactions within guilds are assumed to be stronger than those between members of different guilds (Pianka, 1980). The generally recognised low food availability, particularly in oligotrophic regions such as the deep Mediterranean (Cartes and Sarda, 1992), may also contribute to enhance competitive exclusion among species. Resource partitioning of ecologically important groups in trophic networks such as cephalopods (Fanelli et al., 2012; Guerreiro et al., 2015) and elasmobranchs (Albo-Puigserver et al.,

2015; Churchill et al., 2015) has scarcely been studied up to now. Only a few studies have used SIA in combination with SCA to assess the dietary guilds and food partitioning within elasmobranch (Barría et al., 2015; Espinoza et al., 2015) and cephalopod (Rosas-Luis et al., 2016) assemblages. However, these studies are necessary to assess potential ecological impacts on those taxonomic groups (Vaudo and Heithaus, 2011).

In this work, the trophic ecology of cephalopods and elasmobranchs is investigated in order to determine their ecological role in the deep-sea marine ecosystems of the western Mediterranean. To this end, two integrative approaches were used, SCA and SIA of dominant cephalopod and elasmobranch species, in terms of both abundance and biomass, living along the water column on continental slope grounds. Specifically, the main objective of this work has been the analysis of the feeding strategies and trophic relationships of both faunal groups with the main aim of providing insights into their level of food resource partitioning.

7.2 Material and methods

7.2.1 Data source and study area

Samples were collected in the Balearic Sea (NW Mediterranean) during two different scientific surveys: 1) the MEDITS surveys, carried out annually during early summer from 2007 to 2016; and 2) the IDEADOS surveys, conducted in late autumn (December) 2009 and early summer (July) 2010. The sampling detailed methodology can be found in Chapter 2 (section 2.1.1.1).

7.2.2 Diet analyses

Stomach content

Full stomachs of 18 cephalopod species (N=1823 stomachs) belonging to 3 orders (Teuthoidea, Sepioidea and Octopoda), and 5 elasmobranch species (N=1295 stomachs) belonging to 3 orders (Rajiformes, Squaliformes and Carcharhiniformes) were analyzed (SCA) (Table 7.1). Species selected were those dominant in the megafaunal slope assemblage in terms of both abundance and biomass (Ordines et al., 2011; Quetglas et al., 2014). Elasmobranch stomachs were analysed on board, whereas those of cephalopods were preserved in ethanol for later processing in the laboratory. For this study, only individuals caught between 200 and 900 m depth were considered. For comparisons, diets were standardized as percentage of frequency (%F) by prey item. Stomach contents analysis is explained in Chapter 2 (Section 2.2.1).

Despite prey items were identified to the lowest possible taxon, higher taxonomic categories (ordinal level and above) were used for diet description and statistical analyses. For an ecological overview of prey utilization, the most abundant taxonomic categories (fish and decapod crustaceans) were classified, based on known ecological traits, as pelagic, benthopelagic and benthic (Cartes and Carrassón, 2004) (Table 7.1). Any prey item contributing less than 5% to the total frequency across all species was not taken into account (Porifera, Ascideacea,

Algae, Ostracoda, Elasmobranchii, Bryozoa and Octopoda). Unidentified prey items and plastics (Table 7.3) were excluded from all analyses. The resulting prey classification contained 20 categories.

Stable isotopes

Carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope analyses (SIA) were conducted on 5 elasmobranch (N=170 samples) and 18 cephalopod (N=370 samples) species (Table 7.2). In addition, a total of 21 potential prey species (based on stomach data) collected during the IDEADOS and MEDITS surveys were also analyzed.

As lipids are ^{13}C-depleted relative to proteins and carbohydrates (Sweeting et al., 2006), differential lipid contents can bias the interpretation of δ^{13}C values. The potential for lipid content was explored based on the C:N ratios from percent element by weight. Elasmobranchs and cephalopods analyzed in this study generally exhibited low C:N ratios, consistent with relatively low lipid contents (C:N<3.5; Post et al., 2007), therefore the analyses were conducted on uncorrected δ^{13}C values. To avoid potential interference from the chemical treatment, the urea content was not removed.

Details regarding stable isotope sampling and processing are explained in Chapter 2 (Section 2.2.2)

Table 7.1. List of prey species included in prey categories.

Benthic decapoda	*Aegaeon lacazei* Alpheidae *Alpheus glaber* *Alpheus sp.* *Anapagurus laevis* *Atelecyclus rotundatus* Brachyura *Calocaris macandreae* Crangonidae *Ebalia sp.* *Galathea sp.* Galatheidae *Geryon longipes* *Goneplax rhomboides* *Liocarcinus depurator* *Liocarcinus sp.* *Macropipus tuberculatus* *Monodaeus couchii* *Munida intermedia* *Munida rutllanti* *Munida sp.* *Nephrops norvegicus* Paguridae *Pagurus alatus* *Pagurus prideaux* *Philocheras trispinosus* *Polycheles typhlops* *Processa canaliculata* *Processa nouveli nouveli* *Rissoides desmaresti* Scyllaridae *Solenocera membranacea* Xanthidae	Zooplankton	*Cymbulia peronii* Doliolido *Phronima sedentaria* *Phrosina semilunata* *Pyrosoma atlanticum* *Salpa maxima* *Salpa sp.* Salpida Siphonophorae		*Micromesistius poutassou* *Mora moro* *Nezumia aequalis* *Pagellus acarne* *Pagellus erythrinus* *Phycis blennoides* *Spicara smaris* *Aphia minuta* *Boops boops* *Molva dypterigia* *Macroramphosus scolopax*
		Benthic fish	Anguilliformes *Arnoglossus imperialis* *Arnoglossus ruepelli* *Arnoglossus spp* *Bathysolea profundicola* *Callionymus maculatus* *Callionymus sp* *Cataetix alleni* *Deldentosteus quadrimaculatus* *Gnathophis mystax* *Helicolenus dactylopterus* *Lepadogaster sp.* *Lepidorhombus boscii* *Lepidorhombus spp.* *Lesueurigobius friesii* *Lesueurigobius sanzoi* *Leuseurigobius sp.* *Nemichthys scolopaceus* *Nettastoma melanurum* *Ophichthus rufus* *Paraliparis leptochirus* Pleuronectidae *Symphurus ligulatus* *Symphurus nigrescens* *Symphurus sp.* *Synchiropus phaeton* *Trachinus draco*	Pelagic fish	*Arctozemus risso* *Argyropelecus hemigymnus* *Benthosema glaciale* *Ceratoscopelus maderensis* *Chauliodus sloani* Clupeidae *Cyclothone braueri* *Hygophum benoiti* *Hygophum hygomii* *Lampanyctus crocodilus* *Lampanyctus pusillus* *Lestidiops jayakari* *Lobianchia dofleini* *Maurolicus muelleri* Myctophidae *Myctophum punctatum* *Notoscopelus bolini* *Notoscopelus elongatus* *Polyacanthonotus rissoanus* *Stomias boa boa* *Sudis hyalina* *Symbolophorus veranyi* *Trachurus sp.* *Trachurus trachurus*
Benthopelagic decapoda	*Acanthephyra pelagica* *Acanthephyra sp.* *Aristeus antennatus* Pandalidae *Parapenaeus longirostris* *Plesionika antigai* *Plesionika edwardsii* *Plesionika heterocarpus* *Plesionika martia* *Plesionika narval* *Plesionika sp.* *Pontophilus norvegicus* *Pontophilus spinosus*	Benthopelagic fish	*Argentina sphyraena* Argentinidae *Crystallogobius linearis* *Capros aper* *Centracanthus cirrus* *Cepola macrophthalma* *Chlorophthalmus agassizi* *Coelorinchus caelorhincus*		
Pelagic decapoda	*Chlorotocus crassicornis* *Eusergestes arcticus* *Gennadas elegans* *Pasiphaea multidentata* *Pasiphaea sivado* *Pasiphaea sp.* *Sergestes arachnipodus* *Sergestes sp.* Sergestidae *Sergia robusta* *Systellaspis debilis*		*Epigonus denticulatus* *Gadiculus argenteus* *Gaidropsarus biscayensis* *Gaidropsarus megalokynodon* *Glossanodon leioglossus* Gobidae unid. Gobiidae *Lepidion lepidion* *Lepidopus caudatus* *Merluccius merluccius*		

Table 7.2. Cephalopod and elasmobranch species analyzed for stomach content (Sto) and stable isotopes (Iso) of C and N. The depth range (DR) (in metres), data source (DS) and years when the individuals were collected are also specified. Na: not available.

	Species	Code	DR (m)	Iso DS	Years (Iso DS)	Sto DS	Years (Sto DS)
Cephalopoda	Octopoda						
	Bathypolypus sponsalis	B. spo	200-800[a]	Medits & Ideados	2009-2014	Quetglas et al., 2001	1995-1999
	Eledone cirrhosa	E. cir	50-800[a]	Medits	2010	Ideados	2009-2010
	Octopus salutii	O. sal	200-800[a]	Medits & Ideados	2009-2015	Quetglas et al., 2005	1995-2003
	Pteroctopus tetracirrhus	P. tet	100-800[a]	Medits & Ideados	2009-2011	Quetglas et al., 2009	1995-2005
	Scaeurgus unicirrhus	S. uni	100-800[a]	Medits & Ideados	2010-2014	Na	
	Sepioidea						
	Heteroteuthis dispar	H. dis	600-800[a]	Medits & Ideados	2010	Ideados	2010
	Rondeletiola minor	R. min	40-600[b]	Medits & Ideados	2010	Ideados	2009-2010
	Rossia macrosoma	R. mac	100-600[a]	Medits & Ideados	2010-2012	Ideados	2009-2010
	Sepia orbignyana	S. orb	50-600[a]	Medits	2014	Ideados	2010
	Sepietta oweniana	S. owe	100-600[a]	Medits & Ideados	2010	Ideados	2009-2010
	Teuthoidea						
	Abralia veranyi	A. ver	200-800[a]	Medits & Ideados	2010-2013	Ideados	2009-2010
	Ancistroteuthis lichtensteinii	A. lich	200-800[a]	Medits & Ideados	2010-2015	Na	
	Histioteuthis bonnellii	H. bon	200-800[a]	Medits & Ideados	2010-2014	Quetglas et al. 2010 & Ideados	2009-2010
	Histioteuthis reversa	H. rev	200-800[a]	Medits & Ideados	2010-2014	Quetglas et al. 2010 & Ideados	2009-2010
	Illex coindetii	I. coi	100-600[a]	Medits & Ideados	2009-2014	Ideados	2009-2010
	Loligo forbesii	L. for	50-600[a]	Medits & Ideados	2009-2014	Medits (Valls et al. 2015)	2007-2010
	Todarodes sagittatus	T. sag	100-800[a]	Medits & Ideados	2009-2015	Quetglas et al., 1999	1995-1996
	Todaropsis eblanae	T. ebl	200-600[a]	Medits	2014-2015	Na	
Elasmobranch	Squaliformes						
	Etmopterus spinax	E. spi	442-744[b]	Medits & Ideados	2007-2014	Medits & Ideados	2007-2014
	Carcharhiniformes						
	Galeus melastomus	G. mel	328-744[b]	Medits & Ideados	2007-2014	Medits & Ideados	2007-2016
	Scyliorhinus canicula	S. can	45-420[b]	Medits	2007-2009	Valls et al., 2011& Medits	2007-2010
	Rajiformes						
	Dipturus oxyrhinchus	D. oxy	255-660[b]	Medits	2014	Medits	2009-2016
	Raja clavata	R. cla	58-420[b]	Medits & Ideados	2007-2014	Medits & Ideados	2007-2016

[a] Quetglas et al., 2000; [b] Ordines et al., 2011

Statistical analyses

Different statistical analyses were performed to address the following goals: 1) detection of ontogenetic shifts in diet; 2) identification of species feeding strategies and prey item contribution to diet similarities within feeding strategies; 3) determination of the trophic structure; and 4) exploration of isotopic niche overlap among feeding strategies and species.

In order to identify ontogenetic shifts in diet, only those predators meeting the following requirements (considering both isotope and stomach samples) were used: wide species size range, high variation in $\delta^{15}N$ isotopic mean value ($\geq \pm 0.8\text{‰}$) and large sample size (N $_{isotopes}$ >30 and N $_{full\ stomach}$ >140). When those criteria were met, individuals were divided into 5-30 mm size classes, depending on their size range breadth. Length categories were determined using a hierarchical cluster analysis based on the resemblance matrix of $\delta^{15}N$ and $\delta^{13}C$ mean values (untransformed data), using group-average linking of Euclidean distances. The cluster analysis was carried out with the SIMPROF (similarity profiling) routine, which defines statistically significant groups among samples (Clarke and Gorley 2006). The size-classes identified were treated as functionally distinct predators in all posterior analyses.

Hierarchical agglomerative clustering with the SIMPROF routine were carried out on stomach data (squared root transformed, Bray-Curtis similarity measures) to identify feeding strategies (=trophic guilds). Significant trophic guilds were categorized based on predators habitat into three broad feeding strategies: benthic, benthopelagic and pelagic feeders. To illustrate similarities between the groups resulting from the cluster analysis, multidimensional scaling (MDS) analysis was carried out on the same stomach data. Similarity percentage (SIMPER) analysis was used to identify the most important prey items accounting for the similarities within trophic guilds (at 80% cut-off level).

Isotopic differences among feeding guilds were tested using a distance-based permutational analysis of variance (PERMANOVA test) based on Euclidean distances (for untransformed univariate isotope data). In the case of significant results, pair-wise comparisons were carried out. The significance was set at p=0.05 and obtained by using 9999 permutations. Those species with no stomach data (*Ancistroteuthis lichtensteinii*, *Todaropsis eblanae* and *Scaeurgus unicirrhus*) were not considered for the statistical analyses.

Table 7.3. Diet composition (%F) of cephalopod and elasmobranch species from the study area. Sample size (N) and size range (in mm) are also shown. Plank: zooplankton; Ann: Annelida; Am: Amphipoda; B dec: benthic decapod Crustacea; Crust un: Crustacea unidentified; Euph: Euphausiacea; Iso: Isopoda; Mys: Mysidacea; Nat un: Natantia Crustacea unidentified; BP dec: Benthopelagic decapod Crustacea; P dec: Pelagic decapod Crustacea; Ceph un: Cephalopoda unidentified; Sep: Sepioidea; Teut: Teuthoidea; Biv-Gas: Bivalvia and Gastropoda; Oph: Ophiuroidea; B fish: Benthic fish; BP fish: Benthopelagic fish; Fish un: Fish unidentified; P fish: pelagic fish; Un: unidentified.

Prey items (%F)

Species	N	Size range	Plank	Ann	Am	B dec	Crus un	Euph	Iso	Mys	Nat un	BP dec	P dec	Ceph un	Sep	Teut	Biv - Gas	Oph	B fish	BP fish	Fish un	P fish	Un	Plastic
B. spo (juv)	21	25-48			33.				14.		4.8			33.			57.	33.			4.8		28.6	
B. spo	12	50-95			5.8	61.			16.		6.7			35.			50.	35.			8.3		60.0	
E. cir	64	25-145		29.		47.	18.		13.		9.2	4.6		1.5	1.5		1.5		1.5	6.2	4.6		4.6	
O. sal	22	40-165				76.	18.		3.2		23.	0.5		10.					1.4	3.2	20.	1.4	8.1	
P. tet	17	45-140				76.	18.		4.1		20.	0.6		9.3					2.9	1.7	19.	1.2	8.7	
H. dis	15	10-25			6.7			20.						40.							6.7		26.7	
R. ma	31	10-21				37.	2.3				9.3						2.3				44.	4.7	14.0	
R. min	43	15-70							3.2		45.				6.5						9.7		22.6	
S. orb	9	26-92				55.			11.			11.	11.	11.					11.	11.	44.			
S. owe	96	13-33				3.1			5.2		19.		11.	3.1	2.1		1.0				51.	5.2	11.5	
A. ver	58	8-43							8.6		32.				1.7						39.	13.	15.5	
H. bon	24	15-150					19.		3.8		30.				7.7						53.	23.	3.8	
H. rev	17	11-120					5.6	7.3			24.	0.6	2.8	4.0		1.1					47.	14.	9.6	
I. coin	16	24-245		0.6		0.6	7.3	14.	2.4		1.2		10.	6.7		6.1			1.2		30.	30.		
L. for (juv)	17	38-139				0.6	4.5	41.		12.	0.6	1.1	1.1	1.7			0.6		2.8	30.	30.	2.8	1.7	
L. for	27	140-451				0.4	2.5	16.	1.4		2.2	8.3	2.5	8.3		0.7	1.4		4.0	28.	39.	29.	0.7	
T. sag (juv)	91	110-268				4.4			6.6		33.	14.	3.3	13.	8.8	9.9					8.8	46.	44.	1.
T. sag	61	273-418				9.8			1.6		37.	21.	4.9	11.	6.6	16.					21.	49.	39.	1.
E. spi (juv)	15	102-216	3.2				8.3	22.		1.3			23.	16.	15.	35.					14.	13.		6.
E. spi	73	220-483	4.2	1.4		1.4	4.2	7.0	1.4			2.8	23.	22.	22.	38.					19.	16.	4.2	4.
G. mel (juv)	43	100-410	6.2	0.6	1.0	2.3	4.1	53.	1.0	1.9		0.8	26.	11.	4.8	11.			0.6	0.6	13.	10.	1.9	6.
G. mel	20	416-682	12.	0.5		5.4	2.9	39.	0.5	0.5		1.0	39.	18.	5.9	22.			1.0	2.9	17.	16.	6.4	
S. can	18	100-500	4.7	12.	0.5	20.	3.6	43.	13.	6.8		1.0	18.	14.	2.6	5.2			2.1	0.5	23.	1.6	5.7	
D. oxy	45	170-1080				64.	2.2	26.			60.		15.	22.	2.2		2.2		2.2		2.2			
R. cla (juv)	14	130-590		0.8	18.	80.	6.0	6.0	15.	30.			6.0	36.	3.0	3.0	1.5		3.0	27.	10.	0.8		
R. cla	55	593-910		1.8		87.		5.5	7.3	9.1			5.5	29.	7.3	3.6	9.1		23.	63.	36.	3.6		

In order to give an overview of the food web structure of deep sea cephalopods and elasmobranchs, hierarchical classification analysis and multidimensional scaling (MDS) were carried out on the bivariate isotopic data (untransformed, Euclidean distances). A $\delta^{15}N$–$\delta^{13}C$ scatter plot using mean values was made for all predators and potential prey (based on stomach data) analyzed in this study. All multivariate analyses were performed with PRIMER 6 + PERMANOVA software package from Plymouth Marine Laboratory, UK (Anderson et al., 2008).

The isotopic niche and overlap among feeding guilds (based on stomach data) and species were calculated using the SIAR library (Parnell and Jackson, 2013). The smallest convex hull that contains all individual $\delta^{13}C$ and $\delta^{15}N$ values within a species or group (TA, total area) can be converted as a proxy of the isotopic trophic diversity (Layman et al., 2007). This gives an incomparable measure of niche area when applied to different sample sizes, since the convex hull area generally increases with sample size (Jackson et al., 2011). Consequently, standard ellipse area (SEA), which represents a measure of the mean core population isotopic niche, and standard ellipse area corrected for sample size (SEAc) (Jackson et al., 2011), were also calculated. In addition to these species-specific analyses, niche overlap among feeding guilds SEAc were also calculated. Isotopic niche overlap was measured as the area of the shared SEAc and categorized as high (overlap ≥50%) or low (overlap <50%) (Guerreiro et al., 2015).

7.3 Results

Ontogenic shifts

Of the 23 species considered, cluster analysis was applied to five species (*Raja clavata*, *Etmopterus spinax, Galeus melastomus, Todarodes sagittatus* and *Bathypolypus sponsalis*) and ontogenetic shifts in diet were identified at specific size thresholds (TL). When no significance was detected through SIMPROF test, separations between size classes at a distance >1.5 were considered. Species were divided into two size categories (juveniles *vs* adults) (Fig. 7.2): *R. clavata* (TL= 590 mm; p>0.05), *E. spinax* (TL= 220 mm; p>0.05), *G. melastomus* (TL= 410 mm; p<0.05), *T. sagittatus* (ML= 271 mm; p<0.05) and *B. sponsalis* (ML= 50 mm; p<0.05). A final matrix of 28 size classes (23 species, 5 of them subdivided in two size classes) was obtained (Table 7.3 and 7.4).

Table 7.4. Mean (±sd) of stable isotope values (δ^{13}C and δ^{15}N), sample size (N), size range (mm) and C:N ratio (mean ± sd), along with species (with N>10) isotopic niche width (SEAc: standard ellipse area corrected for sample size) of elasmobranchs and cephalopods examined. See species codes in Table 7.2.

Species	N	Size range	δ^{13}C	δ^{15}N	C:N	SEAc
B. spo (juv)	11	20-49	-19.14 ± 0.40	7.48 ± 0.33	3.57 ± 0.10	0.31
B. spo	19	50-78	-18.55 ± 0.28	8.90 ± 0.72	3.36 ± 0.15	0.51
E. cir	3	85-140	-18.19 ± 0.24	8.83 ± 0.29	3.41 ± 0.09	-
O. sal	23	40-125	-18.65 ± 0.29	8.42 ± 0.68	3.39 ± 0.15	0.52
P. tet	12	85-140	-18.33 ± 0.36	8.49 ± 0.56	3.23 ± 0.12	0.57
S. uni	2	75-80	-18.72 ± 0.28	8.02 ± 0.42	3.18 ± 0.07	-
H. dis	18	15-25	-20.02 ± 0.25	8.40 ± 0.94	3.51 ± 0.20	0.50
R. ma	20	11-18	-19.58 ± 0.19	8.23 ± 0.58	3.56 ± 0.10	0.56
R. min	31	22-67	-19.20 ± 0.37	8.17 ± 0.57	3.36 ± 0.11	0.32
S. orb	3	26-37	-18.74 ± 0.39	7.28 ± 0.24	3.29 ± 0.06	-
S. owe	31	13-30	-19.57 ± 0.42	8.90 ± 0.73	3.57 ± 0.15	0.69
A. ver	13	30-44	-19.74 ± 0.47	9.39 ± 0.77	3.71 ± 0.19	1.12
A. lich	8	13-155	-19.61 ± 0.33	8.69 ± 1.30	3.20 ± 0.11	-
H. bon	12	19-140	-19.83 ± 0.50	10.01 ± 0.59	2.95 ± 0.37	0.83
H. rev	49	21-145	-20.16 ± 0.31	10.02 ± 0.58	3.12 ± 0.25	0.56
I. coin	18	109-206	-19.10 ± 0.61	8.86 ± 0.54	3.36 ± 0.07	0.84
L. for	34	150-475	-19.31 ± 0.42	9.37 ± 0.75	3.38 ± 0.18	0.87
T. ebl	5	125-200	-19.11 ± 0.11	9.59 ± 0.38	3.40 ± 0.06	-
T. sag (juv)	32	114-270	-19.46 ± 0.69	8.43 ± 0.47	3.26 ± 0.08	0.85
T. sag	26	271-400	-19.14 ± 0.63	9.17 ± 0.36	3.23 ± 0.10	0.73
E. spi (juv)	5	143-219	-18.19 ± 0.25	8.81 ± 0.17	2.61 ± 0.06	-
E. spi	26	220-462	-17.96 ± 0.37	10.32 ± 0.55	2.60 ± 0.05	0.54
G. mel (juv)	51	120-409	-18.14 ± 0.34	9.01 ± 0.62	2.57 ± 0.11	0.59
G. mel	31	410-610	-17.71 ± 0.24	10.64 ± 0.40	2.47 ± 0.05	0.29
S. can	19	280-490	-18.20 ± 0.19	9.35 ± 0.63	2.42 ± 0.08	0.29
D. oxy	7	400-1112	-17.50 ± 0.31	9.16 ± 0.42	2.57 ± 0.09	-
R. cla (juv)	16	200-589	-17.84 ± 0.20	8.98 ± 0.49	2.46 ± 0.04	0.26
R. cla	15	620-910	-17.62 ± 0.29	10.77 ± 0.46	2.44 ± 0.07	0.44
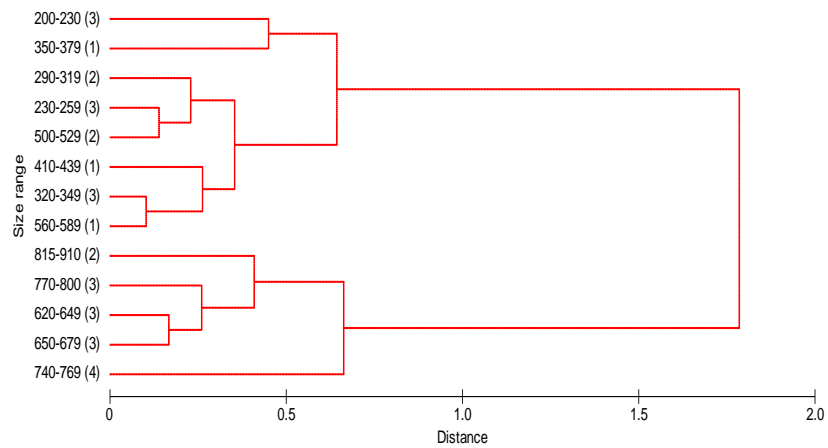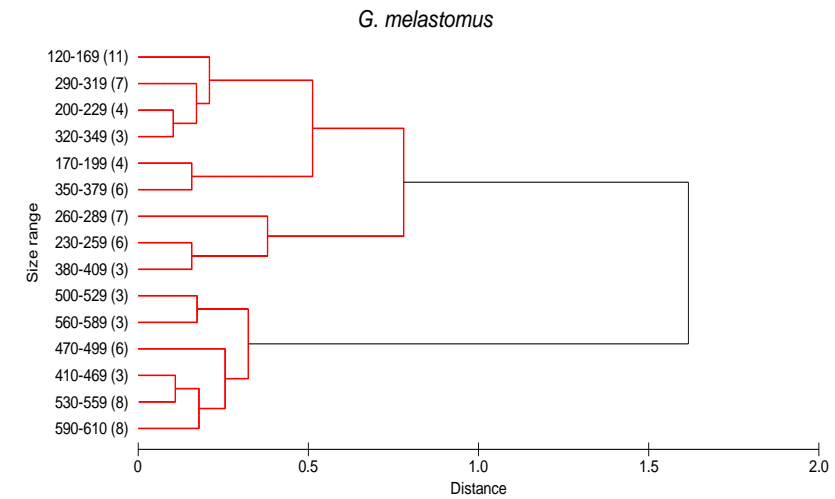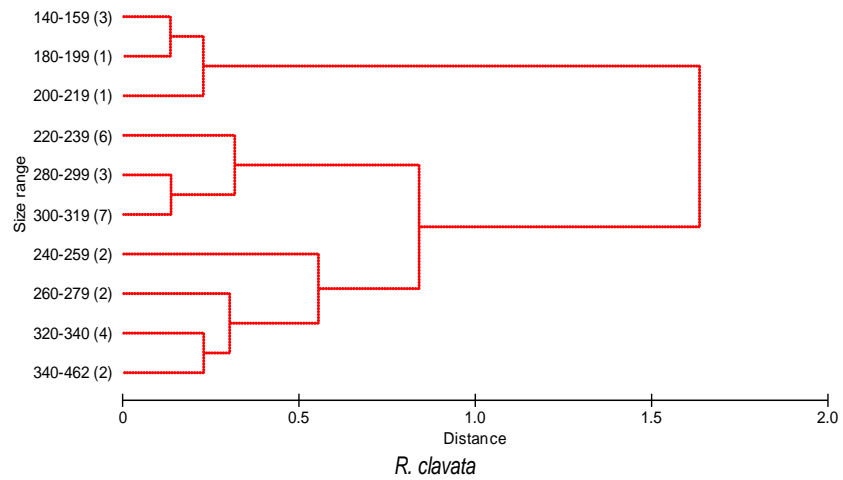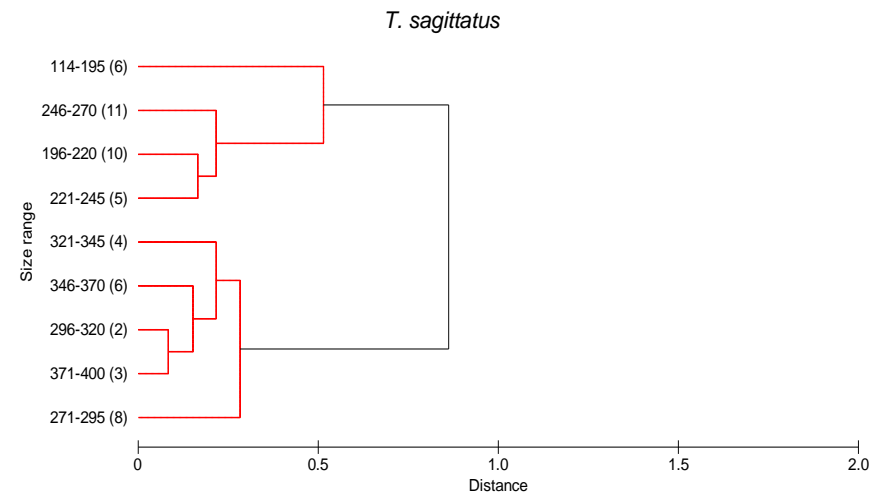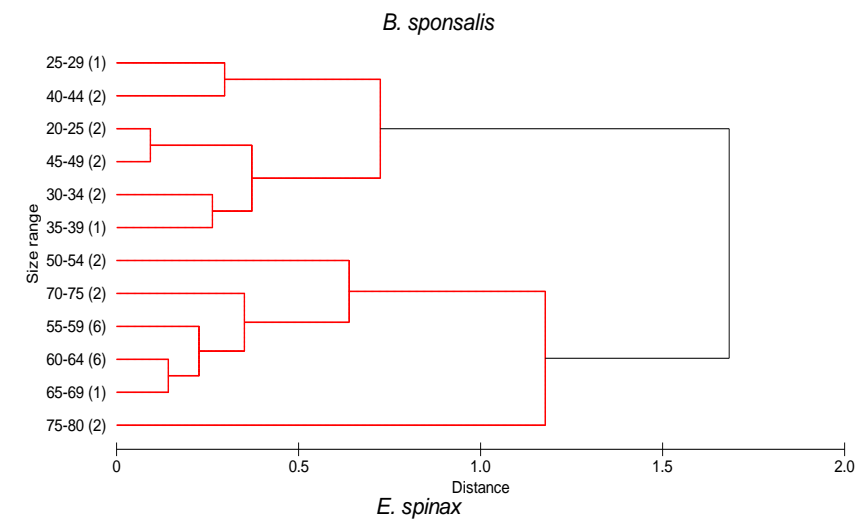
Figure 7.2. Cluster analysis applied on the Euclidean distance for some cephalopod and elasmobranch species (based on δ15N and δ13C mean values). Red lines indicate homogeneous size groups (p<0.05). The number of individuals analyzed within each size interval (in milimetres) is shown within brackets.

