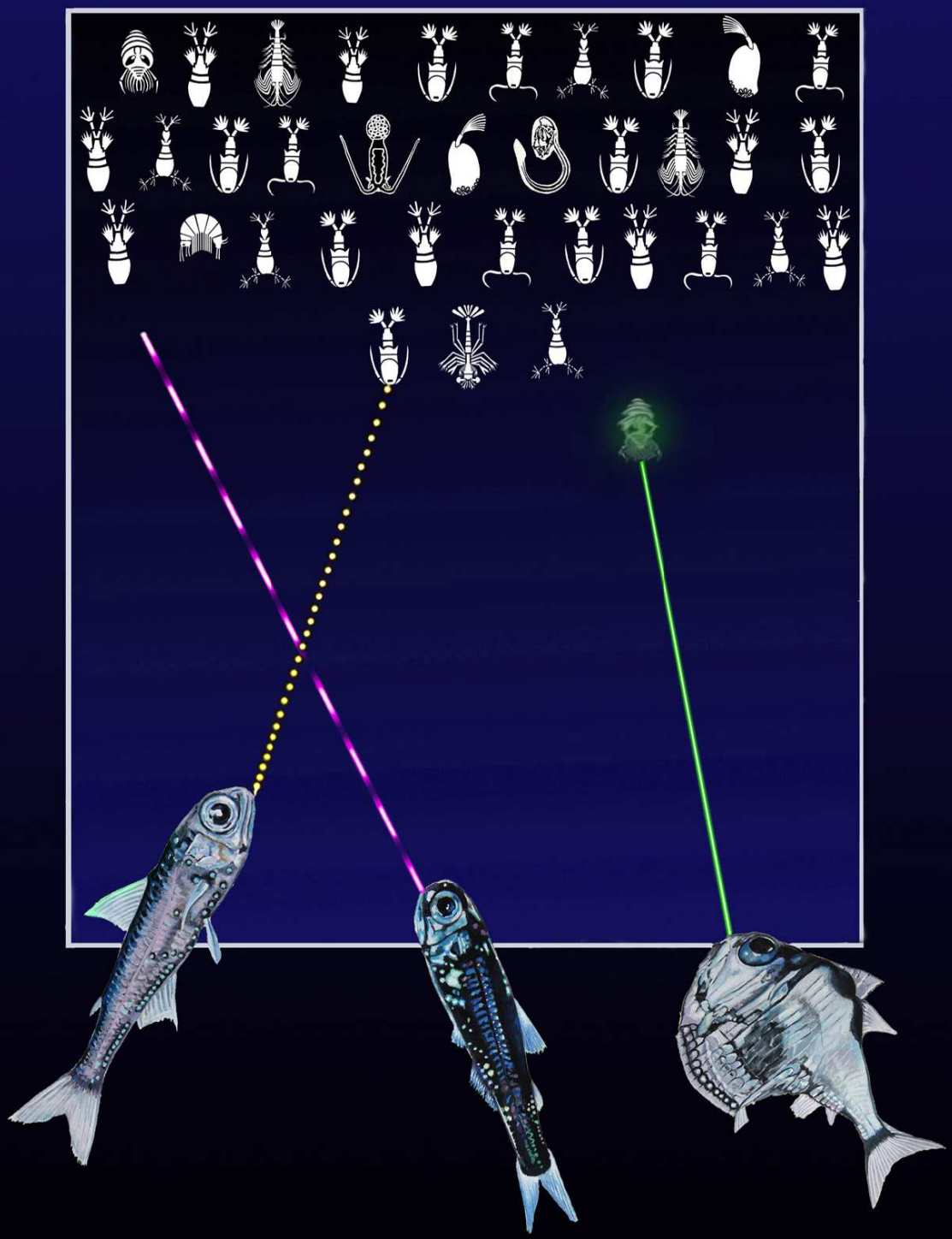
Feeding ecology and community structure of mesopelagic fishes in the western Mediterranean



Ainhoa Bernal Bajo

PhD thesis

FEEDING ECOLOGY AND COMMUNITY STRUCTURE OF MESOPELAGIC FISHES IN THE WESTERN MEDITERRANEAN

Ecología trófica y estructura de la comunidad de peces mesopelágicos del Mediterráneo Occidental

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A Sergi, siempre incondicional.

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La mer est ton miroir; tu contemples ton âme
Dans le déroulement infini de sa lame,
Et ton esprit n'est pas un gouffre moins amer.