Diet and feeding strategies

The hierarchical classification analysis of stomach contents identified seven trophic guilds (A to F) and four species with unique dietary composition based on the SIMPROF routine (p<0.01) (Fig. 7.3). Trophic guilds were further aggregated into three broad trophic categories (at a 45% similarity): benthic, benthopelagic and pelagic feeders (Fig. 7.4).

The benthic and pelagic feeders groups included exclusively cephalopod species, whereas the benthopelagic feeders group included both cephalopods and elasmobranchs. The most important prey items contributing to the diet similarity within trophic guilds are shown in Table 7.5. Based on prey diversity, two main groups were identified within the benthic feeders. The first benthivore guild A (Av sim: 90.80%) consisted of *Bathypolypus sponsalis* (juveniles and adults), which consumed a mixture of bivalves, gastropods, benthic decapods, cephalopods and ophiurans (each of these prey groups contributed ≈ 20% to the intra-guild similarity; Table 7.5). The second benthivore guild B (Av sim: 96.95%) included the octopods *Octopus salutii* and *Pteroctopus tretracirrhus*, which preyed mainly on decapod crustaceans (accounting for 42.5% of diet similarity). *Eledone cirrhosa* and *Rossia macrosoma* also preyed frequently on benthic decapod crustaceans (F=47.7% and 37.2% respectively), but the former also preyed on annelids (F=29%) and the latter on fish (F=44.2%), whereby they were classified as unique groups within the benthic feeders (Fig. 7.3).
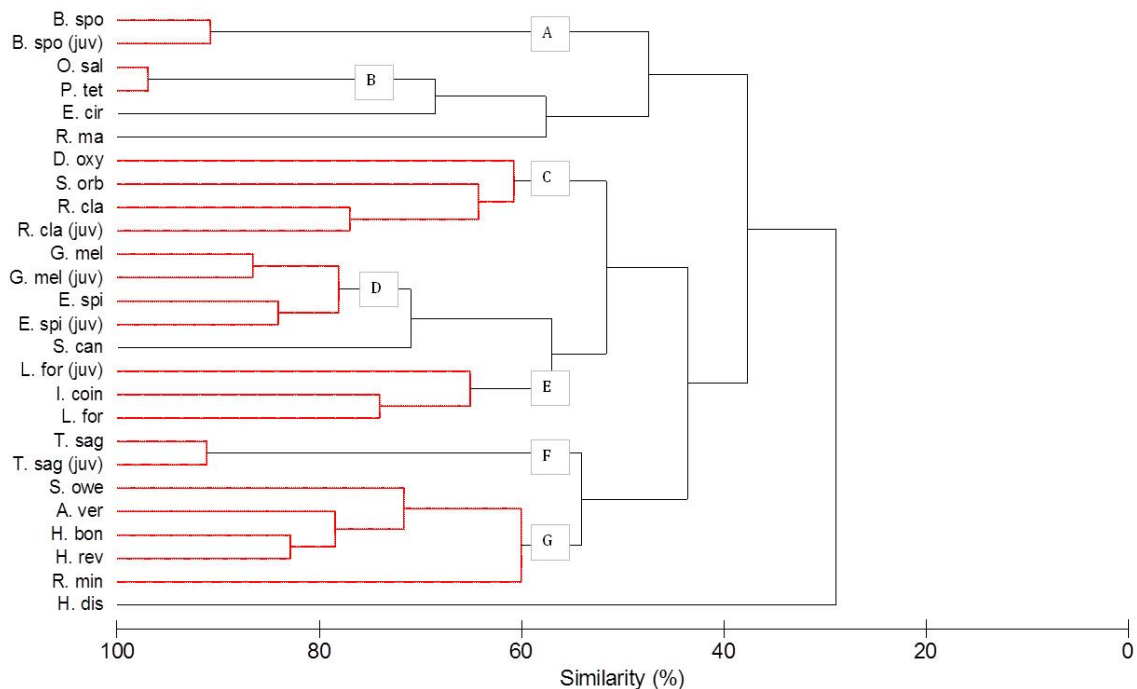
Figure 7.3. Cluster with SIMPROF test (p<0.01) showing predators grouped into seven feeding guilds (red lines) (A to G) and four species with unique dietary composition.

The benthopelagic feeders group, which consumed a mixture of fish, cephalopods and euphausiids in varying proportions, was further subdivided into three trophic guilds. The guild C (Av sim: 64.68%), which included the ray species *Raja clavata*

(juveniles and adults) and *Dipturus oxyrhinchus,* together with *Sepia orbignyana*, had a diet based on decapod crustaceans and fish, contributing to 50.87% and 22.68% respectively to the guild similarity. Both juveniles and adults of the sharks *Galeus melastomus* and *Etmopterus spinax* clustered together in guild D (Av sim: 80.53%); a mixture of cephalopods (%cum=32.1), pelagic crustaceans (cum%=26) and fish (cum%=22) contributed to the similarity of this guild. *Scyliorhinus canicula* also exhibited preference for crustaceans, fish and cephalopods, but the consumption of annelids (F=12%) and isopods (F=13%), led to a unique dietary classification within the benthopelagic feeders group. The guild E (Av sim: 68.11%) consisted of the squids *Loligo forbesii* (juveniles and adults) and *Illex coindetii*, with fish and euphausiids contributing to the 50% and 17% of the intra-guild similarity.

Finally, the pelagic feeders group included two trophic guilds. The squid *Todarodes sagittatus* (juveniles and adults) constituted the guild F (Av sim: 91.15%), consuming mainly fish (e.g. pelagic fish F≈41%), decapods crustaceans (e.g. benthopelagic decapods F≈17%) and squids (F≈13%) and accounting for the 72% to the intra-guild similarity. The guild G (Av sim: 69.51%) comprised two sepiolids (*Rondeletiola minor* and *Sepietta oweniana*) and three squids (*Abralia veranyi*, *Histioteuthis reversa* and *H. bonnellii*), with fish, decapod crustaceans and euphausiids contributing to about 90% of the group similarity. The sepiolid *Heteroteuthis dispar,* which fed frequently on pelagic decapods crustaceans (F= 40%) and euphausiids (F=20%), clustered apart.
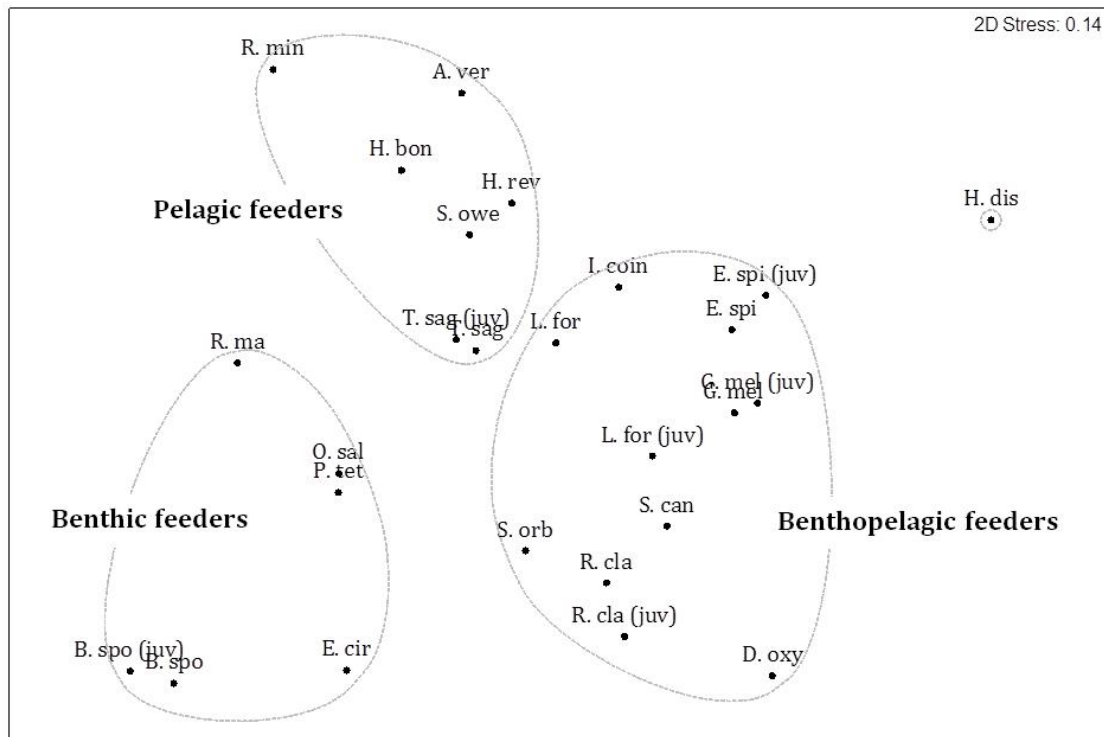
Figure 7.4. Multidimensional scale (MDS) results based on stomach content (%F) data of elasmobranchs and cephalopods from the western Mediterranean. Circles define broad feeding strategies (pelagic, benthic and benthopelagic feeders) at 45% similarity level based on the cluster shown in Figure 7.3. For species codes see Table 7.2.

Table 7.5. Percentage contribution (SIMPER analysis; cut-off for low contribution at 80%) of typifying prey by feeding guild (A to G). Average abundance (Av. Ab), average similarity (Av. Sim) and standard deviation (SD), percentage contribution to the similarity (Contrib%) and percentage contribution to the accumulated similarity (Cum%) are shown.

	Av.Ab	Av.Sim	Sim/SD	Contrib%	Cum%
BENTHIC PREDATORS					
Guild A (Av sim: 90.80%)					
Biv - Gast	7.34	19.85		21.86	21.86
Benthic decapoda	6.81	16.07		17.7	39.56
Cephalopoda unid.	5.84	16.07		17.7	57.26
Ophiuroidea	5.84	16.07		17.7	74.96
Isopoda	3.93	10.53		11.6	86.56
Guild B (Av sim: 96.95%)					
Benthic decapoda	8.74	27.16		28.02	28.02
Natantia unid.	4.66	14.04		14.48	42.50
Pisces unid.	4.51	13.86		14.3	56.80
Crustacea unid.	4.31	13.44		13.86	70.66
Cephalopoda unid.	3.11	9.5		9.8	80.46
BENTHOPELAGIC PREDATORS					
Guild C (Av sim: 64.68%)					
Benthic decapoda	8.45	17.82	11.1	27.55	27.55
Pelagic decapoda	4.86	9.22	6.96	14.25	41.80
Pisces unid.	4.36	6.29	1.6	9.73	51.53
Euphausiacea	3.32	5.87	3.31	9.07	60.60
Benthopelagic decapoda	3.02	5.87	3.31	9.07	69.67
Benthic fish	2.85	4.23	2.57	6.55	76.22
Benthopelagic fish	4.13	4.14	0.9	6.4	82.62
Guild D (Av sim: 80.53%)					
Pelagic decapoda	5.29	11.93	28.44	14.81	14.81
Teuthoidea	5.06	10.41	3.6	12.92	27.73
Euphausiacea	5.23	9.59	2.68	11.91	39.64
Pisces unid.	4.01	9.2	19.21	11.42	51.06
Cephalopoda unid.	4.12	9.17	8.61	11.38	62.44
Pelagic fish	3.75	8.49	9.96	10.54	72.98
Sepiida	3.32	6.27	3.33	7.79	80.77
Guild E (Av sim: 68.11%)					
Pisces unid.	5.78	16.45	20.49	24.16	24.16
Euphausiacea	4.79	11.63	25.41	17.07	41.23
Pelagic fish	4.21	8.67	1.38	12.73	53.96
Crustacea unid.	2.13	5.29	4.32	7.76	61.72
Cephalopoda unid.	2.26	5.14	2.44	7.55	69.27
Benthopelagic fish	3.61	5.09	0.58	7.48	76.74
Benthic fish	1.59	3.82	4.44	5.61	82.35
PELAGIC PREDATORS					
Guild F (Av sim: 91.15%)					
Pisces unid.	6.91	15.55		17.06	17.06
Pelagic fish	6.45	14.34		15.73	32.79
Natantia unid.	5.94	13.14		14.42	47.21
Benthopelagic decapoda	4.2	8.65		9.49	56.70
Cephalopoda unid.	3.51	7.76		8.51	65.21
Teuthoidea	3.6	7.2		7.9	73.11
Benthopelagic fish	3.79	6.79		7.45	80.55
Guild G (Av sim: 69.51%)					
Pisces unid.	6.16	22.64	3.99	32.57	32.57
Natantia unid.	5.48	22.49	4.44	32.35	64.92
Euphausiacea	2.33	9.08	6.78	13.06	77.98
Pelagic fish	2.93	7.4	1.07	10.64	88.62

Trophic structure

Cluster analysis and MDS plot based on $\delta^{13}C$ and $\delta^{15}N$ values revealed the separation of species into five trophic groups at Euclidean distance of 1.3 (Fig. 7.5). These groups were labeled with roman numerals, from I to V, in ascending order through the food web. Group-I, the most ^{15}N-depleted, corresponded to *B. sponsalis* (juveniles) and *S. orbignyana* (mean $\delta^{13}C$=-18.94±0.28‰ and $\delta^{15}N$=7.38±0.13‰).

Species with intermediate $\delta^{15}N$ values were further divided into two subgroups owing to marked $\delta^{13}C$ differences; group-II (mean $\delta^{15}N$=8.77±0.52‰) comprised squid (i.e. *L. forbesii*, *T. sagittatus*) and cuttlefish (i.e. *R. minor*, *R. macrosoma*) species displaying low mean $\delta^{13}C$ values (-19.38±0.35‰), whereas group-III (mean $\delta^{15}N$=8.88±0.29‰) grouped juvenile (*R. clavata*, *E. spinax*, *G. melastomus*) and adult (*S. canicula*, *D. oxyrhinchus*) elasmobranch species together with octopus species (*O. salutii*, *P. tetracirrhus*, *S. unicirrhus*, *B. sponsalis*) showing high mean $\delta^{13}C$ values (-18.18±0.34‰).

At upper levels, group-IV included the two pelagic *Histioteuthis* species showing the lowest mean $\delta^{13}C$ (−19.99±0.23‰) and highest mean $\delta^{15}N$ (10.02‰) values among the cephalopod community. Finally, group-V was occupied by adult individuals of the sharks *E. spinax* and *G. melastomus* and the ray *R. clavata*, which displayed the highest $\delta^{15}N$ (mean $\delta^{15}N$= 10.72±0.06‰) and $\delta^{13}C$ values (-17.61±0.10‰).
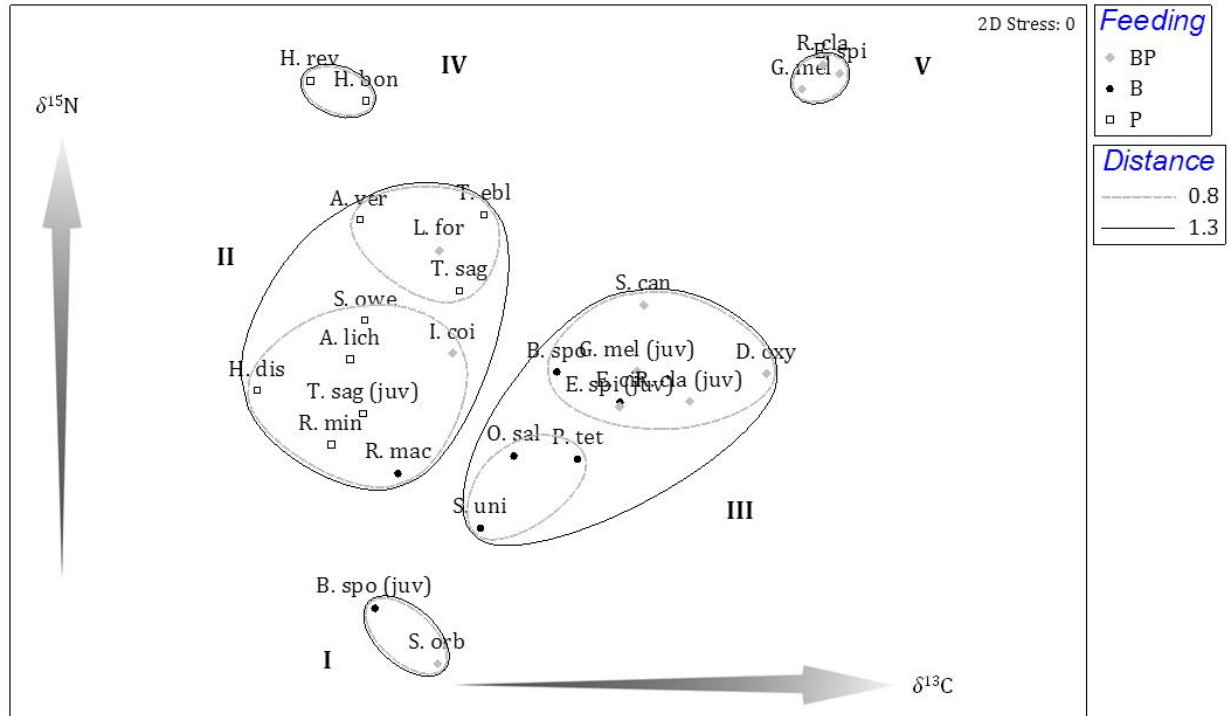
Figure 7.5. Multidimensional scale (MDS) results based on mean $\delta^{13}C$ and $\delta^{15}N$ data (%) of elasmobranchs and cephalopods from the western Mediterranean. Trophic groups (I-V) are defined at Euclidean distance of 1.3. Broad feeding strategies (BP: benthopelagic, B: benthic and P: pelagic feeders) are also shown. For species codes see Table 7.2.

Stable isotopes analysis and feeding strategies

For statistical comparisons and based on dietary similarity (Fig. 7.3), *Rossia macrosoma* and *Eledone cirrhosa* were grouped within the guild B, *Scyliorhinus canicula* was considered within the guild D and *Heteroteuthis dispar* was considered in a unique guild labelled H. Species carbon and nitrogen isotopic ratios differed significantly among feeding guilds identified by stomach data (guilds A to H) (δ^{13}C: pseudo-F= 42.95, p<0.001 and δ^{15}N: pseudo-F= 24.72, p<0.001). The pelagic trophic guild H (mean δ^{13}C=-20.03±0.26‰) was the most ^{13}C-depleted whereas the trophic guild C (rays) (mean δ^{13}C=-17.78±0.40‰) was the most ^{13}C-enriched. Regarding δ^{15}N, the trophic guild D (sharks) (mean δ^{15}N=9.71±0.90‰) showed the highest values, and trophic guild B (mean δ^{15}N= 8.34±0.61‰), which grouped *O. salutii*, *P. tetracirrhus*, *E. cirrhosa* and *R. macrosoma*, displayed the lowest values. Pair-wise comparisons (Table 7.6) indicated that there were no isotopic differences (both δ^{13}C and δ^{15}N values) between octopus trophic guilds A and B (mean δ^{13}C=-18.76±0.43‰, mean δ^{15}N=8.41±0.92‰, and mean δ^{13}C=-18.83±0.51‰, mean δ^{15}N=8.34±0.61‰, respectively). No δ^{13}C-differences were detected between squids trophic guilds E and F (mean δ^{13}C=-19.24±0.50‰ and -19.32±0.46, respectively) nor between trophic guilds G and H (mean δ^{13}C=-19.85±0.45‰ and -20.03±0.26‰, respectively). In contrast to δ^{13}C, a higher number of pair-wise δ^{15}N guilds comparisons did not differ: A (mean δ^{15}N=8.41±0.92‰) *vs* B (mean δ^{15}N=8.34±0.61‰), H (mean δ^{15}N=8.40±0.94‰) and F (mean δ^{15}N=8.77±0.77‰); C *vs* E (mean δ^{15}N =9.19±0.72‰), D (mean δ^{15}N =9.71±0.90‰) and G (mean δ^{15}N=9.38±0.94‰); G *vs* E, and H *vs* B and F.

Stable isotope niches and trophic overlap

Squids had the largest isotopic niche among the cephalopod and elasmobranch species analyzed (Table 7.4). Pelagic squid such as *Abralia veranyi*, *Loligo forbesii* and *Illex coindetii* had the largest isotopic niche width, while juveniles of *Raja clavata* together with carcharhiniformes sharks had the smallest ones. Among 28 pair-wise combinations between trophic guilds, isotopic overlap was only found for 8 pairs (Table 7.6; Fig 7.7a), overall with low SEAc overlap values (<50%). Both octopods feeding guilds (A and B) and squids feeding guilds E and F, showed the highest isotopic overlap (35.6% and 31.4% respectively). Low overlap was also found between both elasmobranch trophic guilds C and D (19.7%). Trophic guild G exhibited similar low overlap with guild H (12.1%) and guild E (11.1%). Very little isotopic overlap was observed between demersal sharks (guild D) and octopods (guild A) trophic guilds (1.4%), as well as between guild F (*T. sagittatus*) and B (octopods) (2.1%), and between F and G (pelagic cephalopods) (6.1%).

At species level, isotopic niche overlap was examined for the species having a sample size higher than 10 individuals and belonging to the same trophic groups identified by both SCA (Fig. 7.3) and SIA (at Euclidean distance of 0.8; Fig. 7.5). All pair-wise comparisons showed low trophic niche overlap (Table 7.8; Fig. 7.7b).

Table 7.6. Results of PERMANOVA pair-wise tests comparing the isotopic ratios of the main feeding guilds obtained from the cluster analysis shown in Figure 2. SEAc overlap (%) between feeding guilds is also shown. See Table 7.5 for feeding guild codes. ns: no significant.

Feeding guilds	$\delta^{13}C$			$\delta^{15}N$			SEAc Overlap (%)
	t	P(perm)	Unique perms	t	P(perm)	Unique perms	
A, B	0.6688	ns	9827	0.4586	ns	9834	35.6
A, C	9.7221	0.0001	9845	4.3864	0.0001	9826	-
A, D	9.6	0.0001	9851	6.9851	0.0001	9821	1.4
A, E	4.3754	0.0001	9836	4.2115	0.0002	9819	-
A, F	5.463	0.0001	9816	1.9329	ns	9839	-
A, G	11.843	0.0001	9821	5.0343	0.0001	9785	-
A, H	11.22	0.0001	9833	3.3125	ns	9834	-
B, C	11.229	0.0001	9846	7.0959	0.0001	9846	-
B, D	12.956	0.0001	9849	1.1195	0.0001	9827	-
B, E	4.415	0.0001	9831	6.9749	0.0001	9823	-
B, F	5.5922	0.0001	9838	3.5194	0.0008	9804	2.1
B, G	14.275	0.0001	9844	8.2141	0.0001	9793	-
B, H	9.6685	0.0001	9823	0.3483	ns	9813	-
C, D	3.7559	0.0005	9807	0.9069	ns	9838	19.7
C, E	15.224	0.0001	9845	1.8217	ns	9842	-
C, F	17.196	0.0001	9837	3.978	0.0004	9846	-
C, G	26.02	0.0001	9831	0.9211	ns	9838	-
C, H	21.912	0.0001	9829	3.6928	0.0006	9836	-
D, E	18.463	0.0001	9827	3.6886	0.0006	9847	-
D, F	20.89	0.0001	9831	6.8264	0.0001	9852	-
D, G	35.93	0.0001	9821	2.8171	0.0072	9819	-
D, H	22.963	0.0001	9832	5.7368	0.0001	9807	-
E, F	0.8605	ns	9828	2.9193	0.0037	9850	31.4
E, G	7.9401	0.0009	9820	1.3159	ns	9834	11.1
E, H	6.4106	0.0001	9831	3.6844	0.0006	9836	6.1
F, G	7.3233	0.0084	9833	4.2842	0.0001	9823	-
F, H	6.2413	0.0001	9854	1.6948	ns	9821	-
G, H	1.6434	ns	9856	4.1421	0.0001	9851	12.1

Table 7.7. $\delta^{15}N$, $\delta^{13}C$ and C:N ratio (mean ± sd) of elasmobranchs and cephalopods prey, collected during the IDEADOS ([a]) and the MEDITS ([b]) surveys. Prey habitat and their predators are also shown. For predators codes see Table 7.2.

Prey species	Habitat	Code	N	$\delta^{13}C$	$\delta^{15}N$	C:N	Predator
Geryon longipes [a]	Benthic	Ger	4	-17.57 ± 0.29	9.94 ± 0.34	3.15 ± 0.03	O. sal, P. tet
Philocheras echinulatus [a]	Benthic	Phil	26	-18.00 ± 0.47	9.15 ± 0.68	3.16 ± 0.06	B. spo, S. can
Alpheus glaber [a]	Benthic	Alph	6	-18.10 ± 0.32	7.31 ± 0.71	3.17 ± 0.05	B. spo, O. sal, T. sag, P. tet, G. mel, R. cla, S. can
Pandalina profunda [a]	Benthic	Pan	5	-18.24 ± 0.53	8.00 ± 0.81	3.20 ± 0.06	S. can
Procesa canaliculata [ab]	Benthic	Pro	10	-18.53 ± 0.62	8.41 ± 0.54	3.18 ± 0.10	B. spo, O. sal, T. sag, D. oxy, S. can, R. cla, G. mel
Solenocera membranacea [a]	Benthic	Sol	4	-18.60 ± 0.14	8.38 ± 0.56	3.13 ± 0.04	T. sag, R. cla, S. can
Callocaris macandrae [a]	Benthic	Mac	4	-19.09 ± 0.53	6.44 ± 0.83	3.48 ± 0.08	B. spo, O. sal, P. tet
Stomias boa [a]	Pelagic	Sb	5	-19.13 ± 0.76	8.79 ± 0.42	3.26 ± 0.09	T. sag, H. bon, S. can, G. mel, E. spi
Pasiphaea multidentata [ab]	Pelagic	Pmul	16	-19.21 ± 0.38	7.36 ± 0.86	3.18 ± 0.08	T. sag, H. rev, E. spi, G. mel
Hygophum benoiti [a]	Pelagic	Hb	4	-19.33 ± 0.06	7.87 ± 0.52	3.47 ± 0.13	H. rev, I. coin, E. spi, G. mel
Arctozenus rissoi [a]	Pelagic	Ar	7	-19.49 ± 1.36	7.73 ± 0.69	4.05 ± 0.64	T. sag, G. mel
Myctophum punctatum [a]	Pelagic	Mp	6	-19.56 ± 0.85	7.93 ± 0.93	3.57 ± 0.25	E. spi, G. mel, H. rev
Hygophum hygomii [a]	Pelagic	Hh	5	-19.71 ± 0.78	9.12 ± 0.78	3.39 ± 0.03	T. sag, H. rev
Maurolicus muelleri [a]	Pelagic	Mm	6	-19.91 ± 0.33	8.40 ± 0.78	3.78 ± 0.53	T. sag, I. coin, L. forb, A. ver, G. mel
Notoscopelus elongatus [a]	Pelagic	Ne	14	-19.92 ± 0.74	8.97 ± 0.76	4.08 ± 0.50	T. sag, A. ver, I. coin, E. spi
Ceratoscopelus maderensis [a]	Pelagic	Cm	20	-19.96 ± 0.85	8.20 ± 0.76	3.68 ± 0.40	T. sag, O. sal, P. tet, H. rev, A. ver, I. coin, E. spi
Symbolophorus veranyi [a]	Pelagic	Sv	7	-20.02 ± 1.15	8.11 ± 0.98	3.80 ± 0.67	T. sag
Lampanyctus crocodilus [a]	Pelagic	Lc	10	-20.19 ± 0.52	7.94 ± 0.50	3.48 ± 0.26	T. sag, H. rev, S. owe, A. ver, E. spi, G. mel
Meganychtiphanes norvegica [ab]	Pelagic	Euph	16	-20.23 ± 0.43	6.32 ± 0.63	3.46 ± 0.23	see table 3 (Euphausiacea)
Lampanyctus pusillus [a]	Pelagic	Lp	4	-20.28 ± 0.67	8.70 ± 0.56	3.94 ± 0.38	T. sag, E. spi
Pasiphaea sivado [ab]	Pelagic	Psiv	6	-20.76 ± 0.48	6.63 ± 0.42	3.15 ± 0.06	T. sag, H. rev, E. spi, S. can

7.4 Discussion

Using stomach contents and stable isotopes (δ^{13}C and δ^{15}N) data, we examine the trophic relationships of five elasmobranch (rays and sharks) and eighteen cephalopod (squids, octopuses and cuttlefishes; 'cuttlefishes' is used '*senso lato*') deep-sea species of the western Mediterranean. Ontogenetic shifts in diet and habitat play an important role in individual growth and survival, species interactions and community structure (Werner and Gilliam, 1984). However, relatively few studies and food web models take into account ontogenetic shifts in feeding strategies, especially in the Mediterranean (Bǎnaru et al., 2013; Coll et al., 2006). Although the relationship between body size and δ^{15}N is not always evident in elasmobranch species (Barría et al., 2015; Churchill et al., 2015; Vaudo and Heithaus, 2011), ontogenetic shifts in diet were determined in this study by means of stable isotope data for *Galeus melastomus, Etmopterus spinax* and *Raja clavata*. Despite juvenile and adult elasmobranchs displayed the same feeding strategy (rays: guild C; sharks: guild D), they were isotopically segregated (Fig. 7.5), indicating that both size groups would consume different size classes of the same prey. Individual size has already been reported to influence the diet of elasmobranchs such as *Scyliorhinus canicula*, *G. melastomus* and *R. clavata* in the Mediterranean (Macpherson, 1980b; Saglam and Bascinar, 2008; Valls et al., 2011). This also applies to other Mediterranean deep-sea fish species, which display partition of food according to predator and prey size (Carrassón and Cartes, 2002; Macpherson, 1981).
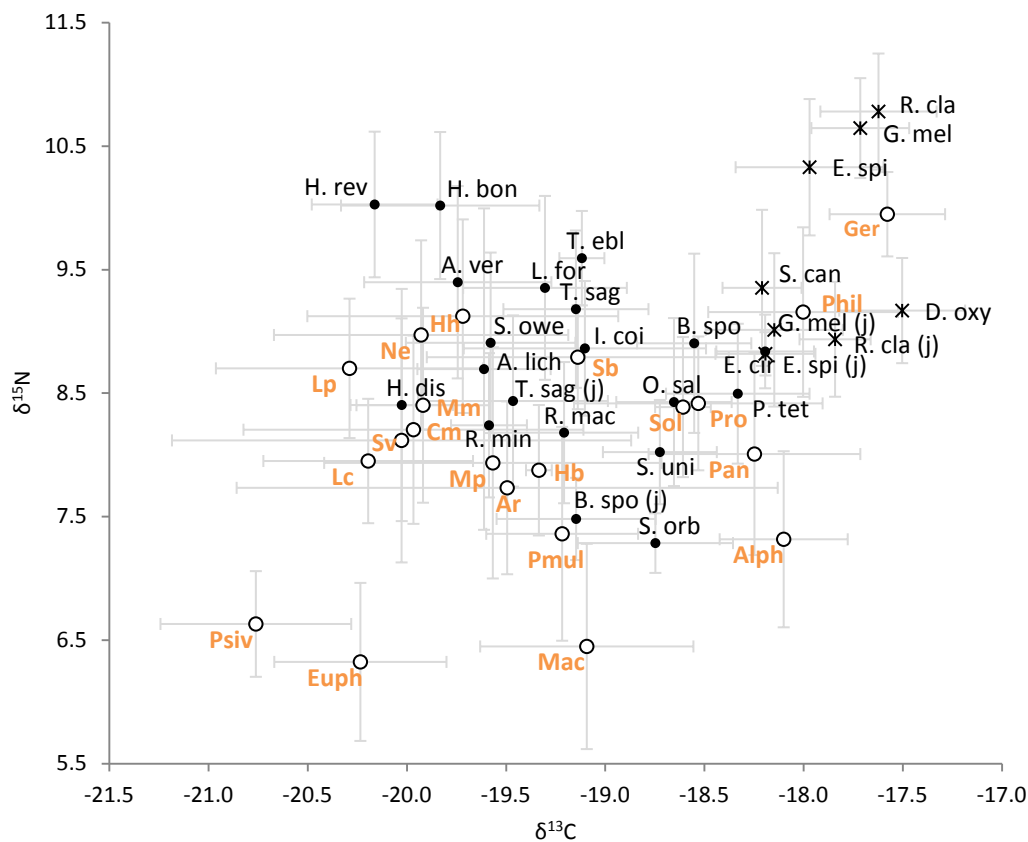
Figure 7.6. Scatterplot of δ^{13}C and δ^{15}N values (mean ± SD) of cephalopods (black dots), elasmobranchs (asterisks) and common prey (white dots) based on stomach content data. For species codes see Tables 7.2 and 7.7.

Regarding cephalopods, little work has been done to date comparing the diet of deep-sea species across different life stages (Hoving et al., 2014). As in elasmobranchs, the octopus *Bathypolypus sponsalis* and the squid *Todarodes sagittatus* displayed size-related isotopic shifts (Fig. 7.2), in spite of being grouped within the same feeding guild (Fig. 7.3). Differences in both δ^{13}C and δ^{15}N values between *B. sponsalis* size-classes reflected their diet differences, as juveniles exhibited a higher preference for amphipods, whereas adults frequently preyed on benthic decapods and juveniles did not. The burrowing shrimp *Calocaris macandreae* was among the most abundant (in number) suprabenthic species along the slope (Ramón et al., 2014) and the main crustacean preyed by *B. sponsalis* in the western Mediterranean (Fanelli et al., 2012; Quetglas et al., 2001). The absence of this prey, which uses a complex configuration of tunnels between 10 and 21 cm depth (Nash et al., 1984), in juveniles, suggests that they forage less actively than adults. This is in accordance with an increasing feeding activity with growth already reported for other cephalopods (Castro and Guerra, 1990; Rocha et al., 1994). Regarding *T. sagittatus,* stomach data showed an increase in benthopelagic prey (fish and decapod crustaceans) as they grow, indicating an ontogenetic shift in foraging behaviour from pelagic to benthopelagic prey. Such a shift in food sources is coherent with a higher abundance of adults in bottom trawl samples compared to midwater trawl samples in the study area (Quetglas et al., 2014). Ontogenetic shifts in diet related to habitat changes have also been reported in other squids using stomach content (Cherel and Duhamel, 2003; Quetglas et al., 1999; Valls et al., 2015) and isotopic (Cherel and Hobson, 2005) data.

As already reported in previous works (Coll et al., 2013; Navarro et al., 2013), cephalopods displayed a high variety of trophic strategies and broad trophic widths. In accordance with their benthic habits, octopuses, which were generally ^{13}C-enriched, exhibited two main feeding strategies. *Bathypolypus sponsalis* (guild A) relied on a wide spectrum of benthic resources (decapod crustaceans, bivalves, gastropods and ophiurans), whereas *Octopus salutii* and *Pteroctopus tetracirrhus* (guild B) preyed mainly on benthic crustaceans. The last octopus species, *Eledone cirrhosa,* displayed a unique dietary composition characterized by the frequent consumption of annelids. Besides, the sepiolid *Rossia macrosoma*, which also preyed on benthic crustaceans, displayed a unique dietary composition within the benthic feeders group characterized by frequent consumption of fish (F=50%). That preference for fish was found to be much important in this study than generally though for sepiolids (Rodhouse and Nigmatullin, 1996). The remaining cephalopod species investigated (ommastrephids, histioteuthids and other sepiolids) are diel migrators, remaining close to the bottom during the daytime but distributing in the water column at night (Quetglas et al., 2014). In spite of this common pattern, they also showed different feeding strategies. The squids *Histioteuthis* spp. and *Abralia veranyi*, and the sepiolids *Sepietta oweniana* and *Rondeletiola minor* (guild G) preyed mainly on pelagic fish and crustaceans characterized by low δ^{13}C values (Table 7.7). As already reported (Fanelli et al., 2012), stable isotope analysis showed that *Histioteuthis* spp. occupied a distinct trophic group feeding at upper levels in the pelagic food chain. Stomach content data also showed different feeding strategies (guilds E and F) within the family Ommastrephidae. In spite of their necktobenthic habits (Quetglas et al., 2014), isotopic data of this family revealed low δ^{13}C values that could be due to the

important contribution of pelagic prey (fish, euphausiids or decapods crustaceans). Mesopelagic fish are important prey of *Illex* spp., *Todarodes* spp. and oceanic cephalopods (Rodhouse and Nigmatullin, 1996). On slope grounds, mesopelagic species concentrate between 400 and 600 m depth forming persistent near bottom aggregations (Olivar et al., 2012). In the study area, they also aggregate at the Benthic Boundary Layer at about 50 m above the bottom (Olivar et al., 2012; Simão et al., 2014) or even closer to the bottom (Abelló et al., 2002; Moranta et al., 2008). Mesopelagic species are thus available for both pelagic and near-bottom dwelling predators. The pelagic prey would be further chased by squids during the prey vertical migration at night (Fanelli et al., 2012; Martínez-Baena et al., 2016; Rosas-luis et al., 2014). This foraging pattern chasing prey into the water column has already been suggested for the benthopelagic squid *Moroteuthis ingens* (Cherel and Duhamel, 2003).
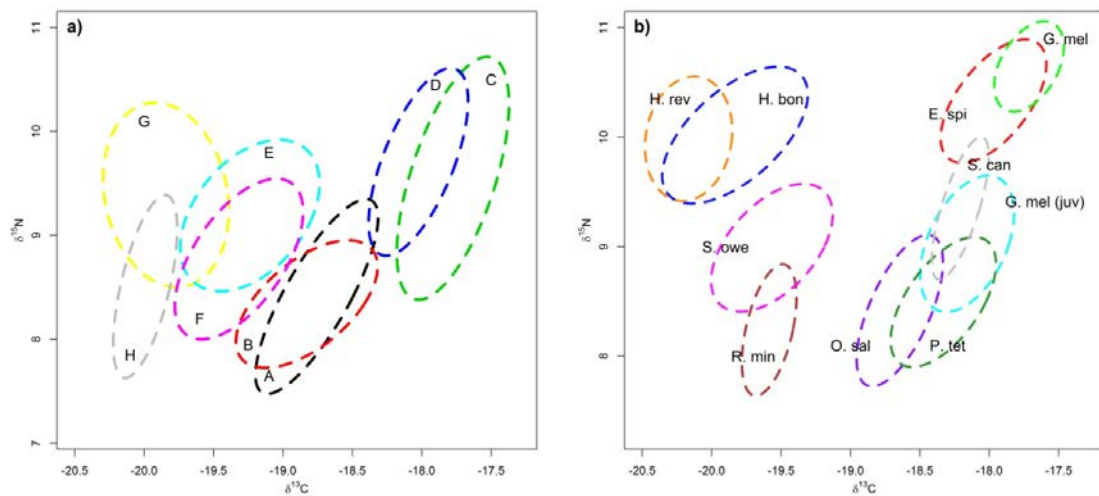
Figure 7.7. Standard ellipse areas corrected for sample size (SEAc) for (a) feeding guilds (A to H) and (b) individual species of cephalopods and elasmobranchs.

Locomotion and environmental adaptations are major factors in species life-history strategies (Pethybridge et al., 2010; Rosa et al., 2005; Seibel, 2007). Species with strong locomotory abilities are characterized by higher metabolic rates than slow-moving species (Childress et al., 1990; Koslow, 1996; Maynou and Cartes, 1998). Accordingly, our results showed that high energy requirements in squids, which generally rely on constant swimming, might be supplied by consuming lipid-rich prey such as mesopelagic fish. By contrast, benthic octopuses, which rely on crypsis and refuge to avoid detection by predators or prey, generally fed on benthic, low $\delta^{15}N$ values prey (Fig. 7.4, Table 7.7).

Regarding elasmobranchs, rays and sharks also exhibited different feeding strategies (trophic guilds C and D, respectively). Despite both groups consumed pelagic crustaceans, rays frequently fed on slow-moving and/or low energetic prey (i.e. benthic crustaceans), whereas sharks foraged upon fast-moving and/or high energetic prey (i.e. cephalopods, euphausiids) (Table 7.3 and 7.7). Along with the swimming performance, the variability in body morphology must be considered for the energetic requirements of a species (Koslow, 1996) and their foraging

behaviour. Compared with shallow species where hydrodynamic and hydrostatic lifts combine to confer buoyancy, deep-sea elasmobranchs have a large, lipid-rich liver allowing them to approach neutral buoyancy by hydrostatic lift alone (Pethybridge et al., 2010; Wetherbee and Nichols, 2000). However, there is no available empirical evidence that benthic deep-sea batoids are neutrally buoyant (Treberg and Speers-roesch, 2016). Batoids lack a propulsive caudal fin and their locomotory efficiency have been suggested to be limited (Di Santo and Kenaley, 2016).

The seven trophic guilds and four species with unique dietary composition identified in this study were further aggregated into three broad strategies: benthic feeders, benthopelagic feeders and pelagic feeders (Fig. 7.4). The benthic and pelagic feeding strategies were constituted exclusively by cephalopod species. Elasmobranchs, together with three cephalopod species, were all grouped within the benthopelagic feeding strategy, but more closely related to pelagic resources than to benthic ones. In oceanic areas (Blaber and Bulman, 1987; Haedrich and Merrett, 1992) and other oligotrophic areas such as the Ionian Sea (Madurell and Cartes, 2005), food webs are more dependent on pelagic than on benthic sources. In this sense, the waters around the Balearic Islands are characterized by its poverty within the general oligotrophy of the Mediterranean Sea (Bosc, 2004; Estrada, 1996). This would explain the high frequency of pelagic prey in the diet of deep-sea elasmobranchs and cephalopods found in this study. Previous works have already highlighted the relevance of mesopelagic prey in fish, cephalopods and crustaceans from the Balearic Islands compared to other Mediterranean (Cartes et al., 2009, 2008; Fanelli and Cartes, 2008) and Atlantic (Valls et al., 2015) areas. Further, food webs over the insular slope were mainly linked to primary production (Fanelli et al., 2013b) displaying a close benthopelagic coupling (Valls et al., 2014b). Due to the low density of benthic food resources around the continental slope of the Balearic Islands (Cartes et al., 2001; Fanelli et al., 2013a, 2009), consumers maximize their caloric intake by consuming highly energetic mesopelagic prey, which has also been suggested for the Ionian Sea fish community (Madurell and Cartes, 2005).

Isotopic niche width (SEAc) revealed interesting features among species sharing the same trophic guild (Fig. 7.3) and trophic group (Fig. 7.5). For example, the two histioteuthid squid species showed similar δ^{15}N but different δ^{13}C range values, indicating that both species fed at the same trophic level but differed in the source of carbon. *Histioteuthis* spp. segregate bathymetrically in the study area (Quetglas et al., 2010) likely reducing competition for food. The same pattern was observed for the deep-sea octopods *O. salutii* and *P. tetracirrhus,* which bathymetric distribution coincides with that of their main prey, the endobenthic shrimp *Alpheus glaber* (Abelló et al., 2002). However, the depths with the highest abundance of those octopods differ (Quetglas et al., 2009, 2005), which would explain their low niche overlap (19%). Differences were also found for the isotopic niches of adult individuals of the sharks *E. spinax* and *G. melastomus*. Both species showed a low trophic overlap (23%) and their SEAc value indicated that the former species displayed a greater degree of trophic diversity. Although previous studies (Macpherson, 1980a; Preciado et al., 2009; Valls et al., 2011) reported diet overlap for those demersal sharks, the data reported here suggest that they occupy different isotopic niches in the study area. Similarly, *S. canicula* and juveniles of *G.*

melastomus, which shared their habitat (Massutí and Moranta, 2003) and main food resources (i.e. euphausiids, pelagic decapods), showed a low isotopic overlap (23%). Size-related and behavioural differences could explain such a low overlap, since the SEAc indicated that *S. canicula* had a more specialized diet.

Table 7.8. Percentage values of overlap of the standard ellipses areas corrected for sample size (SEAc) between pairs of species sharing the same trophic guild (TGd) (see Fig. 7.3) and trophic group (TGp) (see Fig. 7.5). For species codes see Table 7.2.

TGd	TGp	Species	H. bon	H. rev	G. mel	E. spi	G. mel (j)	S. can	O. sal	P. tet	R. min	S. owe
G	IV	H. bon										
G	IV	H. rev	24									
D	V	G. mel	-	-								
D	V	E. spi	-	-	23							
D	III	G. mel (j)	-	-	-	-						
D	III	S. can	-	-	-	-	23					
B	III	O. sal	-	-	-	-	-	-				
B	III	P. tet	-	-	-	-	-	-	19			
G	II	R. min	-	-	-	-	-	-	-	-		
G	II	S. owe	-	-	-	-	-	-	-	-	7	

The structure of cephalopod and elasmobranch food webs revealed a clear isotopic divergence from low to high trophic levels, mainly dictated by differences in their food source (Fig. 7.4; Table 7.6), which would be related to habitat use (Cherel et al., 2009) and food partitioning (Heithaus et al., 2013; Valls et al., 2014b). As expected (France, 1995), the isotopic values showed that the species closely related with the bottom (i.e. octopuses, rays, demersal sharks) were ^{13}C-enriched compared to those having pelagic habits (i.e. squids, cuttlefishes). Large elasmobranch species occupied the trophic group with the highest δ^{13}C and δ^{15}N values. The stable isotopic values of sharks were within the range of those reported in previous studies (Papiol et al., 2013; Polunin et al., 2001) and similar to that of deep-sea demersal fishes (Valls et al., 2014b). Such enriched values did not seem to corroborate sharks stomach content data, which showed frequent consumption of pelagic resources (Table 7.3 and 7.7). Several factors could determine this apparent enrichment compared to squids that also feed on mesopelagic prey. On one hand, it could be explained by large deep-sea sharks exploiting different population fractions with contrasting isotopic signatures. This is the case of the myctophid *Lampanyctus crocodilus*, a prey found in squid (Quetglas et al., 1999; this study) and shark (Valls et al., 2011; this study) stomach contents. Small-sized *L. crocodilus* inhabiting the water column showed lower δ^{13}C values than the large-sized, non-migrant individuals living close to the bottom (Fanelli et al., 2014; Valls et al., 2014a). On the other hand, the quantity and quality of protein that an organism consumes relative to its metabolic needs can generate variation in Δ^{13}C values (i.e. the isotopic difference between consumer tissues and diet; Martínez del Rio et al., 2009). Indeed, sharks following a diet based on squids (high quality and quantity of protein) in a controlled laboratory study showed high Δ^{13}C values (1.7‰; Kim et al., 2012).