- Charles Baudelaire (L'Home et la mer, 1857)

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Summary

Mesopelagic fish populations from the orders Myctophiformes and Stomiiformes were investigated to determine the potential effects of physical and biological factors on their trophic ecology, and characterize the ecological role that have in an oligotrophic system such as the western Mediterranean. The relevance of the mesopelagic fish community is connected to the great biomass that they represent worldwide, constituting common dietary resources for large pelagic fishes of commercial interest, marine birds and marine mammals. Myctophids are also key components of the trophic webs since they perform extensive diel vertical migrations throughout the water column, acting as vectors of matter and energy fluxes.

This study was developed in a region off the Balearic Islands during two seasonal periods, late autumn (water mixing period) and summer (stratification of the water column). The trophic behaviour and vertical distribution of mesopelagic fishes were analysed, and ontogenetic dietary shifts from young individuals to adulthood were described. These aspects have been hardly reported in juveniles and adults of Mediterranean mesopelagic fishes. The present study comprises the most abundant and frequent species of myctophids (*Ceratoscopelus maderensis, Notoscopelus elongatus, Benthosema glaciale, Hygophum hygomii, H. benoiti, Lampanyctus crocodilus, L. pusillus, Lobianchia dofleini,* and *Myctophum punctatum*) and stomiiforms (mainly, the gonostomatid *Cyclothone braueri* and the sternoptychid *Argyropelecus hemigymnus*) of the Mediterranean Sea. The first objective of this thesis was to confirm whether the classical larval identification based on morphological and pigmentation characters, for both myctophids and stomiiforms, was supported by using *barcoding* genetic techniques.

The target species were assigned to two contrasting morphotypes and behavioural dynamics. *C. braueri* has a slender body shape, which could be related with lethargic conducts, since it does not migrate to the surface, whereas migratory myctophids are characterised by a robust musculature and osteological development.

Accordingly with the general pattern observed in other ocean regions, the Mediterranean myctophids stay at deep-water strata during the day and start their diel ascension at dusk, aggregating in the near-surface layers for foraging.

The calculation of the feeding incidence during day and night revealed that adults of most myctophid species were nocturnal feeders, coinciding with the day period in which zooplankton is concentrated in the first hundred metres of the surface. Nevertheless, the stomiiformes *C. braueri* and *A. hemigymus* showed intermittent feeding across day and night-time. It was also observed high feeding incidence at daytime for the oldest and non-migratory individuals of *L. crocodilus*.

This study supports the hypothesis of a night vertical ascension of most myctophid species/individuals towards the epipelagic waters conditioned by the nycthemeral routines of zooplankton and micronekton that myctophids feed on. The implementation of acoustic techniques, by first time in the Mediterranean Sea, to detect mesopelagic organisms, allowed the observation of dense aggregations of myctophids and other organisms near the surface and at ca. 400 m depth (*Deep Scattering Layer*). However, not all the specimens of the myctophid populations responded to this migratory behavior. The oldest stages of *N. elongatus* and *L. crocodilus* were an exception to this pattern, and remain associated to the deepest layers of the water column.

The dietary patterns and shifts throughout the whole ontogenetic development were reported for the myctophid *L. pusillus*, a frequent but barely studied species in the western Mediterranean region. Its larvae were very voracious in comparison with other similar pelagic larvae from the Mediterranean. It was observed, for *L. pusillus*, an ontogenetic trend towards the selection of larger or more nutritive prey such as euphausiids, or certain organisms that could be more easily captured as a result of an improvement of the swimming skills and catchability.

This study has shown that mesopelagic fishes are mainly zooplanktivorous and feed on a wide range of taxa of meso and macrozooplankton. Calanoid copepods were basically the major diet component in most species in terms of number. However, euphausiids, and occasionally, small fishes, were important prey in terms of biomass and carbon content for the adult stages of myctophids.

Despite the considerable dietary overlap among the Mediterranean mesopelagic fishes, the adult individuals of *L. pusillus*, *N. elongatus*, *Hygophum benoiti* and *C. braueri*, showed relatively high prey selectivity. Prey number was quite variable among species, reaching the maximum numbers for the generalist

species *M. punctatum*, whilst, on the contrary, *C. braueri* was characterized by low prey ingestion and high vacuity of the stomach.

Myctophids and stomiiforms showed an increment of both mean carbon content and prey size in their stomachs throughout development, despite non-significant trends were observed of their trophic niche breadths with growth.