Overall, results from both stomach content and stable isotope analyses reflected a clear resource partitioning between and within elasmobranchs and cephalopods from the western Mediterranean deep-sea ecosystems. Ecosystem models should consider diet composition and ontogenetic shifts to define cephalopod and elasmobranch trophic functional groups. Segregation of the isotopic space showed a contrasting food source gradient ($\delta^{13}C$) stretching from pelagic (squids and cuttlefishes) to nektobenthic (octopuses and elasmobranchs) sources. However, deep-sea sharks off the Balearic Islands frequently preyed on mesopelagic species, as did squids and cuttlefishes. Cephalopods, in turn, constituted an important food resource for deep-sea sharks. Benthopelagic cephalopods (Coll et al., 2006; Tsagarakis et al., 2010) and sharks (Moutopoulos et al., 2013; Tecchio et al., 2013) have already been identified as structuring groups in the Mediterranean Sea due to their high biomass and high trophic impact in marine ecosystems. Squids, cuttlefishes and sharks identified as benthopelagic feeders in this study would play an important role by transferring energy/carbon and nutrients from the nycthemeral migrating community to the demersal community. As cephalopods are abundant prey for top predators such as marine mammals, turtles and large pelagic fish (Blanco et al., 2006, 2001; Massutí et al., 1998) they would function as carbon circulators exporting pelagic carbon to the benthic environment, and vice-versa (Cherel and Duhamel, 2003; Guerreiro et al., 2015). By contrast, the small-sized elasmobranchs analyzed here, with some minor exception (i.e. Navarro et al., 2014), do not appear as a main prey of larger predators. Thus, they would transfer energy to the benthic community retaining that carbon into the deep-sea biomass. Trueman et al., (2014) suggested that this biological one-way flux supports as much as 50% of benthic fish production at upper and mid slope depths.
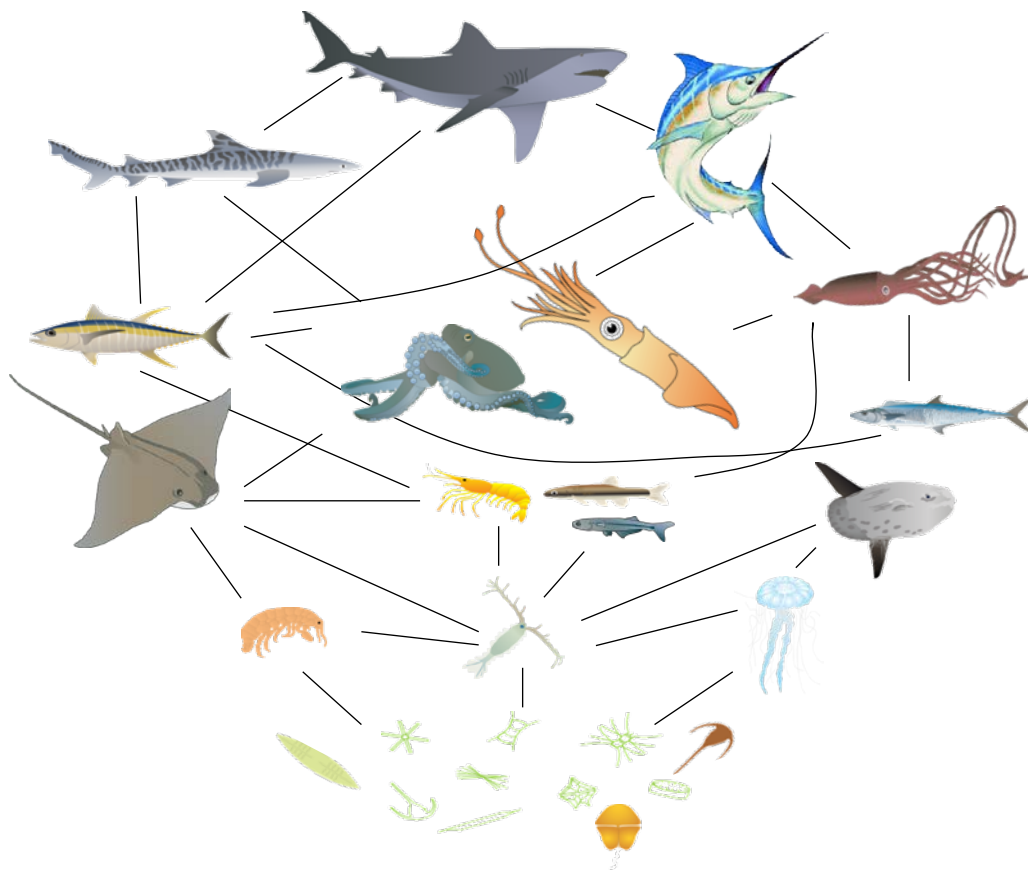
Chapter 8: General Discussion

Chapter 8: General Discussion

Knowing the feeding ecology and trophic interactions of marine fauna is paramount to understand the functioning of an ecological system. This Thesis aimed at providing an integrated picture of the food web structure and dynamics, together with the feeding ecology of individual species, of three key taxonomical groups (cephalopods, elasmobranchs and mesopelagic fishes; see 1.5) in Mediterranean marine ecosystems. To this end, two complementary methodological approaches were used (see 1.6): stable isotope analysis (SIA) and stomach content analysis (SCA). The SIA has been employed to identify energy pathways in trophic networks, to estimate species trophic level and to describe food web structure and dynamics at both the vertical (pelagic–demersal domain) and horizontal (from shelf-break to middle slope) axis, as well as under the influence of contrasting oceanographic scenarios (Algerian *vs* Balearic sub-basins). The SCA, in turn, provides information on prey species composition, revealing predator-prey interactions and resource partitioning among consumers.

Trophic coupling between pelagic and hyperbenthic communities varied with depth in both sub-basins, as the isotopic separation between vertical compartments increased from the shelf-break to the middle slope (Chapter 3). The narrower range of $\delta^{13}C$ values suggested that hyperbenthic shelf-break faunal assemblages are more closely tied to plankton food webs. Zooplankton biomass is higher on the shelf-break than on bathyal depths (Cartes et al., 2008a; Sabatès et al., 1989), which is probably a consequence of the occurrence of frontal systems along the shelf edge (Flexas et al., 2002), becoming available for consumption by hyperbenthic species. Indeed, suprabenthic filter-feeders are almost exclusively found on the upper slope (Cartes et al., 2008a), which indicates their high reliance on the pelagic food web compared to the prevalence of deposit-detritus feeders and carnivores at the deepest slope strata (Madurell et al., 2008). Further, lower $\delta^{13}C$-$\delta^{15}N$ correlations at the shelf-break compared to the slope (Chapter 3) would reflect the isotopic variations of the primary producers (Chapter 6). Our results revealed spatial differences in the strength of the benthopelagic coupling at the deepest slope stratum, since it was higher at the BsB (lower $\delta^{13}C$-separation) compared to the AsB (Chapter 3), indicating that there is a tighter link via mesopelagic prey between surface primary production and hyperbenthic consumers in the former. This higher dependence upon mesopelagic prey seems consistent with higher surface production and zooplankton biomass at the BsB compared to the AsB (Cartes et al., 2008b). By contrast, the higher $\delta^{13}C$ values observed at the AsB indicated that hyperbenthic species were sustained by more reworked and remineralised material of planktonic origin owing to the prevailing hydrodynamic conditions. Indeed, the higher settling velocity (due to water column mixing) at the BsB (Pasqual et al., 2014) results in less degraded

(isotopically depleted) and higher nutritional values of the OM (Pasqual et al., 2015).

In agreement with previous works in the study area (Papiol et al., 2013; Polunin et al., 2001), isotopic values of hyperbenthic fish and sharks from the slope showed high δ^{13}C values (Chapters 3 and 7). Such enrichment, however, would not tally with SCA, which revealed frequent consumption of pelagic resources by those consumers (Chapters 4 and 7). This could be due to differences in food absorption rates since it is not always clear from SCA how much a particular material will contribute to the production of the consumer concerned (Stoner and Zimmerman, 1988). Alternatively, it could be related with migratory differences in terms of size ranges of deep-sea pelagic prey species (Olivar et al., 2012; Quetglas et al., 2014). Hyperbenthic consumers would prey upon mesopelagic prey living close to the sea bottom and therefore being ^{13}C-enriched compared to those inhabiting the water column, as found for the myctophid *Lampanyctus crocodilus* (Chapter 6).

This Thesis also revealed the importance of mesopelagic species as food resources for megafaunal slope assemblages (Chapter 7), such as those of elasmobranchs and cephalopods, from the Balearic Sea. Mesopelagic crustaceans (euphausiids and decapods) constituted a main prey, in terms of biomass, for elasmobranchs (rays and sharks), especially *Scyliorhinus canicula* (both recruits and adults) and recruits of *Etmopterus spinax* and *Galeus melastomus* (Chapter 4). Mesopelagic fish and euphausiids were also important for both recruits and adults of the squids *Loligo forbesii*, *Illex coindetii* and *Todarodes sagittatus*. Important demersal fishing resources, such as the European hake (*Merluccius merluccius*) and the red shrimp (*Aristeus antennatus*), also rely on mesopelagic prey in demersal ecosystems from the Balearic Islands. Euphausiids, mesopelagic decapods and myctophids were more important prey for the red shrimp of the Balearic Sea compared to populations from adjacent areas (Cartes et al., 2008b). Similarly, while hake preys on euphausiids and myctophids in the Archipelago (Cartes et al., 2009), it prefers small-pelagic fish (sardine and anchovy) in the Gulf of Lions (Ferraton et al 2007). In fact, our results are consistent with previous studies reporting that food webs from oligotrophic (Cartes et al., 2009; Madurell and Cartes, 2005b) and open oceanic (Blaber and Bulman, 1987; Koslow, 1997) areas are mainly supported by planktonic sources. The prevalence of mesopelagic species in the diet of the nektobenthic predators analysed in this Thesis indicates that they play a key role for the benthopelagic coupling on the slope food webs from the oligotrophic waters off the Balearic Islands (Bosc, 2004; Estrada, 1996). This agrees with recent research showing that the trophic interactions of mesopelagic and demersal fishes on slope ecosystems play an important role in the carbon cycle, transferring carbon to the deep long-term storage (Trueman et al., 2014).

Despite the recognized key role of mesopelagic organisms in marine food webs, linking lower trophic levels and top predators (Carrassón and Cartes, 2002; Cartes, 1998), the trophic interactions and dynamics within this community have received

little attention to date in the Mediterranean (Bernal, 2014). Results from this Thesis showed homogeneous isotopic (δ^{13}C and δ^{15}N) zooplankton values over the spatial scale sampled (AsB *vs.* BsB, shelf *vs.* slope) but revealed high seasonality in δ^{13}C values (Δ1.2‰; Chapter 6). This would reflect intra-annual changes in species composition of the phytoplankton community (Estrada et al., 1999), with the ^{13}C-depleted dinoflagellates (Fry and Wainright, 1991) dominating over diatoms during the stratification period in the western Mediterranean (Estrada, 1985). In agreement with previous studies (Bode et al., 2007; Koppelmann et al., 2009; Montoya et al., 1992), we observed a narrow ^{15}N range among zooplankton size groups, although it reflected size-based feeding, with higher δ^{15}N on large-sized zooplankton organisms. The mesopelagic fish assemblage also displayed the same seasonal ^{13}C pattern as the zooplankton, demonstrating their role linking primary producers and the nektonic community (Letessier et al., 2012; Miller et al., 2010a). These results highlight the importance of considering spatio-temporal variations on the base of the food chain, linking isotopic variations of consumers to environmental variables, as also reported by previous authors (Cartes et al., 2001; Fanelli and Cartes, 2010, 2008; Jennings and Warr, 2003). If not considered, such variations can effectively lead to misinterpretations in the assessment of the feeding zone, the food partitioning among consumers or the determination of trophic levels from SIA (Chouvelon et al., 2012; Guzzo et al., 2011).

This Thesis extended the study of trophic links from the ecosystem or assemblage level (Chapters 3 and 6) to the taxonomic level, focusing on the diet composition and feeding behaviour of several cephalopod and elasmobranch species (Chapters 4, 5 and 7). The trophic ecology of two high value commercial squid species (*Loligo vulgaris* and *L. forbesii*) was analysed for the first time in the Mediterranean (Chapter 5), revealing a highly piscivorous strategy as it has already been reported in the Atlantic (Collins et al., 1994; Pierce et al., 1994). The diet composition of these two squid differed, reflecting their bathymetric segregation as *L. vulgaris* inhabits shallower waters than *L. forbesii* in the Balearic Sea (Quetglas et al., 2000). Results of the present Thesis revealed seasonal variations in diet for both squid species, coinciding with their main reproduction period. In spring, *L. vulgaris* shifted from a benthopelagic to a more benthic feeding strategy, showing a significant increase in the consumption of high energetic prey such as polychaetes (i.e. nereidids). In turn, the summer and autumn diet of large *L. forbesii* was also characterized by higher consumption rates of migrating and non-migrating mesopelagic fish, indicating predator-prey interactions in the water column or offshore displacements in search of those high caloric content preys. Similar feeding related movements in search of highly energetic prey (i.e. euphausiids, myctophids) have also been reported for the red shrimp (Cartes et al., 2008b) and hake recruits (Cartes et al., 2009) in the study area. These seasonal changes in species diet may be linked to reproductive periods but also to variations in prey availability (Fanelli and Cartes, 2010, 2008; Guijarro et al., 2012).

Our results also revealed an important diet overlap among coexisting elasmobranch species in the study area (Chapter 4). Several studies have found that dietary breadth is inversely related to prey abundances, with predator diets skewed towards abundant prey which leads to a relaxed food competition and high diet similarity (Croxall et al., 1999, Tinker et al., 2008). However, such a high overlap in our samples should be taken with much care owing to the following two main reasons. The most important bias would arise from grouping prey into broad taxonomic categories due to the impossibility of identifying all stomach contents to the species level. Obviously, this grouping would give rise to an erroneously increased diet overlap which would be reduced, or even absent, if prey data was available at the species level. Indeed, δ^{13}C and δ^{15}N signatures of elasmobranch species over the slope indicated a wide range of isotopic values (Chapter 7) consistent with a high diversity of trophic strategies among species (resource partitioning). In the study area, Deudero et al. (2004) also highlighted the usefulness of isotopic analyses to elucidate resource partitioning and differences in feeding niches in closely-related littoral fish. On the other hand, although diet overlap was found among coexisting species, their optimal depths might change, as already reported in our study area (Ordines et al., 2011; Ramírez-Amaro et al., 2015), which would reduce the competition for food.

This Thesis revealed ontogenetic shifts in diet at specific size thresholds, by means of SIA or SCA, for several species: the sharks *G. melastomus* and *E. spinax*, the ray *Raja clavata*, the squids *L. vulgaris*, *L. forbesii* and *Todarodes sagittatus* and the octopus *Bathypolypus sponsalis* (Chapter 5 and 7). In general, small individuals of these species eat smaller prey (e.g. euphausiids, amphipods) with low δ^{15}N values. By contrast, large individuals exploit a wider prey spectrum (e.g. fishes, euphausiids, cephalopods) with enriched δ^{15}N values, indicating that they feed at multiple and higher trophic levels (ontogenetic omnivory; Polis and Strong, 1996).

Apart from size-related shifts in diet, our results also showed ontogenetic shifts in feeding strategy or foraging habitat for several cephalopod and fish species. The shark *S. canicula,* for instance, showed depth-related shifts in diet since it preyed mainly on decapod crustaceans, polychaetes and teleosts over the shelf but switched to an euphausiids-based diet in deeper waters. Changes in the trophic guild may also depend on spatio-temporal changes in the ecological interactions between species (Carrassón and Cartes, 2002) or prey availability (Cartes, 1998). Accordingly, the extensive consumption of euphausiids by *S. canicula*, *G. melastomus* and *L. forbesii* caught between the shelf-break and the upper slope during spring-summer probably coincides with the high abundance of *Meganyctiphanes norvegica* on those grounds (Cartes et al., 2009; Fanelli and Cartes, 2008). Ontogenetic shifts in diet, as those found in most of our study species, can play an important role in determining growth and survival of individuals, species interactions, and community structure (Werner and Gilliam, 1984). Indeed, small and large-sized individuals of most of our study species

occupied different trophic groups (Chapters 3, 6 and 7). In spite of such a generalization of ontogenetic shifts in diet, few studies on trophic web networks take them into account, including those carried out in the Mediterranean (Banaru et al., 2013; Coll et al., 2006).

In summary, this Thesis provided evidence of the importance of the pelagic pathway for the megafaunal demersal communities from the Balearic Sea. Comparisons of two sub-basins with contrasting oceanographic conditions, however, revealed spatial and bathymetric differences in the food webs structure and dynamics and the corresponding benthopelagic coupling. This trophic coupling between the pelagic and hyperbenthic domains was tighter on the sub-basin showing a comparatively higher primary production. In both sub-basins, the benthopelagic coupling decreased with increasing depth. This Thesis also revealed the key role played by mesopelagic organisms as food resources for the megafaunal communities of the continental slope, especially for those living under oligotrophic conditions. The study also characterized the complexity of the trophic links of cephalopod and elasmobranch species in the study area, demonstrated by resource partitioning and changes in diet related to individual size, season and depth. Squid and shark species identified as benthopelagic feeders, play a key role in the transport of energy from midwater regions to the benthos of the continental slope. Altogether, the results of this Thesis revealed that cephalopods played a key role for the benthopelagic coupling, whereas demersal elasmobranchs contributed primarily to a one-way flux accumulating energy resources into deep-sea ecosystems. These results give us a better understanding of the functioning of the trophic networks of the Balearic Islands and will be useful for future studies on ecosystem modeling and to forecast natural and human-induced impacts on Mediterranean marine ecosystems.

Chapter 9: Conclusions

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1. Marine food webs from the shelf-break and continental slope from the Balearic Islands spanned up to four trophic levels.

2. Significant linear relationships between δ^{13}C and δ^{15}N, together with the narrower range of the former compared to the latter, confirm that plankton, and in turn marine snow, constitutes the main source of organic matter for the slope hyperbenthic communities.

3. The wide range of species isotopic values suggests a high diversity of trophic strategies (food partitioning) related with the oligotrophic conditions in waters around the Balearic Islands.

4. Small δ^{13}C differences between the pelagic and the hyperbenthic compartments indicate a close benthopelagic coupling on shelf-break and slope food webs.

5. Benthopelagic coupling varied with depth since it was higher on the shelf-break than on the slope.

6. Benthopelagic coupling was lower at the deepest strata of the Algerian sub-basin compared to the Balearic sub-basin, indicating that deep-sea hyperbenthic species relied on a more reworked and remineralised material of planktonic origin in the former.

7. The δ^{13}C and δ^{15}N values of zooplankton did not vary at the spatial scales sampled in this study, but displayed high ^{13}C-seasonality likely reflecting the shift in phytoplankton species composition.

8. The higher δ^{15}N values of larger sized zooplankton organisms indicated size-related consumption patterns of marine plankton food webs.

9. The trophodynamics of the mesopelagic fishes from the Balearic Sea revealed a close seasonal coupling with planktonic production (δ^{13}C) and minimal spatio-temporal variations of trophic interactions (δ^{15}N).

10. The mesopelagic fish community from the Balearic Islands is constituted mainly by tertiary consumers (Trophic level=3-4), preying on a mixed diet of mesozooplankton, euphausiids and, to a lesser extent, particulate organic matter and other mesopelagic fish.

11. Non-surface-migratory species of the mesopelagic community (e.g. *Cyclotone braueri*, *Argyropelecus hemigymnus*) had lower δ^{15}N values than migrant and more energetically demanding species (e.g. *Lobianchia dofleini*, *Hygophum hygomii*).

12. Despite similar migratory behaviour and overlapping vertical distribution, niche segregation was observed both at interspecific (*Hygophum* and *Lampanyctus*) and intraspecific (*Ceratoscopelus maderensis* and *Notoscopelus elongatus*) levels.

13. Stable isotope analysis was revealed as a good tracker of ontogenetic shifts in diet related to habitat use. Small-sized individuals of *Lampanyctus crocodilus* performing diel migrations, for instance, had lower $\delta^{13}C$ and $\delta^{15}N$ values and higher lipid content than large-sized, non-migrant individuals living close to the bottom.

14. Batoid species from the Balearic Islands, except *Myliobatis aquila*, preyed mainly on natantian crustaceans and teleosts.

15. Demersal shark species from the Balearic Islands based their diets on pelagic prey (decapod crustaceans, fish, euphausiids and cephalopods).

16. Demersal ray species from the Balearic Islands preyed on benthopelagic decapod crustaceans, mysiids and euphausiids.

17. Size was revealed as an important driver of the feeding ecology of elasmobranchs, since both sharks and batoids of most abundant species showed significant ontogenetic shifts in diet.

18. By contrast, differences in diet due to bathymetric changes in elasmobranchs seem to be species specific, as comparisons of *Scyliorhinus canicula* and *Raja clavata* individuals inhabiting the shelf and slope only revealed significant differences in the former.

19. When considering broad taxonomical prey groups, most coexisting elasmobranchs showed significant diet overlaps which might lead to prey competition. However, these results should be treated with caution since they might change with analyses at prey species level.

20. Both stomach and isotope analyses of deep-sea elasmobranch species revealed resource partitioning. Large-sized individuals had wider niche breadths and more enriched $\delta^{15}N$ prey, indicating that they prey on multiple and higher trophic levels than their small-sized counterparts.

21. Although both *Loligo* squid species had a mixed diet including pelagic, benthopelagic and benthic organisms with a clear preference for fish, their prey species composition revealed the bathymetric segregation of both species in the Balearic Islands.

22. Both squid species showed changes in diet related to size and season, but not to sex.

23. *Loligo* squid species showed ontogenetic shifts in diet but, in spite of scarce morphological differences between them, this shift occurred at smaller individual size in *L. forbesii* than in *L. vulgaris* (140 *vs* 210 mm ML).

24. The diet of small-sized individuals of *L. vulgaris* was more dependent on small bottom-living organisms (e.g. mysids) than large individuals that mainly preyed on benthopelagic fish (e.g. sparids).

25. During the reproduction period, the squid *L. vulgaris* prioritized benthic prey (polychaetes and gobiids) over nektonic prey (sparids and cephalopods). Increases in the consumption of the highly nutritive polychaetes might help improving the squid individual body condition during that period.

26. The squid *L. forbesii* showed size-related differences in diet during the reproductive season, with mesopelagic fish and euphausiids being only present in the diet of large individuals. The bathymetric distribution of these preys would indicate movements of large *L. forbesii* to deeper waters for feeding.

27. Isotopic analyses of deep-sea cephalopods and elasmobranchs revealed a clear divergent $\delta^{13}C$ range values indicating food partitioning: Hyperbenthic species (e.g. octopuses, rays, sharks) displayed higher $\delta^{13}C$ values compared to those having pelagic habits (e.g. squids, cuttlefishes).

28. Deep-sea cephalopods and elasmobranchs displayed a high variety of trophic strategies that can be aggregated into three broad groups: benthic, benthopelagic and pelagic feeders. The benthic and pelagic strategies were constituted exclusively by cephalopods, whereas all elasmobranchs, together with some cephalopod species, were grouped within the benthopelagic feeding strategy.

29. Elasmobranchs and squids identified as benthopelagic feeders showed isotopic segregation, indicating that they avoid resource competition by exploiting different mesopelagic prey.

30. Octopus and squid species living on the slope, such as *Bathypolypus sponsalis* and *Todarodes sagittatus,* showed ontogenetic shifts in diet. The former would forage more actively (e.g. burrowing) as they grow, whereas the latter showed a shift in its foraging behaviour from a pelagic to a benthopelagic strategy as they grow.

31. Deep-sea squids and sharks preyed on fast-moving and/or higher energetic prey (i.e. cephalopods, mesopelagic fish), whereas rays and octopuses fed mainly on slow-moving and/or lower energetic prey (i.e. benthic crustaceans).

32. Squids, cuttlefishes and sharks identified as benthopelagic feeders would play an important role by transferring energy/carbon and nutrients from the nycthemeral migrating community to the demersal community.

References

References

Abelló, P., Carbonell, A., Torres, P., 2002. Biogeography of epibenthic crustaceans on the shelf and upper slope off the Iberian Peninsula Mediterranean coasts: implications for the establishment of natural management areas. Sci. Mar. 66, 183–198.

Acosta, J., Canals, M., López-Martínez, J., Muñoz, A., Herranz, P., Urgeles, R., Palomo, C., Casamor, J.L., 2003. The Balearic Promontory geomorphology (western Mediterranean): Morphostructure and active processes. Geomorphology 49, 177–204.

Adloff, F., Somot, S., Sevault, F., Jordà, G., Aznar, R., Déqué, M., Herrmann, M., Marcos, M., Dubois, C., Padorno, E., Alvarez-Fanjul, E., Gomis, D., 2015. Mediterranean Sea response to climate change in an ensemble of twenty first century scenarios. Clim. Dyn. 45, 2775–2802.

Albo-Puigserver, M., Navarro, J., Coll, M., Sáez, R., Cardona, L., Aguzzi, J., 2015. Feeding behaviour and trophic position of three sympatric demersal chondrichthyes in the NW Mediterranean. Environ. Biol. Fishes 524, 255–268.

Amores, A., Monserrat, S., 2014. Hydrodynamic comparison between the north and south of Mallorca Island. J. Mar. Syst. 138, 40–50.

Andersen, V., Sardou, J., 1992. The diel migrations and vertical distributions of Zooplankton and Micronekton in the Northwestern Mediterranean-Sea 1. Euphausiids, Mysids, Decapods and Fishes. J. Plankton Res. 14, 1129–1154.

Andersen, V., Sardou, J., Nival, P., 1992. The Diel Migrations and Vertical Distributions of Zooplankton and Micronekton in the Northwestern Mediterranean Sea 2. Siphonophores, Hydromedusae and Pyrosomids. J. Plankton Res. 14, 1155–1169.

Anderson M.J., Gorley R.N., Clarke K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth.

André, J., Haddon, M., Pecl, G.T., 2010. Modelling climate-change induced nonlinear thresholds in cephalopod population dynamics. Glob. Change Biol. 16, 2866–2875.

Angel, M.V., Boxshall, G.A., 1990. Life in the Benthic Boundary-Layer - Connections to the Mid-Water and Sea-Floor. Philos. T. Roy. Soc. A. 331, 15–28.

Azouz, J., Capapé, C., 1971. Les relations alimentaires entre les Sélaciens et le zoobenthos des côtes nord de la Tunisie. Bull. Inst. Natl. Sci. Tech. Oceanogr. Pêche Salammbo 2, 121–130.

Badcock, J., Merrett, N.R., 1976. Midwater fishes in the eastern North Atlantic–I. Vertical distribution and associated biology in 30°N, 23°W, with developmental notes on certain myctophids. Prog. Oceanogr. 7, 3–58.

Bailey, T.G., Robison, B.H., 1986. Food availability as a selective factor on the chemical compositions of midwater fishes in the eastern North Pacific. Mar. Biol. 91, 131–141.

Baillie, J., Hilton-Taylor, C., Stuart, S.N., 2004. 2004 IUCN red list of threatened species: a global species assessment, Earth.

Ballesteros, E., 1994. The Deep Water *Peyssonnelia* Beds from the Balearic Islands (Western Mediterranean). Mar. Ecol. 15, 233–253.

Ballesteros, E., 1992. Els fons rocosos profunds amb *Osmundaria volubilis* (Linné) R. E. Norris a les Balears. Bolleti la Soc. d'Historia Nat. les Balear.

Baker, R., Buckland, A., Sheaves, M., 2013. Fish gut content analysis: robust measures of diet composition. Fish. Fisher. 15 (1), 170–177.

Bănaru, D., Mellon-Duval, C., Roos, D., Bigot, J.L., Souplet, A., Jadaud, A., Beaubrun, P., Fromentin, J.M., 2013. Trophic structure in the Gulf of Lions marine ecosystem (north-western Mediterranean Sea) and fishing impacts. J. Mar. Syst. 111–112, 45–68

Barría, C., Coll, M., Navarro, J., 2015. Unravelling the ecological role and trophic relationships of uncommon and threatened elasmobranchs in the western Mediterranean Sea. Mar. Ecol. Prog. Ser. 539, 225–240.

Battaglia, P., Andaloro, F., Consoli, P., Esposito, V., Malara, D., Musolino, S., Pedà, C., Romeo, T., 2013. Feeding habits of the Atlantic bluefin tuna, *Thunnus thynnus* (L. 1758), in the central Mediterranean Sea (Strait of Messina). Helgol. Mar. Res. 67, 97–107.

Baustian M.M., Hansen G.J.A., de Kluijver A., Robinson, K., Henry, E. N., Knoll, L. B., Carey, C. C. 2014. Linking the bottom to the top in aquatic ecosystems: mechanisms and stressors of benthic-pelagic coupling. In: Eco-DAS X Symposium Proceedings (Ed Kemp PF), pp. 25–47. Association for the Sciences of Limnology and Oceanography.

Bazairi, H., Ben Haj, S., Boero, F., Cebrian, D., De Juan, S., Limam, A., Lleonart, J., Torchia, G., Rais, C., 2010. The Mediterranean Sea Biodiversity: state of the ecosystems, pressures, impacts and future priorities. Ed. RAC/SPA, Tunis.

Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A., Macleod, H., 2004. Determining trophic niche width: a novel approach using stable isotope analysis. J. Anim. Ecol. 73 (5), 1007–1012.

Bello, G., 2008. Cephalopoda. Biol. Mar. Mediterr. 15, 318–322.

Ben Rais Lasram, F., Guilhaumon, F., Albouy, C., Somot, S., Thuiller, W., Mouillot, D., 2010. The Mediterranean Sea as a "cul-de-sac" for endemic fishes facing climate change. Glob. Chang. Biol. 16, 3233–3245.

Bergman, E., Greenberg, L.A., 1994. Competition between a planktivore, a benthivore, and a species with ontogenetic diet shifts. Ecol. 75, 1233–1245.

Bergmann, M., Dannheim, J., Bauerfeind, E., Klages, M., 2009. Trophic relationships along a bathymetric gradient at the deep-sea observatory HAUSGARTEN. Deep-sea Res. I 56, 408–424.

Bernal, A., Olivar, M.P, Fernandez de Puelles, M.L., 2013. Feeding patterns of *Lampanyctus pusillus* (Pisces: Myctophidae) throughout its ontogenetic development. Mar. Biol. 160, 81–95.

Bertrand, J.A., 2002. Mediterranean marine demersal resources: The Medits international trawl survey (1994-1999) - Foreword. Sci. Mar. 66, 5–7.

Bethoux, J.P., Gentili, B., 1999. Functioning of the Mediterranean sea: Past and present changes related to freshwater input and climate changes. J. Mar. Syst. 20, 33–47.

Beyer, F., 1992. *Meganyctiphanes norvegica* (M-Sars) (Euphausiacea): a voracious predator on *Calanus*, other copepods, and Ctenophores. In: Oslofjorden, Southern Norway. Sarsia 77, 189–206.

Bianchi, N., Morri, C., 2000. Marine biodiversity of the Mediterranean Sea: situation, problems and prospects for future research. Mar. Pollut. Bull. 40, 367–376.

Bicknell, A.W.J., Campbell, M., Knight, M.E., Bilton, D.T., Newton, J., Votier, S.C., 2011. Effects of formalin preservation on stable carbon and nitrogen isotope signatures in calanoid copepods: implications for the use of Continuous Plankton Recorder Survey samples in stable isotope analyses. Rapid Commun. Mass Spectrom. 25, 1794–1800.

Bizzarro, J.J., Robinson, H.J., Rinewalt, C.S., Ebert, D.A., 2007. Comparative feeding ecology of four sympatric skate species off central California, USA. Environ. Biol. Fish. 80, 197–220.

Blaber, S.J.M., Bulman, C.M., 1987. Diets of fishes of the upper continental slope of eastern Tasmania: content, calorific values, dietary overlap and trophic relationships. Mar. Biol. 95, 345–356.

Blanco, C., Raduán, M.Á., Raga, J.A., 2006. Diet of Risso' s dolphin (*Grampus griseus*) in the western Mediterranean Sea. Sci. Mar. 70, 407–411.

Blanco, C., Salomon, O., Raga, J.A., 2001. Diet of the bottlenose dolphin (*Tursiops truncatus*) in the western Mediterranean Sea. J. Mar. Biol. Assoc. U.K. 81, 1053–1058.

Bode, A., Alvarez-Ossorio, M., Cunha, M., Garrido, S., Peleteiro, J., Porteiro, C., Valdés, L., Varela, M., 2007. Stable nitrogen isotope studies of the pelagic food web on the Atlantic shelf of the Iberian Peninsula. Prog. Oceanogr.74, 115–131.

Bode, A., Carrera, P., Lens, S., 2003. The pelagic foodweb in the upwelling ecosystem of Galicia (NW Spain) during spring: natural abundance of stable carbon and nitrogen isotopes. ICES J. Mar. Sci. 60, 11–22.

Boero, F., Belmonte, G., Fanelli, G., Piraino, S., Rubino, F., 1996. The continuity of living matter and the discontinuities of its constituents: Do plankton and benthos really exist? Trends Ecol. Evol. 11, 177–180.

Boldrin, A., Miserocchi, S., Rabitti, S., Turchetto, M.M., Balboni, V., Socal, G., 2002. Particulate matter in the southern Adriatic and Ionian Sea: Characterisation and downward fluxes. J. Mar. Syst. 33-34, 389–410.

Bosc, E., 2004. Seasonal and interannual variability in algal biomass and primary production in the Mediterranean Sea, as derived from 4 years of SeaWiFS observations. Global Biogeochem. Cycles 18, 1–17.

Bosley, K.L., Wainright, S.C., 1999. Effects of preservatives and acidification on the stable isotope ratios ($N^{15}: N^{14}, C^{13}: C^{12}$) of two species of marine animals. Can. J. Fish. Aquat. Sci. 56, 2181–2185.

Boudouresque, C.F., Bernard, G., Pergent, G., Shili, A., Verlaque, M., 2009. Regression of Mediterranean Seagrasses caused by natural processes and anthropogenic disturbances and stress: a critical review. Bot. Mar. 52, 395–418.

Box, A., Deudero, S., Sureda, A., Blanco, A., Alòs, J., Terrados, J., Grau, A.M., Riera, F., 2009. Diet and physiological responses of *Spondyliosoma cantharus* (Linnaeus, 1758) to the Caulerpa racemosa var. cylindracea invasion. J. Exp. Mar. Bio. Ecol. 380, 11–19.

Boyle, P.R., Pierce, G.J., 1994. Fishery biology of northeast Atlantic squid: an overview. Fish. Res. 21, 1–15.

Boyle, P., Rodhouse, P., (Eds.) 2005. Cephalopods as predators, in Cephalopods: Ecology and Fisheries Blackwell Publishing Ltd, Oxford, UK.

Bulman, C.M., He, X., Koslow, J.A., 2002. Trophic ecology of the mid-slope demersal fish community off southern Tasmania, Australia. Mar. Freshwater Res. 53, 59–72.

Bunn, S.E., Barton, D.R., Hynes, H.B.N., Power, G., Pope, M.A., 1989. Stable isotope analysis of carbon flow in a tundra river system. Can. J. Fish. Aquat. Sci. 46, 1769–1775.

Bunn, S.E., Loneragan, N.R., Kempster, M.A., 1995. Effects of acid washing on stable isotope ratios of C and N in penaeid shrimp and seagrass: Implications for food-web studies using multiple stable isotopes. Limnol. Oceanogr. 40, 622–625.

Cabanellas-Reboredo, M., Alós, J., March, D., Palmer, M., Jordà, G., Palmer, M., 2014b. Where and when will they go fishing? Understanding fishing site and time choice in a recreational squid fishery. ICES J. Mar. Sci. 71, 1760–1773.

Cabanellas-Reboredo, M., Alós, J., Palmer, M., March, D., O'Dor, R., 2012. Movement patterns of the European squid *Loligo vulgaris* during the inshore spawning season. Mar. Ecol. Prog. Ser. 466, 133–144.

Cabanellas-Reboredo, M., Calvo-Manazza, M., Palmer, M., Hernández-Urcera, J., Garci, M.E., González, F., Guerra, A., Morales-Nin, B., 2014a. Using artificial devices for identifying spawning preferences of the European squid: Usefulness and limitations. Fish. Res. 157, 70–77.

Cabanellas-Reboredo, M., Deudero, S., Blanco, A., 2009. Stable-isotope signatures (δ^{13}C and δ^{15}N) of different tissues of *Pinna nobilis* Linnaeus, 1758 (Bivalvia): isotopic variations among tissues and between seasons. J. Moll. Stud., eyp021.

Caddy, J.F., Rodhouse, P.G., 1998. Cephalopod and groundfish landings: Evidence for ecological change in global fisheries? Rev. Fish Biol. Fish. 8, 431–444.

Capapé, C., 1976. Etude du régime alimentaire de l'aigle de mer, *Myliobatis aquila* (L. 1758) des côtes tunisiennes. J. Cons. Int. Explor. Mer. 37, 29–35.

Capapé, C., Quignard, J.P., 1974. Dimorphisme sexuel et observations biologiques sur *Myliobatis aquila* (L. 1758). Contribution à l'étude systématique du genre *Myliobatis*, Cuvier, 1817. Ann. Mus. Civ. St. Nat. Genova 80, 1–27.

Carafa, R., Dueri, S., Zaldívar, J. M., 2007. Linking terrestrial and aquatic ecosystems: Complexity, persistence and biodiversity in European food webs EUR 22914 EN. Joint Research Center.

Cardona, L., de Quevedo, I.A., Borrell, A., Aguilar, A., 2012. Massive consumption of gelatinous plankton by Mediterranean apex predators. PloS One 7(3), e31329.

Cardona, L., Revelles, M., Sales, M., Aguilar, A., Borrell, A., 2007. Meadows of the seagrass *Posidonia oceanica* are a significant source of organic matter for adjoining ecosystems. Mar. Ecol. Prog. Ser. 335, 123–131.

Carlson, J.K., Goldman, K.J., Lowe, C.G., 2004. Metabolism, energetic demand, and endothermy. In: Carrier J.C., Musick J.A., Heithaus, M.R. (eds.), Biology of Sharks and Their Relatives. CRC Press, Boca Raton, pp. 203–224.

Carrassón, M., Cartes, J.E., 2002. Trophic relationships in a Mediterranean deep-sea fish community: Partition of food resources, dietary overlap and connections within the benthic boundary layer. Mar. Ecol. Prog. Ser. 241, 41–55.

Carrassón, M., Stefanescu, C., Cartes, J.E., 1992. Diets and Bathymetric Distributions of 2 Bathyal Sharks of the Catalan Deep-Sea (Western Mediterranean). Mar. Ecol. Prog. Ser. 82, 21–30.

Cartes, J.E., 1998. Dynamics of the bathyal Benthic Boundary Layer in the northwestern Mediterranean: Depth and temporal variations in macrofaunal-megafaunal communities and their possible connections within deep-sea trophic webs. Prog. Oceanogr. 41, 111–139.

Cartes, J.E., 1998. Feeding strategies and partition of food resources in deep-water decapod crustaceans (400-2300 m). J. Mar. Biol. Ass. U.K. 78, 509–524.

Cartes, J.E., Abelló, P., Lloris, D., Carbonell, A., 2002. Feeding guilds of western Mediterranean demersal fish and crustaceans : an analysis based on a spring survey *. Sci. Mar. 66, 209–220.

Cartes, J.E., Carrassón, M., 2004. Influence of trophic variables on the depth-range

distributions and zonation rates of deep-sea megafauna: The case of the Western Mediterranean assemblages. Deep. Res. Part I Oceanogr. Res. Pap. 51, 263–279.

Cartes, J.E., Fanelli, E., Papiol, V., Maynou, F., 2010. Trophic relationships at intrannual spatial and temporal scales of macro and megafauna around a submarine canyon off the Catalonian coast (western Mediterranean). J. Sea Res. 63, 180–190.

Cartes, J.E., Hidalgo, M., Papiol, V., Massutí, E., Moranta, J., 2009. Changes in the diet and feeding of the hake *Merluccius merluccius* at the shelf-break of the Balearic Islands: Influence of the mesopelagic-boundary community. Deep. Res. Part I Oceanogr. Res. Pap. 56, 344–365.

Cartes, J.E., Madurell, T., Fanelli, E., López-Jurado, J.L., 2008a. Dynamics of suprabenthos-zooplankton communities around the Balearic Islands (western Mediterranean): Influence of environmental variables and effects on the biological cycle of *Aristeus antennatus*. J. Mar. Syst. 71, 316–335.

Cartes, J.E., Mamouridis, V., Fanelli, E., 2011. Deep-sea suprabenthos assemblages (Crustacea) off the Balearic Islands (western Mediterranean): Mesoscale variability in diversity and production. J. Sea Res. 65, 340–354.

Cartes, J.E., Maynou, F., 1998. Food consumption by bathyal decapod crustacean assemblages in the Western Mediterranean: Predatory impact of megafauna and the food consumption-food supply balance in a deep-water food web. Mar. Ecol. Prog. Ser. 171, 233–246.

Cartes, J.E., Maynou, F., Morales-Nin, B., Massutí, E., Moranta, J., Massuti, E., Moranta, J., 2001. Trophic structure of a bathial benthopelagic boundary layer community south of the Balearic Islands (southwestern Mediterranean). Mar. Ecol. Prog. Ser. 215, 23–35.

Cartes, J.E., Papiol, V., Guijarro, B., 2008b. The feeding and diet of the deep-sea shrimp *Aristeus antennatus* off the Balearic Islands (Western Mediterranean): Influence of environmental factors and relationship with the biological cycle. Prog. Oceanogr. 79, 37–54.

Cartes, J.E., Sarda, F., 1992. Abundance and diversity of decapod crustaceans in the deep-Catalan Sea (Western Mediterranean). J. Nat. Hist. 26, 1305–1323.

Cartes, J.E., Sorbe, J.C., Sardà, F., 1994. Spatial distribution of deep-sea decapods and euphausiids near the bottom in the northwestern Mediterranean. J. Exp. Mar. Biol. Ecol. 179, 131–144.

Castro, B.G., Guerra, Á., 1990. The diet of *Sepia officinalis* (Linnaeus, 1758) and *Sepia elegans* (D'Orbigny, 1835) (Cephalopoda, Sepioidea) from the Ria de Vigo (NW Spain). Sci. Mar. 54, 375–388.

Caut, S., Angulo, E., Courchamp, F., 2009. Variation in discrimination factors (Δ^{15}N and Δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. J. Appl. Ecol. 46, 443–453.

Caut, S., Angulo, E., Courchamp, F., Figuerola, J., 2010. Trophic experiments to estimate isotope discrimination factors. J. Appl. Ecol. 47, 948–954.

Champalbert, G.A., Kouame, B., Pagano, M., Marchal, E., 2008. Feeding behavior of adult *Vinciguerria nimbaria* (Phosichthyidae), in the tropical Atlantic (0º-4º N, 15º W). Mar. Biol. 156, 79–95.

Cherel, Y., Ducatez, S., Fontaine, C., Richard, P., Guinet, C., 2008. Stable isotopes reveal the trophic position and mesopelagic fish diet of female southern elephant seals breeding on the Kerguelen Islands. Mar. Ecol. Prog. Ser. 370, 239–247.

Cherel, Y., Duhamel, G., 2003. Diet of the squid *Moroteuthis ingens* (Teuthoidea : Onychoteuthidae) in the upper slope waters of the Kerguelen Islands Mar. Ecol. Prog. Ser. 250, 197–203.

Cherel, Y., Fontaine, C., Richard, P., Labat, J.P., 2010. Isotopic niches and trophic levels of myctophid fishes and their predators in the Southern Ocean. Limnol. Oceanogr. 55, 324–332.

Cherel, Y., Hobson, K., 2005. Stable isotopes, beaks and predators: a new tool to study the trophic ecology of cephalopods, including giant and colossal squids. Proc. Biol. Sci. 272, 1601–7.

Cherel, Y., Hobson, K.A., 2007. Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. Mar. Ecol. Prog. Ser. 329, 281–287.

Cherel, Y., Kernaléguen, L., Richard, P., Guinet, C., 2009. Whisker isotopic signature depicts migration patterns and multi-year intra-and inter-individual foraging strategies in fur seals. Biol. Lett. 5, 830–832.

Cherel, Y., Ridoux, V., Spitz, J., Richard, P., 2009. Stable isotopes document the trophic structure of a deep-sea cephalopod assemblage including giant octopod and giant squid. Biol. Lett. 5, 364–367.

Childress, J.J., Nygaard, M.H., 1973. Chemical composition of midwater fishes as a function of depth of occurrence off southern California. Deep-Sea Res. 20, 1093–1109.

Childress, J.J., Price, M.H., Favuzzi, J., Cowles, D., 1990. Chemical composition of midwater fishes as a function of depth of occurrence off the Hawaiian Islands: food availability as a selective factor? Mar. Biol. 105, 235–246.

Childress, J.J., Thuesen, E. V., 1995. Chapter 9 metabolic potentials of deep-sea fishes: A comparative approach. Biochem. Mol. Biol. Fishes 5, 175–196.

Chouvelon, T., Spitz, J., Caurant, F., Mendez-Fernandez, P., Chappuis, A., Laugier, F., Le Goff, E., Bustamante, P., 2012. Revisiting the use of δ^{15}N in meso-scale studies of marine food webs by considering spatio-temporal variations in stable isotopic signatures. The case of an open ecosystem: the Bay of Biscay. Prog. Oceanogr. 101, 95–102.

Chouvelon, T., Spitz, J., Cherel, Y., Caurant, F., Sirmel, R., Mèndez-Fernandez, P., Bustamante, P., 2011. Inter-specific and and ontogenic differences in δ^{13}C and δ^{15}N values and Hg and Cd concentrations of cephalopods. Mar. Ecol. Prog. Ser. 433, 107–120.

Choy, C.A., Davison, P.C., Drazen, J.C., Flynn, A., Gier, E.J., Hoffman, J.C., McClain-Counts, J.P., Miller, T.W., Popp, B.N., Ross, S.W., Sutton, T.T., 2012. Global trophic position comparison of two dominant mesopelagic fish families (Myctophidae, Stomiidae) using amino acid nitrogen isotopic analyses. PloS One 7, e50133.

Christensen, N.L., Bartuska, A.M., Brown, J.H., Carpenter, S., D'Antonio, C., Francis, R., Franklin, J.F., MacMahon, J.A., Noss, R.F., Parsons, D.J., Peterson, C.H., Turner, M.G., Woodmansee, R.G., 1996. The report of the ecological society of america committee on the scientific basis for ecosystem management. Ecol. Appl. 6, 665–691.

Christensen, V., Pauly, D., 1992. ECOPATH II - a software for balancing steady-state ecosystem models and calculating network characteristics. Ecol. Modell. 61, 169–185.

Christensen, V., Walters, C.J., 2004. Ecopath with Ecosim: Methods, capabilities and limitations. Ecol. Modell.

Churchill, D.A., Heithaus, M.R., Vaudo, J.J., Grubbs, R.D., Gastrich, K., Castro, J.I., 2015. Trophic interactions of common elasmobranchs in deep-sea communities of the Gulf

of Mexico revealed through stable isotope and stomach content analysis. Deep. Res. Part II Top. Stud. Oceanogr. 115, 92–102.

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Aus. J. Ecol. 18, 117-143.

Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.

Clarke, K.R., Warwick, R.M., 2001. Change in marine communities: an approach to statistical analysis and interpretation, 2nd edn. PRIMER-E, Plymouth.

Clarke, M.R., Kristensen, T.K., 1980. Cephalopod beaks from the stomachs of two northern bottlenosed whales (*Hyperoodon ampullatus*). J. Mar. Biol. Assoc. U.K. 60, 151–156.

Coelho, M., Domingues, P., Balguerias, E., Fernandez, M., Andrade, J.P., 1997. A comparative study of the diet of *Loligo vulgaris* (Lamarck, 1799) (Mollusca: Cephalopoda) from the south coast of Portugal and the Saharan Bank (Central-East Atlantic). Fish. Res. 29, 245–255.

Coll, M., Navarro, J., Olson, R.J., Christensen, V., 2013. Assessing the Trophic position and ecological role of squids in marine ecosystems by means of food-web models. Deep. Res. Part II Top. Stud. Oceanogr. 95, 21–36.

Coll, M., Palomera, I., Tudela, S., Dowd, M., 2008. Food-web dynamics in the South Catalan Sea ecosystem (NW Mediterranean) for 1978-2003. Ecol. Model. 217, 95–116.

Coll, M., Palomera, I., Tudela, S., Sardà, F., 2006. Trophic flows, ecosystem structure and fishing impacts in the South Catalan Sea, Northwestern Mediterranean. J. Mar. Syst. 59, 63–96.

Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Lasram, F.B.R., Aguzzi, J., Ballesteros, E., Bianchi, and others, 2010. The biodiversity of the Mediterranean Sea: Estimates, patterns, and threats. PLoS One 5 (8), e11842.

Collins, M.A., De Grave, S., Lordan, C., Burnell, G.M., Rodhouse, P.G., 1994. Diet of the squid *Loligo forbesi* Steenstrup (Cephalopoda: Loliginidae) in Irish waters. ICES J. Mar. Sci. 51, 337–344.

Collins, M.A., Rodhouse, P.G.K., 2006. Southern Ocean Cephalopods. Adv. Mar. Biol. 50, 191–265.

Colloca, F., Cardinale, M., Maynou, F., Giannoulaki, M., Scarcella, G., Jenko, K., Bellido, J.M., Fiorentino, F., 2013. Rebuilding Mediterranean fisheries: A new paradigm for ecological sustainability. Fish Fish. 14, 89–109.

Collwell, R.K., Futuyma, D.J., 1971. On the measurement of niche breadth and overlap. Ecology 52, 567–576.

Connan, M., Cherel, Y., Mayzaud, P., 2007. Lipids from stomach oil of procellariiform seabirds document the importance of myctophid fish in the Southern Ocean. Limnol. Oceanogr. 52, 2445–2455.

Cortes, E., 1997. A critical review of methods of studying fish feeding based on analysis of stomach contents: Application to elasmobranch fishes. Can. J. Fish. Aquat. Sci. 54, 726–738.

Cortes, E., 1999. Standardized diet compositions and trophic levels of sharks. ICES J. Mar. Sci. 56, 707–717.

Costalago, D., Navarro, J., Alvarez-Calleja, I., Palomera, I., 2012. Ontogenetic and seasonal changes in the feeding habits and trophic levels of two small pelagic fish species. Mar. Ecol-Prog. Ser. 460, 169–181.

Croxall, J.P., Reid, K., Prince, P.A., 1999. Diet, provisioning and productivity responses of marine predators to differences in availability of Antarctic krill. Mar. Ecol. Prog. Ser. 177, 115–131.

Danovaro, R., Dinet, A., Duineveld, G., Tselepides, A., 1999. Benthic response to particulate fluxes in different trophic environments: A comparison between the Gulf of Lions-Catalan Sea (western-Mediterranean) and the Cretan Sea (eastern-Mediterranean), in: Progress in Oceanography. pp. 287–312.

Darnaude, A.M., Salen-Picard, C., Polunin, N.V.C., Harmelin-Vivien, M.L., 2004. Trophodynamic linkage between river runoff and coastal fishery elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). Oecologia 138 (3), 325–32

Davenport, S., Bax, N. J., 2002. A trophic study of a marine ecosystem off southern Australia using stable isotopes of carbon and nitrogen. Can. J. Fish. Aquat. Sci. 59, 514–530.

Deming, J.W., Tabor, P.S., Colwell, R.R., 1981. Barophilic Growth of Bacteria from Intestinal Tracts of Deep-sea Invertebrates. Microb. Ecol. 7, 85–94.

Deniro, M.J., Epstein, S., 1978. Influence of diet on the contribution of carbon isotopes in animals. Geochim. Cosmochim. Ac. 42 (5), 495–506.

Deniro, M.J., Epstein, S., 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Ac. 45 (3), 341–351.

Deudero, S., Pinnegar, J.K., Polunin, N.V.C., Morey, G., Morales-Nin, B., 2004. Spatial variation and ontogenic shifts in the isotopic composition of Mediterranean litoral fishes. Mar. Biol. 145 (5), 971–981.

Di Santo, V., Kenaley, C.P., 2016. Skating by: low energetic costs of swimming in a batoid fish. J. Exp. Biol. 219, 1804–1807.

D'Onghia, G., Matarrese, A., Tursi, A., Sion, L., 1995. Observations on the depth distribution pattern of the small-spotted catshark in the North Aegean Sea. J. Fish Biol. 47, 421–426.

Doubleday, Z.A., Prowse, T.A.A., Arkhipkin, A., Pierce, G.J., Semmens, J., Steer, M., Leporati, S.C., Lourenço, S., Quetglas, A., Sauer, W., Gillanders, B.M., 2016. Global proliferation of cephalopods. Curr. Biol. 26, R406–R407.

Doucett, R.R., Booth, R.K., Power, G., McKinley, R.S., 1999. Effects of the spawning migration on the nutritional status of anadromous Atlantic salmon (*Salmo salar*): insights from stable isotope analysis. Can. J. Fish Aquat. Sci. 56, 2172–2180.

Dulvy, N.K., Allen, D.J., Ralph, G.M., Walls, R.H.L., 2016. The conservation status of Sharks, Rays and Chimaeras in the Mediterranean Sea [Brochure]. Málaga, Spain.

Dulvy, N.K., Fowler, S.L., Musick, J.A., Cavanagh, R.D., Kyne, M., Harrison, L.R., Carlson, J.K., Davidson, L.N.K., Sonja, V., 2014. Extinction risk and conservation of the world's sharks and rays. Elife 3, 1–35.

Dunton, K.H., Saupe, S.M., Golikov, A.N., Schell, D.M., Schonberg, S.V., 1989. Trophic Relationships and Isotopic Gradients Among Arctic and Subarctic Marine Fauna. Mar. Ecol. Prog. Ser. 56, 89–97.

Eardly, D.F., Carton, M.W., Gallagher, J.M., Patching, J.W., 2001. Bacterial abundance and activity in deep-sea sediments from the eastern North Atlantic. Prog. Oceanogr. 50, 245–259.

Ellis, J.R., Pawson, M.G., Shackley, S.E., 1996. The comparative feeding ecology of six species of shark and four species of ray (elasmobranchii) in the north-east Atlantic. J. Mar. Biol. Ass. U.K. 76, 89–106.

Emig, C.C., 1989. Distributional Patterns Along the Mediterranean Continental–Margin (Upper Bathyal) Using *Gryphus Vitreus* (Brachiopoda) Densities. Palaeogeogr. Palaeocl. 71, 253–256.

Espinoza, M., Munroe, S.E.M., Clarke, T.M., Fisk, A.T., Wehrtmann, I.S., 2015. Feeding ecology of common demersal elasmobranch species in the Pacific coast of Costa Rica inferred from stable isotope and stomach content analyses. J. Exp. Mar. Bio. Ecol. 470, 12–25.

Estrada, M., 1996. Primary production in the northwestern Mediterranean. Sci. Mar. 60, 55–64.