Seasonal changes did not showed significant effects on the feeding patterns of mesopelagic fishes. However, it was observed a dissimilar contribution of some prey groups to the diets of the most generalist fish species (e.g. *C. maderensis*) between autumn and summer, which reflects seasonal variations of these prey groups in the pelagic environment.

The data on the feeding patterns and diet composition of the fish species studied in this thesis were highly valuable, since they could be required for modeling some ecological aspects of the biological communities in the western Mediterranean. Moreover, the results of fish diets and vertical distributions are of importance in the study of the downward transport of organic matter throughout the water column, which is widely unknown at global scale.

Resumen

En esta tesis doctoral se ha estudiado la relación de las poblaciones de peces mesopelágicos pertenecientes a los órdenes Myctophiformes y Stomiiformes con el medio pelágico, analizando el posible efecto de factores físicos y biológicos en su ecología trófica, y caracterizando su papel ecológico en un ecosistema oligotrófico de la cuenca occidental del Mar Mediterráneo. La importancia de estos peces mesopelágicos subyace a su elevada biomasa en todos los océanos, constituyendo una base alimenticia para peces pelágicos de carácter comercial, así como aves y mamíferos marinos. Los mictófidos son elementos claves de las cadenas tróficas al efectuar extensas migraciones diarias verticales, actuando como vectores de flujo de materia orgánica y energía.

El presente estudio se desarrolló en las proximidades del archipiélago balear en dos períodos, final del otoño (período de mezcla de la columna de agua) y en verano (estratificación). Se ha investigado el comportamiento trófico y la distribución vertical de peces mesopelágicos, describiendo los cambios en la dieta a lo largo del desarrollo ontogénico. Estos aspectos han sido apenas estudiados en juveniles y adultos de los peces mesopelágicos en el Mediterráneo occidental. El estudio abarca las especies más abundantes y frecuentes de mictófidos (*Ceratoscopelus maderensis, Notoscopelus elongatus, Benthosema glaciale, Hygophum hygomii, H. benoiti, Lampanyctus crocodilus, L. pusillus, Lobianchia dofleini, y Myctophum punctatum*) y stomiiformes (principalmente el gonostomátido *Cyclothone braueri* y el sternoptíquido *Argyropelecus hemigymnus*). El primer objetivo de esta tesis consistió en comprobar si la clásica identificación de estadios larvarios de mictófidos y stomiiformes en base a caracteres morfológicos y de pigmentación era confirmada utilizando técnicas moleculares de *barcoding*.

Las especies objeto de estudio responden a principalmente a dos morfotipos y dinámicas diferentes. El cuerpo de *C. braueri* tiene una morfología estilizada y grácil que podría relacionarse con un comportamiento letárgico, a diferencia de las especies de mictófidos que realizan migraciones verticales diarias, lo que adaptativamente requiere una musculatura y un desarrollo osteológico más robustos.

Siguiendo el patrón general observado en otras regiones y océanos, durante el día, los mictófidos del mediterráneo occidental permanecen en aguas profundas

y comienzan a ascender al atardecer y a lo largo de la noche, concentrándose en las capas superficiales para alimentarse. El cálculo de la incidencia alimentaria diurna y nocturna determinó que los adultos de todas las especies de mictófidos tienen hábitos principalmente nocturnos, coincidiendo con el momento en que el plancton está más concentrado en la superficie. En cambio, los stomiiformes *C. braueri* y *A. hemigymus* mostraron hábitos de alimentación intermitente tanto de día como de noche. También se observó alta incidencia alimentaria en los individuos adultos de más edad y no migradores de *L. crocodilus*, los cuales capturan sus presas cerca del fondo adecuándose a hábitos bentopelágicos.

Este estudio apoya la hipótesis de una ascensión vertical de la mayoría de especies o individuos de peces mesopelágicos, hacia las aguas superficiales, condicionada por el régimen nictimeral del zooplancton y micronecton del cual se alimentan. La utilización de técnicas acústicas para la detección de estos organismos mesopelágicos, por primera vez en el Mar Mediterráneo, permitió observar la formación de densas agregaciones de mictófidos y otros organismos en la superficie y próximas a los 400 m de profundidad (capa de reflexión profunda), aunque no todos los individuos de la población respondieron a esta dinámica migratoria. Los estadios de mayor edad de *N. elongatus* y *L. crocodilus* constituyeron una excepción a este patrón, permaneciendo asociados a las capas más profundas de la columna de agua.

Se ha encontrado una elevada heterogeneidad en la composición dietética intraspecífica junto a un amplio solapamiento interespecífico. La mayor parte de las especies exhibieron estrategias de alimentación mixtas con un patrón generalista, pero revelando un cierto grado de selectividad hacia determinados tipos de presa en función de su morfología y tamaño.