Estrada, M., 1985. Deep Phytoplankton and Chlorophyll Maxima in the Western Mediterranean, in: Moraitou-Apostolopoulou, M., Kiortsis, V. (Eds.), Mediterranean Marine Ecosystems. Springer US, Boston, MA, pp. 247–277.

Estrada, M., Varela, R.A., Salat, J., Cruzado, A., Arias, E., 1999. Spatio-temporal variability of the winter phytoplankton distribution across the Catalan and North Balearic fronts (NW Mediterranean). J. Plankton Res. 21, 1–20.

EUROMODEL Group, 1995. Progress from 1989 to 1992 in understanding the circulation of the Western Mediterranean. Oceanol. Acta 18 (2), 255–271.

Fanelli, E., Cartes, J.E., 2008. Spatio-temporal changes in gut contents and stable isotopes in two deep Mediterranean pandalids: influence on the reproductive cycle. Mar. Ecol. Prog. Ser. 355, 219–233.

Fanelli, E., Cartes, J.E., 2010. Temporal variations in the feeding habits and trophic levels of three deep-sea demersal fishes from the western Mediterranean Sea, based on stomach contents and stable isotope analyses. Mar. Ecol. Prog. Ser. 402, 213–232.

Fanelli, E., Cartes, J.E., Papiol, V., 2011a. Food web structure of deep-sea macrozooplankton and micronekton off the Catalan slope: Insight from stable isotopes. J. Mar. Syst. 87, 79–89.

Fanelli, E., Cartes, J.E., Papiol, V., 2012. Assemblage structure and trophic ecology of deep-sea demersal cephalopods in the Balearic basin (NW Mediterranean). Mar. Freshw. Res. 355, 219–233.

Fanelli, E., Cartes, J.E., Papiol, V., López-Pérez, C., 2013a. Environmental drivers of megafaunal assemblage composition and biomass distribution over mainland and insular slopes of the Balearic Basin (Western Mediterranean). Deep. Res. Part I Oceanogr. Res. Pap. 78, 79–94.

Fanelli, E., Cartes, J.E., Rumolo, P., Sprovieri, M., 2009. Food-web structure and trophodynamics of mesopelagic-suprabenthic bathyal macrofauna of the Algerian Basin based on stable isotopes of carbon and nitrogen. Deep. Res. Part I Oceanogr. Res. Pap. 56 (9), 1504–1520.

Fanelli, E., Papiol, V., Cartes, J., Rumolo, P., Brunet, C., Sprovieri, M., 2011b. Food web structure of the epibenthic and infaunal invertebrates on the Catalan slope (NW Mediterranean): Evidence from δ^{13}C and δ^{15}N analysis. Deep-Sea Res. I 58, 98–109.

Fanelli, E., Papiol, V., Cartes, J.E., Rumolo, P., López-Pérez, C., 2013b. Trophic webs of deep-sea megafauna on mainland and insular slopes of the NW Mediterranean: a comparison by stable isotope analysis. Mar. Ecol. Prog. Ser. 490, 199–221.

Farias, I., Figueiredo, I., Moura, T., Gordo, L.S., Neves, A., Serra-Pereira, B., 2006. Diet comparison of four ray species (*Raja clavata*, *Raja brachyura*, *Raja montagui* and *Leucoraja naevus*) caught along the Portuguese continental shelf. Aquat. Living Resour. 19, 105–114.

Fenchel, T., 1988. Marine Plankton Food-Chains. Annu. Rev. Ecol. Syst. 19, 19–38.

Fernández de Puelles, M.L., Macias, M.L., Vicente, L., 2014. Seasonal spatial pattern and community structure of zooplankton in waters off the Baleares archipelago (Central Western Mediterranean). J. Mar. Syst. 138, 82–94.

Fernández de Puelles, M.L., Pinot, J.M., Valencia, J., 2003. Seasonal and interannual variability of zooplankton community in waters off Mallorca island: 1994-1999. Oceanol. Acta 26, 673-686

Ferraton, F., Harmelin-Vivien, M., Mellon-Duval, C., Souplet, A., 2007. Spatio-temporal variation in diet may affect condition and abundance of juvenile European hake in the Gulf of Lions (NW Mediterranean). Mar. Ecol. Prog. Ser. 337, 197–208.

Ferry, L.A., Cailliet, G.M., 1996. Sample size sufficiency and data anlaysis. Are we characterizing and comparing diet properly? In: MacKinlay D, Shearer K (Eds). Feeding ecology and nutrition in fish. Int. Cong. Biol. Fish. San Francisco, p 71–80.

Field, J.C., Baltz, K.E.N., Walker, W.A., 2007. Range Expansion and Trophic Interactions of the Jumbo Squid *Dosidicus Gigas* in the California Current. Calif. Coop. Ocean. Fish. Investig. Reports 48, 131–146.

Flexas, M.M., Durrieu de Madron, X., Garcia, M.A., Canals, M., Arnau, P., 2002. Flow variability in the Gulf of Lions during the MATER HFF experiment (March-May 1997). J. Mar. Syst. 33-34, 197–214.

Flynn, A., Kloser, R., 2012. Cross-basin heterogeneity in lanternfish (family Myctophidae) assemblages and isotopic niches (δ^{13}C and δ^{15}N) in the southern Tasman Sea abyssal basin. Deep-Sea Res. I 69, 113–127.

Font, J., Millot, C., Salas, J., Julià, A., Chic, O., 1998. The drift of Modified Atlantic Water from the Alboran Sea to the eastern Mediterranean. Sci. Mar. 62, 211–216.

France, R. L. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Mar. Ecol. Prog. Ser. 124, 307–312.

Freire, J., Carabel, S., Verísimo, P., Bernárdez, C., Fernández, L., 2009. Patterns of juvenile habitat use by the spider crab *Maja brachydactyla* as revealed by stable isotope analyses. Sci. Mar. 73 (1), 39–49.

Fry, B., 1988. Food Web Structure on Georges Bank from Stable C, N, and S Isotopic Compositions. Limnol. Oceanogr. 33, 1182–1190.

Fry, B., Quiñones, R.B., 1994. Biomass spectra and stable-isotope indicators of trophic level in zooplankton of the northwest Atlantic. Mar. Ecol-Prog. Ser. 112, 201–204.

Fry, B., Sherr, E.B., 1984. δ^{13}C Measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib. Mar. Sci. 27, 13–47.

Fry, B., Wainright, S.C., 1991. Diatom sources of ^{13}C-rich carbon in marine food webs. Mar. Ecol-Prog. Ser. 76, 149–157.

Gage, J.D., Tyler, P.A., 1991. Deep-sea biology: a natural history of organisms at the deep-sea floor. Cambridge University Press.

Gallagher, A.J., Skubel, R.A., Pethybridge, H.R., Hammerschlag, N., 2017. Energy metabolism in mobile, wild-sampled sharks inferred by plasma lipids. Conserv. Physiol. 5, cox002.

Galvan, D., Sweeting, C.J., Polunin, N.V.C., 2012. Methodological uncertainty in resource mixing models for generalist fishes. Oecologia 169, 1083–1093.

Galvan, D.E., Sweeting, C.J., Reid, W.D.K., 2010. Power of stable isotope techniques to detect size-based feeding in marine fishes. Mar. Ecol-Prog. Ser. 407, 271–278.

García-Alonso, J., Müller, C.T., Hardege, J.D., 2008. Influence of food regimes and seasonality on fatty acid composition in the ragworm. Aquat. Biol. 4, 7-13.

García-Lafuente, J., López-Jurado, J., Lucaya, N., Yanez, M., Garcia, J., 1995. Circulation of water masses through the ibiza channel. Oceanol. Acta 18, 245–254.

Gartner, J.V.J., Hopkins, T.L., Baird, R.C., Milliken, D.M., 1987. The lanternfishes (Pices: Myctophidae) of the Eastern Gulf of Mexico. Fish. Bull. 35, 81–98.

Gartner Jr., J.V., Crabtree, R.E., Sulak, K.J., 1997. Feeding at depth. In: Randall, D.J., Farrell, A.P. (Eds.), Deep-sea fishes. Academic Press, San Diego, pp. 115–193.

Garrison L.P., Link J.S., 2000. Dietary guild structure of the fish community in the Northeast United States continental shelf ecosystem. Mar. Ecol. Prog. Ser. 202, 231–240.

Gasalla, M.A., Rodrigues, A.R., Postuma, F.A., 2010. The trophic role of the squid *Loligo plei* as a keystone species in the South Brazil Bight ecosystem. ICES J. Mar. Sci. 67, 1413–1424.

Gaskett, A.C., Bulman, C., He, X., Goldsworthy, S.D., 2001. Diet composition and guild structure of mesopelagic and bathypelagic fishes near Macquarie Island, Australia. New Zeal. J. Mar. Fresh. 35, 469–476.

Gili, J.M., Coma, R., 1998. Benthic suspension feeders: Their paramount role in littoral marine food webs. Trends Ecol. Evol. 13 (8), 316–21.

Giller, P.S., Gee, J.H.R., 1987. The analysis of community organisation: the influence of equilibrium, scale and terminology. In Gee, J.H.R. and Giller, P.S. (Eds), Organisation of communities, past and present. Blackwell Scientific, Oxford, pp. 519–542.

Giorgi, F., 2006. Climate change hot-spots. Geophys. Res. Lett. 33.

Gomes, T.M., Sola, E., Gros, M.P., Menezes, G., Pinho, M.R., 1998. Trophic relationships and feeding habits of demersal fishes from the Azores: importance to multispecies assessment. ICES J. Mar. Sci. 35, 7–21.

Gjøsaeter, J., Kawaguchi, K., 1980. A review of the world resources of mesopelagic fish. FAO Fish. Tech. Pap. 193, 123–134.

Golani, D., 1993. Trophic adaptation of Red Sea fishes to the eastern Mediterranean environment—review and new data. Isr. J. Zool. 39, 391–402.

Gooday, A.J., 1988. A response by benthic Foraminifera to the deposition of phytodetritus in the deep-sea. Nature 332, 70–73.

Gooday, A.J., Turley, C.M., 1990. Responses by benthic organisms to inputs of organic material to the ocean floor: a review. Philos. Trans. R. Soc. Lond. A. 331, 119–138

Goodyear, R.H., Gibbs, R.H., Roper, C.F.E., Kleckner, R.C., Sweeney, M.J., 1972. Mediterr. Biol. Stud. Final Rep., 2, Smithsonian Institution, Washington, DC.

Graeb, B.D.S., Mangan, M.T., Jolley, J.C., Wahl, D.H., Dettmers, J.M., 2006. Ontogenetic changes in prey preference and foraging ability of yellow perch: Insights based on relative energetic return of prey. Trans. Am. Fish. Soc. 135, 1493–1498.

Graf, G. 1992. Benthic-pelagic coupling: a benthic view. *Oceanogr. Mar. Biol. Ann. Rev.*, 30, 149–190.

Griffiths, J. R., Kadin, M., Nascimento, F. J. A., Tamelander, T., Törnroos, A., Bonaglia, S., Bonsdorff, E., Brüchert, V., Gårdmark, A., Järnström, M., Kotta, J., Lindegren, M., Nordström, M. C., Norkko, A., Olsson, J., Weigel, B., Žydelis, R., Blenckner, T., Niiranen,

S. and Winder, M., 2017. The importance of benthic–pelagic coupling for marine ecosystem functioning in a changing world. Glob Change Biol, 23: 2179–2196.

Grober-Dunsmore, R., Wooninck, L., Field, J., Ainsworth, C., Beets, J., Berkeley, S., Bohnsack, J., Boulon, R., Brodeur, R., Brodziak, J., Crowder, L., Gleason, D., Hixon, M., Kaufman, L., Lindberg, B., Miller, M., Morgan, L., Wahle, C., 2008. Vertical Zoning in Marine Protected Areas: Ecological Considerations for Balancing Pelagic Fishing with Conservation of Benthic Communities. Fisheries 33, 598–610.

Guerra, A., Rocha, F., 1994. The life history of *Loligo vulgaris* and *Loligo forbesi* (Cephalopoda: Loliginidae) in Galician waters (NW Spain). Fish. Res. 21, 43–69.

Guerreiro, M., Phillips, R.A., Cherel, Y., Ceia, F.R., Alvito, P., Rosa, R., Xavier, J.C., 2015. Habitat and trophic ecology of Southern Ocean cephalopods from stable isotope analyses. Mar. Ecol. Prog. Ser. 530, 119–134.

Guijarro, B., Fanelli, E., Moranta, J., Cartes, J.E., Massutí, E., 2012. Small-scale differences in the distribution and population dynamics of pandalid shrimps in the western Mediterranean in relation to environmental factors. Fish. Res. 120, 33–47.

Guzzo, M.M., Haffner, G.D., Sorge, S., Rush, S.A., Fisk, A.T., 2011. Spatial and temporal variabilities of $\delta^{13}C$ and $\delta^{15}N$ within lower trophic levels of a large lake: Implications for estimating trophic relationships of consumers. Hydrobiologia 675, 41–53.

Haedrich, R.L., Merrett, N.R., 1992. Production/Biomass Ratios, Size Frequencies and Biomass Spectra in Deep-Sea Demersal Fishes, in: Rowe, G.T., Pariente, V. (Eds.), Deep-Sea Food Chains and the Global Carbon Cycle. Springer Netherlands, Dordrecht, pp. 157–182.

Haedrick, R.L., Merrett, N.R., 1997. Deep-Sea demersal fish and fisheries. Chapman and Hall, London.

Hall, S.J., Raffaelli, D.G., 1993. Food Webs-Theory and Reality. In Begon, M., Fitter, A.H. (Eds.), Advances in Ecological Research. T. J. Press Padstow, Cornwall, Vol. 24, pp. 187–239.

Hanson, J.M., Chouinard, G.A., 2002. Diet of Atlantic cod in the southern Gulf of St Lawrence as an index of ecosystem change, 1959-2000. J. Fish Biol. 60, 902–922.

Harrigan, P., Zieman, J.C., Macko, S.A., 1989. The base of nutritional support for the gray snapper *(Lutjanus griseus)*: an evaluation based on combined stomach content and stable isotope analysis. Bul.l Mar. Sci. 44, 65–77.

Hedd, A., Montevecchi, W.A., 2006. Diet and trophic position of Leach's storm-petrel *Oceanodroma leucorhoa* during breeding and moult, inferred from stable isotope analysis of feathers. Mar. Ecol-Prog. Ser. 322, 291–301.

Heithaus, M.R., 2004. Predator - Prey interactions. In: Carrier J.C., Musick J.A., Heithaus, M.R. (Eds.), *Biology of Sharks and Their Relatives*. CRC Press, Boca Raton, pp. 487-521

Heithaus, M.R., Vaudo, J.J., Kreicker, S., Layman, C.A., Krützen, M., Burkholder, D.A., Gastrich, K., Bessey, C., Sarabia, R., Cameron, K., Wirsing, A., Thomson, J.A., Dunphy-Daly, M.M., 2013. Apparent resource partitioning and trophic structure of large-bodied marine predators in a relatively pristine seagrass ecosystem. Mar. Ecol. Prog. Ser. 481, 225–237.

Heupel, M.R., Knip, D.M., Simpfendorfer, C.A., Dulvy, N.K., 2014. Sizing up the ecological role of sharks as predators. Mar. Ecol. Prog. Ser. 495, 291–298.

Hidalgo, M., Massutí, E., Moranta, J., Cartes, J.E., Lloret, J., Oliver, P., Morales-Nin, B., 2008. Seasonal and short spatial patterns in European hake (*Merluccius merluccius* L.) recruitment process at the Balearic Islands (western Mediterranean): The role of

environment on distribution and condition. J. Mar. Syst. 71, 367–384.

Hobson, K.A., Ambrose, W.G., Renaud, P.E., 1995. Sources of primary production, benthic-pelagic coupling, and trophic relationships within the Northeast Water Polynya: Insights from δ^{13}C and δ^{15}N analysis. Mar. Ecol. Prog. Ser. 128, 1–10.

Hoffman, J.C., Sutton, T.T., 2010. Lipid correction for carbon stable isotope analysis of deep-sea fishes. Deep-Sea Res. I 57, 956–964.

Holden, M.J., Tucker, R.N., 1974. Food of *Raja clavata* Linnaeus 1758, *Raja montagui* Fowler 1910, *Raja naevus* Muller and Henle 1841 and *Raja brachyura* Lafont 1873 in British Waters. J. Cons. 35, 189–193.

Holling 1973. Resilience and Stability of Ecological Systems. Annual Review of Ecology and Systematics. Vol. 4, 1-23.

Hopkins, T.S. 2006. Physics of the sea. In: Margalef, R. (Ed.), Key Environments: Western Mediterranean. Pergamon Press, New York, pp. 100-125.

Hoving, H.J.T., Perez, J.A.A., Bolstad, K.S.R., Braid, H.E., Evans, A.B., Fuchs, D., Judkins, H., Kelly, J.T., Marian, J.E.A.R., Nakajima, R., Piatkowski, U., Reid, A., Vecchione, M., Xavier, J.C.C., 2014. The study of deep-sea cephalopods. Adv. Mar. Biol. 67, 235–359.

Hulley, P.A. 1984. Myctophidae. In: Whitehead, P., Bauchot, M.L., Hureau, J.C., Nielsen, J., Tortonese, E. (Eds.), Fishes of the north-eastern Atlantic and the Mediterranean. UNESCO, pp. 429–483.

Hulley, P.A. 1986. Myctophidae. In: Smith, M.M., Heemstra, P.C. (Eds.), Smiths' sea fishes. Springer-Verlag, Berlin, pp. 282–321.

Hulley, P.A. 1990. Myctophidae. In Quero, J.C., Hureau, J.C., Karrer, C., Post, A., Saldanha, L. (Eds.), Check-list of the fishes of the eastern tropical Atlantic. UNESCO, Paris, Vol. 1, pp. 398–467.

Hulley, P.A., 1981. Results of the Research Cruises of Frv Herwig, Walther to South-America. 58. Family Myctophidae (Osteichthyes, Myctophiformes). Archiv. Fischereiwissenschaft 31, 1–300.

Hurlbert, S.H. - 1978. Measurement of niche overlap and some relatives. Ecology 59, 67-77.

Hussey, N.E., Brush, J., McCarthy, I.D., Fisk, A.T., 2010. ^{15}N and ^{13}C diet-tissue discrimination factors for large sharks under semi controlled conditions. Comp. Biochem. Physiol. Part A, 445–453.

Hussey, N.E., Dudley, S.F.J., McCarthy, I.D., Cliff, G., Fisk, A.T., 2011. Stable isotope profiles of large marine predators: viable indicators of trophic position, diet and movement in sharks? Can. J. Fish. Aquat. Sci. 68, 2029–2045.

Hussey, N.E., Macneil, M.A., Mcmeans, B.C., Olin, J.A., Dudley, S.F.J., Cliff, G., Wintner, S.P., Fennessy, S.T., Fisk, A.T., 2014. Rescaling the trophic structure of marine food webs. Ecol. Lett. 17, 239–250.

Hyslop, E.J., 1980. Stomach contents analysis-a review of methods and their application. J. Fish Biol. 1741, 1–429.

Iglesias, M., Massutí, E., Reñones, O., Morales-Nin, B., 1994. Three small-scale fisheries based on the island of Majorca (NW Mediterranean). Monogr. Boll. Soc. Hist. Nat. Balears 37, 35–58.

Iglesias, M., Morales-Nin, B., 2001. Life cycle of the pelagic goby *Aphia minuta* (Pisces: Gobiidae). Sci. Mar. 65, 183–192.

Iken, K., Bluhm, B.A., Gradinger, R., 2005. Food web structure in the high Arctic Canada Basin: evidence from $\delta^{13}C$ and $\delta^{15}N$ analysis. Polar Biol. 28, 238–249.

Iken, K., Brey, T., Wand, U., Voigt, J., Junghans, P., 2001. Food web structure of the benthic community at the Porcupine Abyssal Plain (NE Atlantic): a stable isotope analysis. Prog. Oceanogr. 50, 383–405.

IPCC, 2013. Working Group I Contribution to the IPCC Fifth Assessment Report, Climate Change 2013: The Physical Science Basis. Ipcc AR5, 2014.

Irigoien, X., Klevjer, T.A., Røstad, A., Martinez, U., Boyra, G., Acuña, J.L., Bode, A., Echevarria, F., Gonzalez-Gordillo, J.I., Hernandez-Leon, S., Agusti, S., Aksnes, D.L., Duarte, C.M., Kaartvedt, S., 2014. Large mesopelagic fishes biomass and trophic efficiency in the open ocean. Nature Communications 5. doi: 10.1038/ncomms4271

Ishihara, H., 1990. The skates and rays of the western North Pacific: an overview of their fisheries, utilization, and classification. In: Pratt H.L., Gruber S.H., Taniuchi T. (Eds.), *Elasmobranches as living resources: Advances in the biology, ecology, systematics and the status of the fisheries*. NOAA Tech. Rep. 90, pp. 485-497.

Jackson, A.L., Inger, R., Parnell, A.C., Bearhop, S., 2011. Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. J. Anim. Ecol. 80 (3), 595–602.

Jackson, G.D., Bustamante, P., Cherel, Y., Fulton, E.A., Grist, E.P.M., Jackson, C.H., Nichols, P.D., Pethybridge, H., Phillips, K., Ward, R.D., Xavier, J.C., 2007. Applying new tools to cephalopod trophic dynamics and ecology: perspectives from the Southern Ocean Cephalopod Workshop, February 2-3, 2006. Rev. Fish. Biol. Fish. 17, 79–99.

Jardas, I., Santic, M., Pallaoro, A., 2004. Diet composition of the eagle ray, *Myliobatis aquila* (Chondrichthyes : Myliobatidae), in the eastern Adriatic Sea. Cybium 28, 372–374.

Jennings, S., Reñones, O., Morales-Nin, B., Polunin, N.V.C., Moranta, J., Coll, J., 1997. Spatial variation in the ^{15}N and ^{13}C stable isotope composition of plants, invertebrates and fishes on Mediterranean reefs: Implications for the study of trophic pathways. Mar. Ecol. Prog. Ser. 146, 109–116.

Jennings, S., Warr, K.J., 2003. Environmental correlates of large-scale spatial variation in the delta N-15 of marine animals. Mar. Biol. 142, 1131–1140.

Jereb. P., Roper, C., 2010. Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. Myopsid and Oegopsid Squids. FAO species Catalogue for Fishery Purposes, n4 vol 2. FAO, Rome.

Jeschke, J.M., Kokko, H., 2009. The roles of body size and phylogeny in fast and slow life histories. Evol. Ecol. 23, 867–878.

Johnson, A.F., Valls, M., Moranta, J., Jenkins, S.R., Hiddink, J.G., Hinz, H., 2012. Effect of prey abundance and size on the distribution of demersal fishes. Can. J. Fish. Aquat. Sci. 69, 191–200.

Jordà, G., Marbà, N., Duarte, C.M., 2013. Climate warming and Mediterranean seagrass. Nat. Clim. Chang. 3, 3–4.

Karpov, K.A., Cailliet, G.M., 1979. Prey composition of the market squid, *Loligo opalescens* Berry, in relation to depth and location of capture, size of squid, and sex of spawning squid. Calif. Coop. Ocean. Fish. Invest. Rep. 20, 51–57.

Kelly, J.F., 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can. J. Zool. 78, 1–27.

Keller, S., Bartolino, V., Hidalgo, M., Bitetto, I., Casciaro, L., Cuccu, D., Esteban, A., Garcia, C.,

Garofalo, G., Josephides, M., Jadaud, A., Lefkaditou, E., Maiorano, P., Manfredi, C., Marceta, B., Massutí, E., Micallef, R., Peristeraki, P., Relini, G., Sartor, P., Spedicato, M.T., Tserpes, G., Quetglas, A., 2016. Large-scale spatio-temporal patterns of mediterranean cephalopod diversity. PLoS One 11, 1–19.

Kim, S.L., Casper, D.R., Galván-Magaña, F., Ochoa-Díaz, R., Hernández-Aguilar, S.B., Koch, P.L., 2012. Carbon and nitrogen discrimination factors for elasmobranch soft tissues based on a long-term controlled feeding study. Environ. Biol. Fishes 95, 37–52.

Koppelmann, R., Bottger-Schnack, R., Mobius, J., Weikert, H., 2009. Trophic relationships of zooplankton in the eastern Mediterranean based on stable isotope measurements. J. Plankton Res. 31, 669–686.

Koslow, J.A., 1996. Energetic and life-history patterns of deep-sea benthic, benthopelagic and seamount-associated fish. J. Fish Biol. 49, 54–74.

Koslow, J.A., 1997. Seamounts and the Ecology of Deep-Sea Fisheries. Am. Sci. 85, 168–176.

Krebs, C.J., 1989. *Ecological Methodology*. 2nd. ed. Addison-Welsey Educational Publishers, Inc., Menlo Park, CA.

Laptikhovsky, V., Arkhipkin, A., Brickle, P., 2010. Squid as a resource shared by fish and humans on the Falkland Islands' shelf. Fish. Res. 106, 151–155.

Lassalle, G., and others. 2011. Lower trophic levels and detrital biomass control the Bay of Biscay continental shelf food web: Implications for ecosystem management. Progr. Oceanogr. 91, 561-575

Layman, C.A., Arrington, D.A., Montana, C.G., Post, D.M., 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88, 42–48.

Le Loc'h, F., Hily, C., Grall, J., 2008. Benthic community and food web structure on the continental shelf of the Bay of Biscay (North Eastern Atlantic) revealed by stable isotopes analysis. J. Mar. Syst. 72, 17–34.

Lea, M.A., Nichols, P.D., Wilson, G., 2002. Fatty acid composition of lipid-rich myctophids and mackerel icefish (*Champsocephalus gunnari*) - Southern ocean food-web implications. Polar Biol. 25, 843–854.

Lehmann, M.F., Bernasconi, S.M., Barbieri, A., McKenzie, J., 2002. Preservation of organic matter and alteration of its carbon and nitrogen isotope composition during simulated and in situ early sedimentary diagenesis. Geochim. Cosmochim. Acta 66 (20), 3573–3584.

Lejeusne, C., Chevaldonné, P., Pergent-Martini, C., Boudouresque, C.F., Pérez, T., 2010. Climate change effects on a miniature ocean: the highly diverse, highly impacted Mediterranean Sea. Trends Ecol. Evol. 25, 250–260.

Letessier, T., Pond, D.W., McGill, R.A., Reid, W.D., Brierley, A.S., 2012. Trophic interaction of invertebrate zooplankton on either side of the Charlie Gibbs Fracture Zone/Subpolar Front of the Mid-Atlantic Ridge. J. Mar. Syst. 94, 174–184.

Linde, M., Grau, A. M., Riera, F., Massutí-Pascual, E., 2004. Analysis of trophic ontogeny in *Epinephelus marginatus* (Serranidae). Cybium, 28 (1), 27–35.

Lombarte, A., Chic, Ò., Parisi-Baradad, V., Olivella, R., Riera, J., García-Ladona, E., 2006. A web-based environment from shape analysis of fish otoliths. The AFORO database. Sci. Mar. 70, 147–152.

López-Jurado, J.L., Marcos, M., Monserrat, S., 2008. Hydrographic conditions affecting two fishing grounds of Mallorca island (Western Mediterranean): during the IDEA Project (2003–2004). J. Mar. Syst. 71, 303–315.

Macko, S.A., Estep, M.L.F., 1984. Microbial alteration of stable nitrogen and carbon isotopic compositions of organic matter. Org. Geochem. 6, 787–790.

Macpherson, E., 1979. Ecological overlap between macrourids in the western Mediterranean Sea. Mar. Biol. 53, 149–159.

Macpherson, E., 1980. Régime alimentaire de *Galeus melastomus* Rafinesque, 1810, *Etmopterus spinax* (L., 1788) et *Scymnorhinus licha* (Bonnaterre, 1788) en Mediterranée Occidentale. Vie Mil. 30, 139-148.

Macpherson, E., 1981. Resource partitioning in a Mediterranean Demersal Fish Community Mar. Ecol. Prog. Ser. 4, 183-193.

Macy, W.K., 1982. Feeding Patterns of the Long-Finned Squid, *Loligo pealei*, in New England Waters. Biol. Bull. 162, 28–38.

Madurell, T., Cartes, J.E., 2005. Trophodynamics of a deep-sea demersal fish assemblage from the bathyal eastern Ionian Sea (Mediterranean Sea). Deep-sea Res. I 52, 2049–2064.

Madurell, T., Cartes, J.E., Labropoulou, M., 2004. Changes in the structure of fish assemblages in a bathyal site of the Ionian Sea (eastern Mediterranean). Fish. Res. 66, 245–260.

Madurell, T., Fanelli, E., Cartes, J.E., 2008. Isotopic composition of carbon and nitrogen of suprabenthic fauna in the NW Balearic Islands (western Mediterranean). J. Mar. Syst. 71, 336–345.

Maltby E., 2000. Ecosystem approach: from principle to practice. Ecosystem Service and Sustainable Watershed Management in North China International Conference, Beijing, P.R. China, August 23 - 25, 2000.

Mangold-Wirz, K., 1963. Biologie des céphalopodes benthiques et nectoniques de la Mer Catalane. Vie Milieu (Suppl) 13, 1–285.

Marasco R.J, Goodman D., Grimes C.B., Lawson P.W., Punt A.E., Quinn T.J.I., 2007. Ecosystem-based fisheries management: some practical suggestions. Can. J. Fish Aquat. Sci. 64, 928–939.

Martínez-Baena, F., Navarro, J., Albo-Puigserver, M., Palomera, I., Rosas-Luis, R., 2016. Feeding habits of the short-finned squid *Illex coindetii* in the western Mediterranean sea using combined stomach content and isotopic analysis. J. Mar. Biol. Assoc. U.K. 96, 1235–1242.

Martínez Del Rio, C., Wolf, N., Carleton, S.A., Gannes, L.Z., 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biol. Rev. 84 (1), 91–111.

Maruyama, A., Yamada, Y., Yuma, M., Rusuwa, B., 2001. Stable nitrogen and carbon isotope ratios as migration tracers of a landlocked goby, *Rhinogobius* sp (the orange form), in the Lake Biwa water system. Ecol. Res. 16, 697–703.

Massutí, E., Deudero, S., Sánchez, P., Morales-Nin, B., 1998. Diet and feeding of dolphin (*Coryphaena hippurus*) in Western Mediterranean waters. Bull. Mar. Sci. 63 (2), 329–341.

Massutí, E., Gordon, J.D.M., Moranta, J., Swan, S.C., Stefanescu, C., Merrett, N.R., 2004. Mediterranean and Atlantic deep-sea fish assemblages : differences in biomass composition and size-related structure. Sci. Mar. 68, 101–115.

Massutí, E., Monserrat, S., Oliver, P., Moranta, J., López-Jurado, J. L., Marcos, M., ... , Pereda, P., 2008. The influence of oceanographic scenarios on the population dynamics of demersal resources in the western Mediterranean: hypothesis for hake and red shrimp off Balearic Islands. J. Mar. Syst., *71*(3), 421-438.

Massutí, E., Moranta, J., 2003. Demersal assemblages and depth distribution of elasmobranchs from the continental shelf and slope off the Balearic Islands (western Mediterranean). ICES J. Mar. Sci. 60, 753–766.

Massutí, E., Olivar, M.P., Monserrat, S., Rueda, L., Oliver, P., 2014. Towards the understanding of the influence of the environmental conditions on the demersal resources and ecosystems in the western Mediterranean: Motivations, aims and methods of the IDEADOS project. J. Mar. Syst. 138, 3–19

Massutí, M., Oliver, M., 1948. Estudio de la biometría y biología de la sardina de Mahón (Baleares) especialmente de su alimentación. Bol. Inst. Esp. Oceanogr. 3, 15 p.

Massutí, E., Reñones, O., 2005. Demersal resource assemblages in the trawl fishing grounds off the Balearic Islands (western Mediterranean). Sci. Mar. 69, 167–181.

Maynou, F., Cartes, J.E., 1998. Daily ration estimates and comparative study of food consumption in nine species of deep-water decapod crustaceans of the NW Mediterranean. Mar. Ecol. Prog. Ser. 171, 221–231.

Maynou, F., Cartes, J.E., 2000. Community structure of bathyal decapod crustaceans off south-west Balearic Islands (western Mediterranenan): seasonality and regional patterns in zonation. J. Mar. Biol. Assoc. U.K. 80, 789–798.

McCutchan, J.H., Lewis, W.M., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102, 378–390.

Mees, J., Jones, M.B., 1997. The hyperbenthos. Oceanogr. Mar. Biol. 35, 221–255.

MerMex-Group, 2011. Marine ecosystems' responses to climatic and anthropogenic forcings in the Mediterranean. Prog. Oceanogr. 91, 97–166.

Meyer, J.L., Schultz, E.T., Helfman, G.S., 1983. Fish Schools - An Asset to Corals. Science 220, 1047–1049.

Miller, T.W., Bosley, K.L., Shibata, J., Brodeur, R.D., Omori, K., Emmett, R., 2013. Contribution of prey to Humboldt squid *Dosidicus gigas* in the northern California Current, revealed by stable isotope analyses. Mar. Ecol. Prog. Ser. 477, 123–134.

Miller, M.J., Chikaraishi, Y., Ogawa, N.O., Yamada, Y., Tsukamoto, K., Ohkouchi, N., 2012. A low trophic position of Japanese eel larvae indicates feeding on marine snow. Biol. Lett. 20120826.

Miller, T.W., Brodeur, R.D., Rau, G., Omori, K., 2010. Prey dominance shapes trophic structure of the northern California Current pelagic food web: evidence from stable isotopes and diet analysis. Mar. Ecol. Prog. Ser. 420, 15–26.

Miller, T.W., Brodeur, R.D., Rau, G.H., 2008. Carbon stable isotopes reveal relative contribution of shelf-slope production to the northern California Current pelagic community. Limnol. Oceanogr. 53, 1493–1503.

Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between δ^{15}N and animal age. Geochim. Cosmochim. A. 48, 1135–1140.

Miquel, J.C., Fowler, S.W., Larosa, J., Buatmenard, P., 1994. Dynamics of the Downward Flux of Particles and Carbon in the Open Northwestern Mediterranean Sea. Deep-sea Res. I 41, 243–261.

Miserocchi, S., Faganeli, J., Balboni, V., Heussner, S., Monaco, A., Kerherve, P., 1999. Characteristics and sources of the settling particulate organic matter in the South Adriatic basin, in: Organic Geochemistry. pp. 411–421.

Moloney, C. L., St John, M.A., Denman, K.L., Karl, D.M., Köster, F.W., Sundby, S., Wilson, R.P., 2011. Weaving marine food webs from end to end under global change. J. Mar. Syst. 84 (3), 106–116.

Montoya, J.P., Wiebe, P.H., McCarthy, J.J., 1992. Natural abundance of ^{15}N in particulate nitrogen and zooplankton in the Gulf Stream region and warm-core ring 86A. Deep-sea Res. Part A. Oceanogr. Res. Pap. 39, S363–S392.

Morales-Nin, B., Moranta, J., 1997. Life history and fishery of the common dentex (*Dentex dentex*) in Mallorca (Balearic Islands, western Mediterranean). Fish. Res., 30 (1-2), 67–76.

Morales-Nin, B., Moranta, .J, García, C., Tugores, M.P., Grau, A.M., Riera, F., Cerdà, M., 2005. The recreational fishery off Majorca Island (western Mediterranean): some implications for coastal resource management. ICES J. Mar. Sci. 62, 727–739.

Moranta, J., Quetglas, A., Massutí, E., Guijarro, B., Hidalgo, M., Díaz, P., 2008. Spatio-temporal variations in deep-sea demersal communities off the Balearic Islands (western Mediterranean). J. Mar. Syst. 71, 346–366.

Moranta, J., Stefanescu, C., Massutí, E., Morales-Nin, B., Lloris, D., 1998. Fish community structure and depth-related trends on the continental slope of the Balearic Islands (Algerian basin, western Mediterranean). Mar. Ecol. Prog. Ser. 171, 247–259.

Morato, T., E. Sola, M.P. Gros and G. Menezes - 2003. Diets of thornback ray (*Raja clavata*) and tope shark (*Galeorhinus galeus*) in the bottom longline fishery of the Azores, northeastern Atlantic. Fish. Bull. 101, 590–602.

Moreno A, Boavida-Portugal J, Pimentel M, Pereira J, Rosa R (2013) *Loligo vulgaris*, European squid. In: Rosa R, O'Dor R, Pierce G (eds) Advances in squid biology, ecology and fisheries, Part I. Nova Science Publisher, New York, NY, p 3–32.

Motta, P.J., 2004. Prey capture behavior and feeding mechanics of elasmobranchs. In: Carrier J.C., Musick J.A., Heithaus, M.R. (Eds.), *Biology of Sharks and Their Relatives*. CRC Press, Boca Raton, pp. 165–202.

Moutopoulos, D.K., Libralato, S., Solidoro, C., Stergiou, K.I., 2013. Toward an ecosystem approach to fisheries in the Mediterranean Sea: Multi-gear/multi-species implications from an ecosystem model of the Greek Ionian Sea. J. Mar. Syst. 113–114, 13–28.

Myers, N., Fonseca, G. a B., Mittermeier, R. a, Fonseca, G. a B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.

Nader, M., Indary, S., Boustany, L., 2012. The puffer fish *Lagocephalus sceleratus* (Gmelin, 1789) in the eastern Mediterranean. EastMed Technical Documents 10, EastMed, Food and Agriculture Organization of the United Nations (FAO).

Nash, R.D.M., Chapman, C.J., Atkinson, R.J.A., Morgan, P.J., 1984. Observations on the burrows and burrowing behaviour of *Calocaris macandreae* (Crustacea: Decapoda: Thalassinoidea). J. Zool. 202, 425–439.

Navarro, F.P., 1926. Estudios sobre clupeidos de Baleares. 1Estados jóvenes de la sardina. Notas Resum. Inst. Esp. Oceanogr. 9, 25 p.

Navarro, F.P., 1927. Estudios sobre clupeidos de Baleares. 2 La alacha. Notas Resum. Inst. Esp. Oceanogr. 21, 33 p.

Navarro, J., Coll, M., Louzao, M., Palomera, I., Delgado, A., Forero, M.G., 2011. Comparison of ecosystem modelling and isotopic approach as ecological tools to investigate food webs in the NW Mediterranean Sea. J. Exp. Mar. Biol. Ecol. 401, 97–104.

Navarro, J., Coll, M., Somes, C.J., Olson, R.J., 2013. Trophic niche of squids: Insights from isotopic data in marine systems worldwide. Deep. Res. Part II Top. Stud. Oceanogr. 95, 93–102.

Navarro, J., López, L., Coll, M., Barría, C., Sáez-Liante, R., 2014. Short- and long-term importance of small sharks in the diet of the rare deep-sea shark *Dalatias licha*. Mar. Biol. 161, 1697–1707.

Navarro, J., Louzao, M., Arcos, J.M., Delgado, A., Hobson, K.A., Oro, D., Genovart, M., Igual, M., Forero, M.G., 2009. Seasonal changes in the diet of a critically endangered seabird and the importance of trawling discards. Mar. Biol. 156, 2571–2578.

Navia, A.F., Cortés, E., Mejía-Falla, P.A., 2010. Topological analysis of the ecological importance of elasmobranch fishes: A food web study on the Gulf of Tortugas, Colombia. Ecol. Modell. 221, 2918–2926.

Nilsen, M., Pedersen, T., Nilssen, E.M., Fredriksen, S., 2008. Trophic studies in a high-latitude fjord ecosystem - a comparison of stable isotope analyses (δ^{13}C and δ^{15}N) and trophic-level estimates from a mass-balance model. Can. J. Fish Aquat. Sci. 65, 2791–2806.

Nixon, M., 1987. Cephalopod diets, in: Boyle, P.R. (Ed.), Cephalopod Life Cycles, Vol. II. Academic Press, London, Orlando, pp. 201–220.

O'Dor, R.K., Wells, M.J., 1987. Energy and nutrient flow in cephalopods, in: Cephalopod Life Cycles, Vol. II. pp. 109–133.

Olaso, I., Velasco, F., Perez, N., 1998. Importance of discarded blue whiting (*Micromesistius poutassou*) in the diet of lesser spotted dogfish (*Scyliorhinus canicula*) in the Cantabrian Sea. ICES J. Mar. Sci. 55, 331-341.

Olaso, I., Velasco, F., Sánchez, F., Serrano, A., Rodríguez-Cabello, C., Cendrero, O., 2005. Trophic relation of lesser spotted catshark (*Scyliorhinus canicula*) and blackmouth catshark (*Galeus melastomus*) in the Cantabrian Sea. J. Northwest Atl. Fish. Soc. 35, 481–494.

Olivar, M.P., Bernal, A., Molí, B., Peña, M., Balbín, R., Castellón, A., Miquel, J., Massutí, E., 2012. Vertical distribution, diversity and assemblages of mesopelagic fishes in the western Mediterranean. Deep-Sea Res. I 62, 53–69.

Oliver, M., Massutí, M., 1952. El raó *Xyrichtys novacula* (Fam. Labridae). Notas biológicas y biométricas. Bol. Inst. Esp. Oceanogr. 48, 15 p.

Olson, R.J., Popp, B.N., Graham, B.S., López-Ibarra, G.A., Galvan-Magana, F., Lennert-Cody, C.E., Bocanegra-Castillo, N., Wallsgrove, N.J., Gier, E., Alatorre-Ramirez, V., Ballance, L.T., Fry, B., 2010. Food-web inferences of stable isotope spatial patterns in copepods and yellowfin tuna in the pelagic eastern Pacific Ocean. Prog. Oceanogr. 86, 124–138.

Ordines, F., Massutí, E., Moranta, J., Quetglas, A., Guijarro, B., Fliti, K., 2011. Balearic Islands *vs* Algeria: two nearby western Mediterranean elasmobranchs populations with different oceanographic scenarios and fishing histories. Sci. Mar. 75, 707–717.

Ordines, F., Massutí, E., 2009. Relationships between macro-epibenthic communities and fish on the shelf grounds of the western Mediterranean. Aquat. Conserv. Mar. Freshw. Ecosyst. 19, 370–383.

O'Reilly, C.M., Hecky, R.E., Cohen, A.S., Plisnier, P.D., 2002. Interpreting stable isotopes in food webs: Recognizing the role of time averaging at different trophic levels. Limnol. Oceanogr. 47, 306–309.

Pakhomov, E.A., Bushula, T., Kaehler, S., Watkins, B.P., Leslie, R.W., 2006. Structure and distribution of the slope fish community in the vicinity of the sub-Antarctic Prince Edward Archipelago. J. Fish. Biol. 68, 1834–1866.

Pakhomov, E.A., Perissinotto, R., 1996. Antarctic neritic krill *Euphausia crystallorophias*: Spatio-temporal distribution, growth and grazing rates. Deep-sea Res. I 43, 59–87.

Palma, S., 1990. Stomach Contents of *Cyclothone braueri* Jespersen et Taning 1926 (Gonostomatidae) in the Ligurian Sea, Western Mediterranean. J. Plankton Res. 12, 519–534.

Papiol, V., Cartes, J. E., Fanelli, E., Rumolo, P., 2013. Food web structure and seasonality of slope megafauna in the NW Mediterranean elucidated by stable isotopes: Relationship with available food sources. J. Sea Res. 77, 53–69.

Papiol, V., Cartes, J., Fanelli, E., Maynou, F., 2012. Influence of environmental variables on the spatio-temporal dynamics of bentho-pelagic assemblages in the middle slope of the Balearic Basin (NW Mediterranean). Deep-Sea Res. I 61, 84–99.

Parnell, A.C., Inger, R., Bearhop, S., Jackson, A.L., 2010. Source partitioning using stable isotopes: Coping with too much variation. PloS one 5 (3), e9672.

Parnell, A.C., Jackson, A.L., 2013. SIAR: Stable isotope analysis in R. R package version 4.2. Available from https//cran.r-project.org/web/packages=siar.

Pasqual, C., Amores, A., Flexas, M.M., Monserrat, S., Calafat, A., 2014. Environmental factors controlling particulate mass fluxes on the Mallorca continental slope (western Mediterranean Sea). J. Mar. Syst. 138, 63–69.

Pasqual, C., Calafat, A., Lopez-Fernandez, P., Pusceddu, A., 2015. Organic carbon inputs to the sea bottom of the Mallorca continental slope. J. Mar. Syst. 148, 142–151.

Pauly, D., Christensen, V., 1995. Primary production required to sustain global fisheries. Nature 374, 255–257.

Peña, M, M. P. Olivar, R. Balbín, J.L. López-Jurado, M. Iglesias, J. Miquel. 2014. Acoustic detection of mesopelagic fishes in scattering layers of the Balearic Sea (western Mediterranean). Can. J. Fish. Aquat. Sci. 71 (8), 1186–1197.

Pergent, G., Bazairi, H., Bianchi, C.N., Boudouresque, C.F., Buia, M. C., Calvo, S., P. Clabaut, P., Harmelin-Vivien, M., Mateo, M.A., Montefalcone, M., Morri, C., Orfanidis, S., Pergent-Martini, C., Semroud, R., Serrano, O., Thibaut, T., Tomasello, A., Verlaque, M., 2014. Climate change and Mediterranean seagrass meadows: a synopsis for environmental managers. Med. Mar. Sci. 15, 462–473.

Personnic, S., Boudouresque, C. F., Astruch, P., Ballesteros, E., Blouet, S., Bellan-Santini, D., Bonhomme, P., Thibault-Botha, D., Feunteun, E., Harmelin-Vivien, M., Pergent, G., Pergent-Martini, C., Pastor, J., Poggiale, J.C., Renaud, F., Thibaut, T., Ruitton, S., 2014. An ecosystem-based approach to assess the status of a Mediterranean ecosystem, the *Posidonia oceanica* seagrass meadow. PloS one 9(6), e98994.

Peterson, B.J., Fry, B., 1987. Stable Isotopes in Ecosystem Studies. Annu. Rev. Ecol. Syst. 18, 293–320.

Pethybridge, H., Daley, R., Virtue, P., Butler, E.C. V, Cossa, D., Nichols, P.D., 2010. Lipid and mercury profiles of 61 mid-trophic species collected off south-eastern Australia. Mar. Freshw. Res. 61 (10), 1092–1108.

Petursdottir, H. Gislason, A., Falk-Petersen, S., Hop, H., Svavarsson, J., 2008. Trophic interactions of the pelagic ecosystem over the Reykjanes Ridge as evaluated by fatty acid and stable isotope analyses. Deep-Sea Res. II 55, 83–93.

Phillips, D.L., 2012. Converting isotope values to diet composition: the use of mixing models. J. Mammal 93, 342–352.

Phillips, D.L., Inger, R., Bearhop, S., Jackson, A.L., Moore, J.W., Parnell, A.C., Semmens, B.X., Ward, E.J., 2014. Best practices for use of stable isotope mixing models in food-web studies. Can. J. Zool. 92, 823–835.

Phillips, K.L., Jackson, G.D., Nichols, P.D., 2001. Predation on myctophids by the squid *Moroteuthis ingens* around Macquarie and Heard Islands: stomach contents and fatty acid analyses. Mar. Ecol-Prog. Ser. 215, 179–189.

Pianka, E.R., 1980. Guild Structure in Desert Lizards. Oikos 35, 194–201.

Piatkowski, U., Pierce, G.J., Morais da Cunha, M., 2001. Impact of cephalopods in the food chain and their interaction with the environment and fisheries: An overview. Fish. Res. 52(1), 5–10.

Pierce, G.J., Allcock, L., Bruno, I., Bustamante, P. González, A., Guerra, A., Jereb, P., Lefkaditou, E., Malham, S., Moreno, A., Pereira, J., Piatkowski, Uwe, Rasero, M., Sánchez, P., Santos, B., Santurtún, M., Seixas, S., Villanueva, R. (Eds.), 2010. *Cephalopod biology and fisheries in Europe* ICES Cooperative Research Report, 303. ICES, Copenhagen.

Pierce, G.J., Boyle P.R., Hastie, L.C., Santos, M.B., 1994. Diets of squid *Loligo forbesi* and *Loligo vulgaris* in the northeast Atlantic. Fish. Res. 21, 149–163.

Pierce, G.J., Valavanis, V.D., Guerra, A., Jereb, P., Orsi-Relini, L., Bellido, J.M., Katara, I., Piatkowski, U., Pereira, J., Balguerias, E., Sobrino, I., Lefkaditou, E., Wang, J., Santurtun, M., Boyle, P.R., Hastie, L.C., MacLeod, C.D., Smith, J.M., Viana, M., González, A.F., Zuur, A.F., 2008. A review of cephalopod-environment interactions in European Seas. Hydrobiologia 612 (1), 49–70.

Pikitch, E.K., Santora, C., Babcock, E.A., Bakun, A., Bonfil, R., Conover, D.O., Dayton, P., Doukakis, P., Fluharty, D., Heneman, B., Houde, E.D., Link, J., Livingston, P.A., Mangel, M., McAllister, M.K., Pope, J., Sainsbury, K.J., 2004. Ecosystem-based fishery management. Science 305, 346–347.

Pimm, S.L., 1982. *Food webs*. Chapman and Hall (Eds.), London.

Pimm, S.L., Lawton, J.H., Cohen, J.E., 1991. Food Web Patterns and Their Consequences. Nature 350, 669–674.

Pinnegar, J.K., Polunin, N.V.C., 1999. Differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. Funct. Ecol. 13, 225–231.

Pinnegar, J.K., Polunin, N.V.C., 2000. Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. Oecologia 122, 399–409.

Pinot, J.M., López-Jurado, J.L., Riera, M., 2002.The CANALES experiment (1996–1998). Interannual, seasonal and mesoescale variability of the circulation in the Balearic Channels. Prog. Oceanogr. 55, 335–370.

Podrazhanskaya, S.G., 1993. Feeding habits of mesopelagic species of fish and estimation of plankton Graze in the Northwest Atlantic. NAFO Sci. Coun. Studies 19, 79–85.

Polis, G.A., Anderson, W.B., Holt, R.D., 1997. Toward an Integration of Landscape and Food Web Ecology: The Dynamics of Spatially Subsidized Food Webs. Annu. Rev. Ecol. Syst. 28, 289–316.

Polis, G.A., Strong, D.R., 1996. Food web complexity and community dynamics. Am. Nat. 147, 813–846.

Polunin, N.V.C., Morales-Nin, B., Pawsey, W.E., Cartes, J.E., Pinnegar, J.K., Moranta, J., 2001. Feeding relationships in Mediterranean bathyal assemblages elucidated by stable nitrogen and carbon isotope data. Mar. Ecol. Prog. Ser. 220, 13–23.

Pomeroy, L.R., 2001. Caught in the food web: complexity made simple? Sci. Mar. 65 (Suppl 2), 31–40.

Pons-Moyà, J., Pons, G.X., García, Ll. i Grau, A.M., 1998. Mol.luscs i decapodes presents en el contingut gastric del rafel, *Trigla lyra* (Linnaeus, 1758) (Osteichthyes, Triglidae) del SW de Mallorca (Mediterrama occidental). Boll. Soc. Hist. Nat. Balears 41, 87–100.

Post, D.M., 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology 83, 703–718.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montana, C.G., 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152, 179–189.

Post, D.M., Pace, M.L., Hairston, N.G., 2000. Ecosystem size determines food-chain length in lakes. Nature 405, 1047–1049.

Preciado, I., Cartes, J.E., Serrano, A., Velasco, F., Olaso, I., Sánchez, F., Frutos, I., 2009. Resource utilization by deep-sea sharks at the Le Danois Bank, Cantabrian Sea, northeast Atlantic Ocean. J. Fish. Biol. 75, 1331–1355.

Preciado, I., Cartes, J.E., Punzón, A., Frutos, I., López-López, L., Serrano, A., 2016. Food web functioning of the benthopelagic community in a deep-sea seamount based on diet and stable isotope analyses. Deep-Sea Res. Part II Top. Stud. Oceanogr., 137, 56–68.

Procaccini, G., Buia M.C., Gambi, M.C., M Perez, M, Pergent, G., Pergent-Martini, C., Romero J., 2003. The seagrasses of the Western Mediterranean. In: E.P. Green and F.T. Short (Eds.), *World atlas of seagrasses*, pp. 48-58. University of California Press, Berkeley, USA.

Puerta, P., Hunsicker, M.E., Quetglas, A., Álvarez-Berastegui, D., Esteban, A., González, M., Hidalgo, M., 2015. Spatially explicit modeling reveals cephalopod distributions match contrasting trophic pathways in the western mediterranean sea. PLoS One 10 (7), e0133439.

Pusch, C., Hulley, P.A., Kock, K.H., 2004. Community structure and feeding ecology of mesopelagic fishes in the slope waters of King George Island (South Shetland Islands, Antarctica). Deep-Sea Res. I 51, 1685–1708.

Quetglas, A., Alemany, F., Carbonell, A., Merella, P., Sanchez, P., 1999. Diet of the European flying squid *Todarodes sagittatus* (Cephalopoda: Ommastrephidae) in the Balearic Sea (western Mediterranean). J. Mar. Biol. Assoc. U.K. 79 (3), 479–486.