El estudio de la dieta se completó a lo largo de todo el ciclo vital de *L. pusillus*, puesto que es una especie muy frecuente en la cuenca mediterránea de hábitos desconocidos. Los resultados revelaron que se trata de uno de los mictófidos más voraces durante el estadio larvario en comparación con otras larvas pelágicas. Se observó una tendencia ontogénica hacia la selección de presas de mayor valor energético, como los eufausiáceos, o cuya captura supone una menor inversión de energía (organismos luminiscentes como ostrácodos o el copépodo *Pleuromamma*), dada la mayor habilidad natatoria y de captura de *L. pusillus*.

Este estudio ha mostrado como los peces mesopelágicos son depredadores que se alimentan de un amplio espectro de taxones del meso y macrozooplancton. Los copépodos calanoides constituyeron prácticamente la base dietética de todos los peces mesopelágicos en términos de abundancia numérica. Sin embargo, los eufausiáceos, y ocasionalmente, pequeños peces cercanos taxonómicamente, fueron presas importantes en términos de biomasa en los estadios adultos de mictófidos.

A pesar de la tendencia al solapamiento de dietas, los adultos de algunas especies como el *L. pusillus, N. elongatus, H. benoiti, C. braueri,* mostraron una dieta más selectiva que otros mictófidos. El número de presas también fue muy variable entre especies, alcanzando valores muy altos en las especie generalista *M. punctatum,* mientras que en el extremo opuesto, *C. braueri* se caracteriza por baja ingestión de presas y alta vacuidad.

Desde el punto de vista ontogénico, mictófidos y stomiiformes adultos aumentaron el contenido medio en carbono, y el tamaño de las presas, respecto a los estadios juveniles, a pesar de que no se observaron tendencias en la amplitud de sus nichos tróficos con la edad.

La variación de las condiciones estacionales no mostró un efecto directo sobre la alimentación de peces mesopelágicos. Sin embargo, se puede observar una contribución diferencial entre otoño y verano a la dieta de los depredadores más generalistas (ej. *C. maderensis*) por parte de ciertos tipos de presa, reflejando así la variación estacional en el medio pelágico de dichas presas.

Los datos específicos de la dieta de las especies estudiadas en esta tesis son valiosos para la aplicación de modelos ecológicos en el Mediterráneo, los cuales carecen de datos suficientes sobre la comunidad de peces mesopelágicos. Por otro lado, los datos dietéticos y de distribuciones verticales son de gran importancia en el estudio del transporte de materia orgánica a través de la columna de agua, aspecto que es ampliamente desconocido a escala global.

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Part I

GENERAL

INTRODUCTION

Environmental characterization of the study area (western Mediterranean)

The Mediterranean Sea has been fairly valued by the exceptional advantages offered to humans, as its waters are easily navigable and the surrounding coastline displays a variety of cultures that make the most of its gentle weather and the exploitation of biological resources. The last decades have seen an explosion in the growth of scientific knowledge, expanding towards the more unexplored offshore and deep waters far apart from the over-populated coasts. It is the less-known open ocean which constitutes the context of the present study. The topography of the Mediterranean basin is logically connected to some physical and hydrological phenomena that explain certain dynamics of its marine ecosystem. This sea has been often conceived as a "small-scale ocean", as it reproduces, on a small scale, certain phenomena that take place in the Atlantic or Pacific Oceans.

The Mediterranean (MS) is a semi-enclosed sea located in a temperate region (Fig. 1) and composed by a string of basins that determine an enlarged shape over the west-east axis. It is exclusively connected by the western- and easternmost ends with the Atlantic Ocean and the Red Sea, through the Strait of Gibraltar and the narrow Suez Channel, respectively. The water exchange through the Strait of Gibraltar is outstandingly greater than that through the Suez Channel with the Indian Ocean. A submarine ridge, extending under the Strait of Sicilia to the Tunisian coast, separates the western Mediterranean (WM) sub-region, which covers about 328100 m^2, from the eastern Mediterranean (Fig. 1). Three basins are confined in the western sub-region: the Algerian, the Balearic and the Tyrrhenian, each of them characterized by particular physical circumstances. The mean estimated depth of the western basin is 1612 m and the

mean surface temperature is 15.5 ºC (coast of the Gulf of Lion), but an isothermal limit of 18 ºC stretches from Gibraltar to the north of Sardinia.