Quetglas, A., Alemany, F., Carbonell, A., Merella, P., Sánchez, P., S??nchez, P., 1998. Biology and fishery of Octopus vulgaris Cuvier, 1797, caught by trawlers in Mallorca (Balearic Sea, Western Mediterranean). Fish. Res. 36, 237–249.

Quetglas, A., Carbonell, A., Sánchez, P., 2000. Demersal Continental Shelf and Upper Slope Cephalopod Assemblages from the Balearic Sea (North-Western Mediterranean). Biological Aspects of Some Deep-Sea Species. Estuar. Coast. Shelf Sci. 50, 739–749.

Quetglas, A., De Mesa, A., Ordines, F., Grau, A., 2010. Life history of the deep-sea cephalopod family Histioteuthidae in the western Mediterranean. Deep-Sea Res. I 57, 999–1008.

Quetglas, A., González, M., Carbonell, A., Sánchez, P., 2001. Biology of the deep-sea octopus *Bathypolypus sponsalis* (Cephalopoda: Octopodidae) from the western Mediterranean Sea. Mar. Biol. 138, 785–792.

Quetglas, A., González, M., Franco, I., 2005. Biology of the upper-slope cephalopod *Octopus salutii* from the western Mediterranean Sea. Mar. Biol. 146, 1131–1138.

Quetglas, A., Guijarro, B., Ordines, F., Massutí, E., 2012. Stock boundaries for fisheries assessment and management in the Mediterranean: the Balearic Islands as a case study. Sci. Mar. 76, 17–28.

Quetglas, A., Ordines, F., González, M., Franco, I., 2009. Life history of the bathyal octopus *Pteroctopus tetracirrhus* (Mollusca, Cephalopoda) in the Mediterranean Sea. Deep-Sea Res. Part I Oceanogr. Res. Pap. 56, 1379–1390.

Quetglas, A., Merino, G., Ordines, F., Guijarro, B., Garau, A., Grau, A.M., Oliver, P., Massutí, E., 2016a. Assessment and management of western Mediterranean small-scale fisheries. Ocean Coast. Manag., 133, 95–104.

Quetglas, A., Rueda, L., Alvarez-Berastegui, D., Guijarro, B., Massutí, E., 2016b. Contrasting responses to harvesting and environmental drivers of fast and slow life history species. PLoS One 11, 1–15.

Quetglas, A., Valls, M., Ordines, F., de Mesa, A., Olivar, M.P., Keller, S., Massutí, E., 2014. Structure and dynamics of cephalopod assemblages in the water column on shelf-break and slope grounds of the western Mediterranean. J. Mar. Syst. 138, 150–159.

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Ragonese, S., Jereb, P., 1986. *Loligo forbesi* Steenstrup 1856 (Cephalopoda: Loliginidae) nel versante siciliano del Mare Pelagico (Canale di Sicilia): nota preliminare sulla distribuzione, composizione per taglia e biologia riproduttiva. Nova Thalassia 8, 529–535.

Ramírez-Amaro, S., Ordines, F., Terrasa, B., Esteban, A., García, C., Guijarro, B., Massutí, E., 2015. Demersal chondrichthyans in the western Mediterranean: Assemblages and biological parameters of their main species. Mar. Freshw. Res. 67, 636–652.

Ramón, M., Abelló, P., Ordines, F., Massutí, E., 2014. Deep epibenthic communities in two contrasting areas of the Balearic Islands (western Mediterranean). J. Mar. Syst. 138, 182–193.

Reglero, P., Ciannelli, L., Alvarez-Berastegui, D., Balbín, R., López-Jurado, J.L., Alemany, F., 2012. Geographically and environmentally driven spawning distributions of tuna species in the western Mediterranean Sea. Mar. Ecol. Prog. Ser. 463, 273–284.

Reid, S.B., Hirota, J., Young, R.E., Hallacher, L.E., 1991. Mesopelagic-boundary community in Hawaii: micronekton at the interface between neritic and oceanic ecosystems. Mar. Biol. 109, 427–440.

Reid, W.D.K., Wigham, B.D., Mcgill, R.A.R., Polunin, N.V.C., 2012. Elucidating trophic pathways in benthic deep-sea assemblages of the Mid-Atlantic Ridge north and south of the Charlie-Gibbs Fracture Zone. Mar. Ecol. Prog. Ser. 463, 89–103.

Relini, G., Bertrand, J.A., Zamboni, A., 1999. Synthesis of the knowledge on bottom fishery resources in Central Mediterranean (Italy and Corsica). Biol. Mar. Medit. 6 (Suppl. 1), 94–98.

Reñones, O., Polunin, N.V.C., Goni, R., 2002. Size related dietary shifts of *Epinephelus marginatus* in a western Mediterranean littoral ecosystem: an isotope and stomach

content analysis. J. Fish Biol. 61, 122–137.

Revill, A.T., Young, J.W., Lansdell, M., 2009. Stable isotopic evidence for trophic groupings and bio-regionalization of predators and their prey in oceanic waters off eastern Australia. Mar. Biol. 156, 1241–1253.

Rinewalt, C.S., Ebert, D.A., Cailliet, G.M., 2007. Food habits of the sandpaper skate, *Bathyraja kincaidii* (Garman, 1908) off central California: seasonal variation in diet linked to oceanographic conditions. Environ. Biol. Fish. 80, 147–163.

Rocha, F., Castro, B.G., Gil, M.S., Guerra, A., 1994. The diets of *Loligo vulgaris* and *L. forbesi* (Cephalopoda: Loliginidae) in northwestern Spanish Atlantic waters. Sarsia 79, 119–126.

Rocha, F., Guerra, A., 1999. Age and growth of two sympatric squid *Loligo vulgaris* and *Loligo forbesi*, in Galician waters (north-west Spain). J. Mar. Biol. Assoc. U.K. 79, 697–707.

Rodhouse, P.G., Nigmatullin, C.M., 1996. Role as consumers. Philos. T. Roy. Soc. B. 351, 1003–1022.

Rodhouse, P.G.K., Pierce, G.J., Nichols, O.C., Sauer, W.H.H., Arkhipkin, A.I., Laptikhovsky, V. V., Lipinski, M.R., Ramos, J.E., Gras, M., Kidokoro, H., Sadayasu, K., Pereira, J., Lefkaditou, E., Pita, C., Gasalla, M., Haimovici, M., Sakai, M., Downey, N., 2014. Environmental effects on cephalopod population dynamics: Implications for management of fisheries. Adv. Mar. Biol. 67, 99–233.

Roe, H.S.J., Badcock, J., 1984. The diel migrations and distributions within a mesopelagic community in the north east Atlantic. 5. Vertical Migrations and Feeding of Fish. Prog. Oceanogr. 13, 389–424.

Romanelli, M., Colasante, A., Scacco, U., Consalvo, I., Finoia, M.G., Vacchi, M., 2007. Commercial catches, reproduction and feeding habits of *Raja asterias* (Chondrichthyes: Rajidae) in a coastal area of the Tyrrhenian Sea (Italy, northern Mediterranean). Acta Adriat. 48, 57–71.

Root, R.B., 1967. The niche exploration pattern of the blue-gray gnatcatcher. Ecol. Monogr. 317–350.

Roper, C.F.E., Sweeney, M.J., Nauen C.E., 1984. FAO species catalogue. Cephalopods of the world. An annotated and illustrated catalogue of species of interest to fisheries. FAO Fish. Synop. 125 (3).

Rosa, R., Dierssen, H.M., Gonzalez, L., Seibel, B.A., 2008. Ecological biogeography of cephalopod molluscs in the Atlantic Ocean: Historical and contemporary causes of coastal diversity patterns. Glob. Ecol. Biogeogr. 17, 600–610.

Rosa, R., Pereira, J., Nunes, M.L., 2005. Biochemical composition of cephalopods with different life strategies, with special reference to a giant squid, *Architeuthis* sp. Mar. Biol. 146, 739–751.

Rosas-Luis, R., Navarro, J., Sánchez, P., Río, J.L. Del, 2016. Assessing the trophic ecology of three sympatric squid in the marine ecosystem off the Patagonian Shelf by combining stomach content and stable isotopic analyses. Mar. Biol. Res. 12, 402–411.

Rosas-luis, R., Villanueva, R., Sánchez, P., 2014. Trophic habits of the Ommastrephid squid *Illex coindetii* and *Todarodes sagittatus* in the northwestern Mediterranean Sea. Fish. Res. 152, 21–28.

Rosenblatt, A., Heithaus, M., Mather, M., Matich, P., Nifong, J., Ripple, W., Silliman, B., 2013. The roles of large top predators in coastal ecosystems: New insights from long term ecological research. Oceanography 26, 156–167.

Ross, S.T., 1977. Patterns of resource partitioning in sea robins (Pisces: triglidae). Copeia 561–571.

Ross, S.W., Quattrini, A.M., Roa-Varon, A.Y., McClain, J.P., 2010. Species composition and distributions of mesopelagic fishes over the slope of the north-central Gulf of Mexico. Deep-Sea Res. II 57, 1926–1956.

Rossberg, A. G., Farnsworth, K. D., 2011. Simplification of structured population dynamics in complex ecological communities. Theor. Ecology 4(4), 449–465.

Rozas, L.P., Odum, W.E., 1988. Occupation of submerged aquatic vegetation by fishes. Test. Roles Food Refug. 77, 101–106.

Sabatès, A., Gili, J.M., Pagès, F., 1989. Relationship between zooplankton distribution, geographic characteristics and hydrographic patterns off the Catalan coast (Western Mediterranean). Mar. Biol. 103, 153–159.

Saglam, H., Bascinar, N.S., 2008. Feeding ecology of thornback ray (*Raja clavata* Linnaeus, 1758) on the Turkish coast of the south-eastern Black Sea. Mar. Biol. Res. 4, 451–457.

Sahling, H., Galkin, S.V., Salyuk, A., Greinert, J., Foerstel, H., Piepenburg, D., Suess, E., 2003. Depth-related structure and ecological significance of cold-seep communities–a case study from the Sea of Okhotsk. Deep-sea Res. I 50, 1391–1409.

Saidi, B., Bradai, M.N., Bouain, A., 2009. Reproductive biology and diet of *Mustelus punctulatus* (Risso, 1826) (Chondrichthyes: Triakidae) from the Gulf of Gabes, central Mediterranean Sea. Sci. Mar. 73 (2), 249–258.

Sala, E., Ballesteros, E., 1997. Partitioning of space and food resources by three fish of the genus *Diplodus* (Sparidae) in a Mediterranean rocky infralittoral ecosystem. Mar. Ecol. Prog. Ser. 152, 273–283.

Sánchez, P., 1986. Distribucion batimetrica y abundancia de algunos cefalopodos del mar Catalan. Investig. Pesq. 50, 237–245.

Sánchez, P., Belcari, P., Sartor, P., 1998. Composition and spatial distribution of cephalopods in two north-western Mediterranean areas. South African J. Mar. Sci. 20, 17–24.

Sardou, J., Etienne, M., Andersen, V., 1996. Seasonal abundance and vertical distributions of macroplankton and micronekton in the Northwestern Mediterranean Sea. Oceanol. Acta 19, 645–656.

Schoener, T.W., 1970. Nonsynchronous spatial overlap of lizards in patchy habitats. Ecology 51, 408.

Schoener, T.W., 1974. Resource Partitioning in Ecological Communities. Science 185, 27–39.

Seibel, B.A, 2007. On the depth and scale of metabolic rate variation: scaling of oxygen consumption rates and enzymatic activity in the Class Cephalopoda (Mollusca). J. Exp. Biol. 210, 1–11.

Seibel, B.A., Goffredi, S.K., Thuesen, E. V., Childress, J.J., Robison, B.H., 2004. Ammonium content and buoyancy in midwater cephalopods. J. Exp. Mar. Bio. Ecol. 313, 375–387.

Serrano, A., Velasco, F., Olaso, I., 2003. Polychaete annelids in the diet of demersal fish from the southern shelf of the Bay of Biscay. J. Mar. Biol. Ass. U.K. 83, 619–623.

Shreeve, R., Collins, M., Tarling, G., Main, C., Ward, P., Johnston, N., 2009. Feeding ecology of myctophid fishes in the northern Scotia Sea. Mar. Ecol. Prog. Ser. 386, 221–236.

Sifner, S., Vrgoc, N., 2004. Population structure, maturation and reproduction of the European squid, *Loligo vulgaris*, in the Central Adriatic Sea. Fish. Res. 69, 239–249.

Simão, D.S., Torres, A.P., Olivar, M.P., Abelló, P., 2014. Vertical and temporal distribution of pelagic decapod crustaceans over the shelf-break and middle slope in two contrasting zones around Mallorca (western Mediterranean Sea). J. Mar. Syst. 138, 139–149.

Simpfendorfer, C.A., Heupel, M.R., White, W.T., Dulvy, N.K., 2011. The importance of research and public opinion to conservation management of sharks and rays: A synthesis. Mar. Freshw. Res. 62, 518–527.

Smith, C.R., Berelson, W., DeMaster, D.J., Dobbs, F.C., Hammond, D., Hoover, D.J., Pope, R.H., Stephens, M., 1997. Latitudinal variations in benthic processes in the abyssal equatorial Pacific: control by biogenic particle flux. Deep-Sea Res. II 44, 2295–2317.

Smith, C.R., Mincks, S., DeMaster, D.J., 2006. A synthesis of bentho-pelagic coupling on the Antarctic shelf: Food banks, ecosystem inertia and global climate change. Deep-Sea Res. II 53, 875–894.

Smith, J.M., Macleod, C.D., Valavanis, V., Hastie, L., Valinassab, T., Bailey, N., Santos, M.B., Pierce, G.J., 2013. Habitat and distribution of post-recruit life stages of the squid *Loligo forbesii*. Deep-Sea Res. II 95, 145–159.

Sokolova, M.N., 1997. Trophic structure of abyssal macrobenthos. Adv. Mar. Biol. 32, 427–525.

Spitz, J., Mourocq, E., Schoen, V., Ridoux, V., 2010. Proximate composition and energy content of forage species from the Bay of Biscay: High- or low-quality food? ICES J. Mar. Sci. 67, 909–915.

Stefanescu, C., Cartes, J., 1992. Benthopelagic habits of adult specimens of *Lampanyctus crocodilus* (Risso, 1810) (Osteichthyes, Myctophidae) in the western Mediterranean deep slope. Sci. Mar. 56, 69–74.

Stergiou, K.I., Karpouzi, V.S., 2002. Feeding habits and trophic levels of Mediterranean fish. Rev. Fish Biol. Fish. 11, 217–254.

Stevens, J.D., Bonfil, R., Dulvy, N.K., Walker, P.A., 2000. The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. ICES J. Mar. Sci. 57, 476–494.

Stoner, A.W., Zimmerman, R.J., 1988. Food pathways associated with penaeid shrimps in a mangrove-fringed estuary. Fish. Bull. 86 (3), 543–552.

Stowasser, G., 2004. Squid and their prey: insights from fatty acid and stable isotope analysis. PhD dissertation, University of Aberdeen, Aberdeen, UK.

Stowasser, G., Pierce, G.J., Moffat, C.F., Collins, M.A., Forsythe, J.W., 2006. Experimental study on the effect of diet on fatty acid and stable isotope profiles of the squid *Lolliguncula brevis*. J. Exp. Mar. Bio. Ecol. 333 (1), 97–114.

Sugisaki, H., Tsuda, A., 1995. Nitrogen and carbon stable isotopic ecology in the ocean: The transportation of organic materials through the food web. In: Sakai, H., Nozaki, Y. (Eds.), Biogeochemical Processes and Ocean Flux in the Western Pacific. Terrapub, Tokio, pp. 307–317.

Suntsov, A.V., Brodeur, R.D., 2008. Trophic ecology of three dominant myctophid species in the northern California Current region. Mar. Ecol. Prog. Ser. 373, 81–96.

Sutton, T.T., 2005. Trophic ecology of the deep-sea fish *Malacosteus niger* (Pisces: Stomiidae): An enigmatic feeding ecology to facilitate a unique visual system? Deep-Sea Res. I 52, 2065–2076.

Sweeting, C.J., Barry, J., Barnes, C., Polunin, N.V.C., Jennings, S., 2007a. Effects of body size and environment on diet-tissue $\delta^{15}N$ fractionation in fishes. J. Exp. Mar. Biol. Ecol. 340, 1–10.

Sweeting, C.J., Barry, J.T., Polunin, N.V.C., Jennings, S., 2007b. Effects of body size and environment on diet-tissue $\delta^{13}C$ fractionation in fishes. J. Exp. Mar. Biol. Ecol. 352, 165–176.

Sweeting, C.J., Polunin, N.V.C., Jennings, S., 2006. Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of sh tissues. Rapid Commun. Mass Spectrom. 20, 595–601.

Takai, N., Sakamoto, W., 1999. Identification of local populations of Lake Biwa catfish *Silurus biwaensis* in Japan on the basis of $\delta^{13}C$ and $\delta^{15}N$ analyses. Can. J. Zool. 77, 258–266.

Tecchio, S., Coll, M., Christensen, V., Company, J.B., Ramírez-Llodra, E., Sarda, F., 2013. Food web structure and vulnerability of a deep-sea ecosystem in the NW Mediterranean Sea. Deep. Res. Part I Oceanogr. Res. Pap. 75, 1–15.

Tecchio, S., van Oevelen, D., Soetaert, K., Navarro, J., Ramírez-Llodra, E., 2013. Trophic dynamics of deep-sea megabenthos are mediated by surface productivity. PLoS One 8, e63796.

ter Braak C.J.F., Smilauer, P., 1998. CANOCO reference manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination. Microcomputer Power, Ithaca, New York.

Thrush, S.F., Dayton, P.K., 2010. What Can Ecology Contribute to Ecosystem–Based Management? Annu. Rev. Mar. Sci. 2, 419–441.

Tinker, M.T., Bentall, G., Estes, J.A., 2008. Food limitation leads to behavioral diversification and dietary specialization in sea otters. Proc. Natl. Acad. Sci. U.S.A. 105, 560–565.

Treberg, J.R., Speers-roesch, B., 2016. Does the physiology of chondrichthyan fishes constrain their distribution in the deep sea? J. Exp. Biol. 219, 615–625.

Trueman, C.N., Johnston, G., O'Hea, B., MacKenzie, K.M., 2014. Trophic interactions of fish communities at midwater depths enhance long-term carbon storage and benthic production on continental slopes. Proc. R. Soc. B 281, 20140669.

Trueman, C.N., McGill, R.A.R., Guyard, P.H., 2005. The effect of growth rate on tissue-diet isotopic spacing in rapidly growing animals. An experimental study with Atlantic salmon (*Salmo salar*). Rapid Commun. Mass Sp. 19, 3239–3247.

Tsagarakis, K., Coll, M., Giannoulaki, M., Somarakis, S., Papaconstantinou, C., Machias, A., 2010. Food-web traits of the North Aegean Sea ecosystem (Eastern Mediterranean) and comparison with other Mediterranean ecosystems. Estuar. Coast. Shelf Sci. 233–248.

Valls, E., Navarro, J., Barría, C., Coll, M., Ferández-Borrás, J., Rotllant, G., 2016. Seasonal, ontogenetic and sexual changes in lipid metabolism of the small-spotted catshark (*Scyliorhinus canicula*) in deep-sea free-living conditions. J. Exp. Mar. Bio. Ecol. 483, 59–63.

Valls, M., Cabanellas-Reboredo, M., Uranga, I., Quetglas, A., 2015. Feeding ecology of two squid species from the western Mediterranean. Mar. Ecol. Prog. Ser. 531, 207–219.

Valls, M., Olivar, M.P., Fernández de Puelles, M.L., Molí, B., Bernal, A., Sweeting, C.J., 2014a. Trophic structure of mesopelagic fishes in the western Mediterranean based on stable isotopes of carbon and nitrogen. J. Mar. Syst. 138, 160–170.

Valls, M., Quetglas, A., Ordines, F., Moranta, J., 2011. Feeding ecology of demersal elasmobranchs from the shelf and slope off the Balearic Sea (western Mediterranean). Sci. Mar. 75, 633–639.

Valls, M., Sweeting, C.J., Olivar, M.P., Fernández de Puelles, M.L., Pasqual, C., Polunin, N.V.C., Quetglas, A., 2014b. Structure and dynamics of food webs in the water column on shelf and slope grounds of the western Mediterranean. J. Mar. Syst. 138, 171–181.

Vander Zanden, M.J., Cabana, G., Rasmussen, J.B., 1997. Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (δ^{15}N) and literature dietary data. Can. J. Fish. Aquat. Sci. 54, 1142–1158.

VanderZanden, M.J., Shuter, B.J., Lester, N., Rasmussen, J.B., 1999. Patterns of food chain length in lakes: A stable isotope study. Am. Nat. 154 (4), 406–416.

Vander Zanden, M.J., Rasmussen, J.B., 2001. Variation in δ^{15}N and δ^{13}N trophic fractionation: Implications for aquatic food web studies. Limnol. Oceanogr. 46, 2061–2066.

Vasilakopoulos, P., Maravelias, C.D., Tserpes, G., 2014. The alarming decline of mediterranean fish stocks. Curr. Biol. 24, 1643–1648.

Vaudo, J.J., Heithaus, M.R., 2011. Dietary niche overlap in a nearshore elasmobranch mesopredator community. Mar. Ecol. Prog. Ser. 425, 247–260.

Vinogradov, V.I., Noskov, A.S., 1979. Feeding of short-finned squid, *Illex illecebrosus*, and long-finned squid, *Loligo pealei*, off Nova Scotia and New England, 1974-1975. ICNAF Sel. Pap. 5, 31–36.

Vovk, A.N., 1985. Feeding spectrum of longfin squid (*Loligo pealei*) in the northwest Atlantic and its position in the ecosystem. Northwest Atl. Fish. Org. Sci. Counc. Stud. 8, 33–38.

Wallace, R.K. - 1981. An assessment of diet-overlap indexes. Trans. Am. Fish. Soc. 110, 72–76.

Watanabe, H., Kawaguchi, K., Hayashi, A., 2002. Feeding habits of juvenile surface-migratory myctophid fishes (family Myctophidae) in the Kuroshio region of the western North Pacific. Mar. Ecol. Prog. Ser. 236, 263–272.

Wangvoralak, S., Hastie, L.C., Pierce, G.J., 2011. Temporal and ontogenetic variation in the diet of squid (*Loligo forbesii* Streenstrup) in Scottish waters. Hydrobiologia 670, 223–240.

Watts, M.C., Etter, R.J., Rex, M.A., 1992. Effects of spatial and temporal scale on the relationship of surface pigment biomass to community structure in the deep-sea benthos. In: Rowe G.T., Pariente, V. (Eds.), Deep-sea food chains and the global carbon cyclying. Kluwer Academic Publishers, Dordrecht, pp 245–254.

Werner, E.E., Gilliam, J.F., 1984. The Ontogenetic Niche and Species Interactions in Size-Structured Populations. Annu. Rev. Ecol. Syst. 15, 393–425.

Wetherbee, B.M., Cortes, E., 2004. Food consumption and feeding habits. In: Carrier J.C., Musick J.A., Heithaus, M.R. (Eds.), Biology of Sharks and Their Relatives. CRC Press, Boca Raton, pp. 225–246.

Wetherbee, B.M., Cortés, E., Bizzarro, J.J., 2012. Food Consumption and Feeding Habits, in: Biology of Sharks and Their Relatives, Second Edition.

Wetherbee, B.M., Nichols, P.D., 2000. Lipid composition of the liver oil of deep-sea sharks from the Chatham Rise, New Zealand. Comp. Biochem. Physiol. - B Biochem. Mol. Biol. 125, 511–521.

Williams, I.D., Polunin, N.V.C., Hendrick, V.J., 2001. Limits to grazing by herbivorous fishes and the impact of low coral cover on macroalgal abundance on a coral reef in Belize. Mar. Ecol. Prog. Ser. 222, 187–196.

Winemiller, K.O., Akin, S., Zeug, S.C., 2007. Production sources and food web structure of a temperate tidal estuary: integration of dietary and stable isotope data. Mar. Ecol. Prog. Ser. 343, 63–76.

Worms, J., 1979. L'utilisation des prises commerciales en biologie des pêches. Application á l'étude d'une population de *Loligo vulgaris* (Cephalopoda, Teuthoidea) du Golfe du Lion. PhD dissertation, University of Montpelier, France.

Wurtz, M., Giuffra, E., 1989. Reproduzione e accrescimiento di *Loligo vulgaris* (Cephalopoda, Myopsida) in Mar Ligure. Oebalia 152, 831–834.

Wurtz, M., Vacchi, M., 1978. Ricerca di cicli nictemerali nel-l'alimentazione di selaci batiali. Quad. Lab. Tecnol. Pesca 3 (Suppl.), 155–164.

Yahel, G., Yahel, R., Katz, T., Lazar, B., Herut, B., Tunnicliffe, V., 2008. Fish activity: a major mechanism for sediment resuspension and organic matter remineralization in coastal marine sediments. Mar. Ecol. Prog. Ser. 372, 195–209.

Yoon, W.D., Nival, P., Choe, S.M., Picheral, M., Gorsky, G., 2007. Vertical distribution and nutritional behaviour of *Cyclothone braueri*, *Nematoscelis megalops*, *Meganyctiphanes norvegica* and *Salpa fusiformis* in the NW Mediterranean mesopelagic zone. ICES CM 2007/F: 03, 1–28.

Young, J. W., Lansdell, M. J., Campbell, R. A., Cooper, S. P., Juanes, F., Guest, M. A., 2010. Feeding ecology and niche segregation in oceanic top predators off eastern Australia. Mar. Biol. 157 (11), 2347–2368.

Young, J.W., Hunt, B.P. V, Cook, T.R., Llopiz, J.K., Hazen, E.L., Pethybridge, H.R., Ceccarelli, D., Lorrain, A., Olson, R.J., Allain, V., Menkes, C., Patterson, T., Nicol, S., Lehodey, P., Kloser, R.J., Arrizabalaga, H., Anela Choy, C., 2015. The trophodynamics of marine top predators: Current knowledge, recent advances and challenges. Deep. Res. Part II Top. Stud. Oceanogr. 113, 170–187.

Young, J.W., Olson, R.J., Rodhouse, P.G.K., 2013. The role of squids in pelagic ecosystems: An overview. Deep. Res. Part II Top. Stud. Oceanogr. 95, 3–6.

Zariquiey, R., 1968. Crustáceos Decápodos Ibéricos. Inv. Pesq. 32, 1–510.