The semi-enclosed condition of the Mediterranean determines it as a water-deficitary concentration basin, where the water inputs from the rivers are significantly lower than water evaporation. Water loss by evaporation is not balanced by rain and river runoffs in the annual cycle, resulting in an enhancement of the salinity (mean around 39), higher in the eastern sub-basin

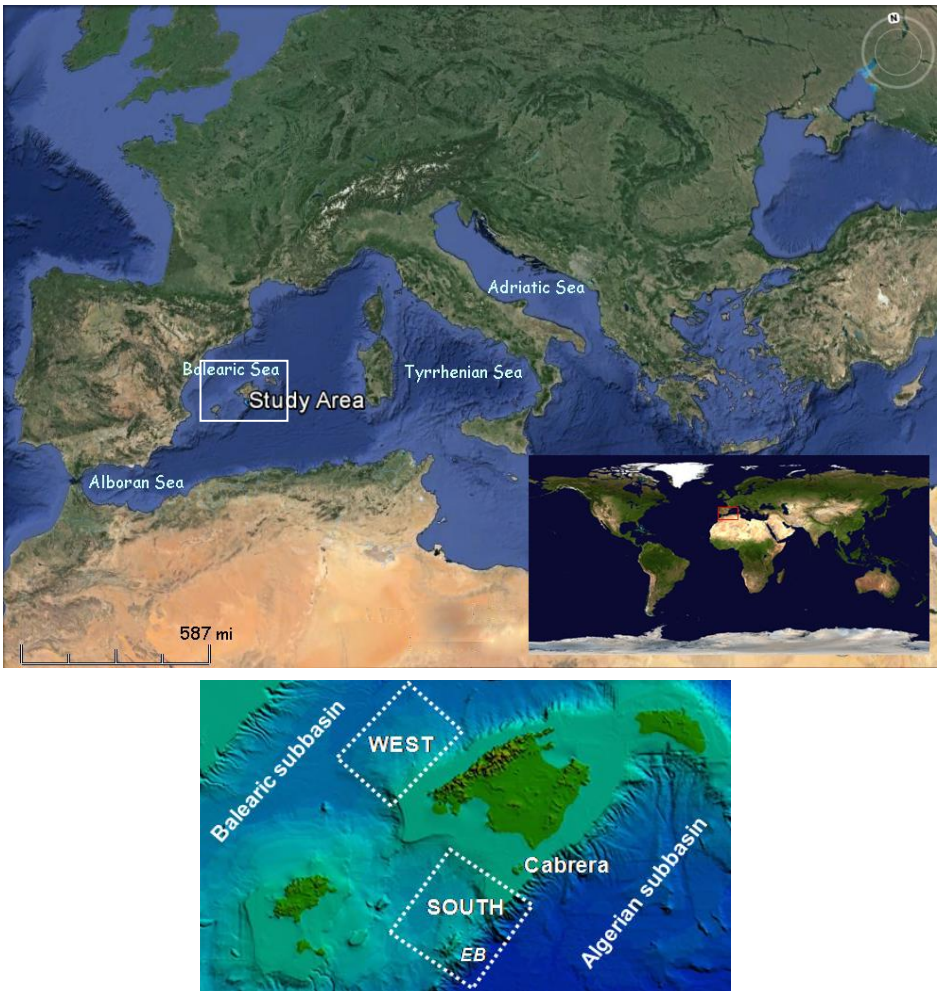


Fig. 1 Map of the Mediterranean Sea showing the area of study in the western basin. The lower image is a magnification of the two fishing grounds, on the west and south off Mallorca Island.

and lower in the western. Moreover, evaporation plays an important role in the dynamics of the MS and enhances the convection processes in the winter season, when the surface waters become warmer than the immediate air. The northern dominant winds, which used to be reported as associated to the high pressure in the NE Atlantic, have a notable incidence in some marine surfaces such as the northern areas of the Balearic archipelago, causing an increasing evaporation and cooling the superficial water masses. Thus, superficial water masses become denser and sink to the depth by convection associated with the permanent circulation of the Northern Current along the continental slope. The water masses drift southwards mixing with waters from different origins and constituting the Deep Water Current. The unbalance between evaporation and water inputs also determines a surface flux that favours the entry of Atlantic Water (AW) through the Strait of Gibraltar (Rodríguez, 1982; Salat, 1995).

Whilst in the coastal areas and continental shelf, winds are determinant factors of variability (Font, 1990), in the central area of the Balearic Islands and surroundings, which comprises the region of study, the sources of variability are

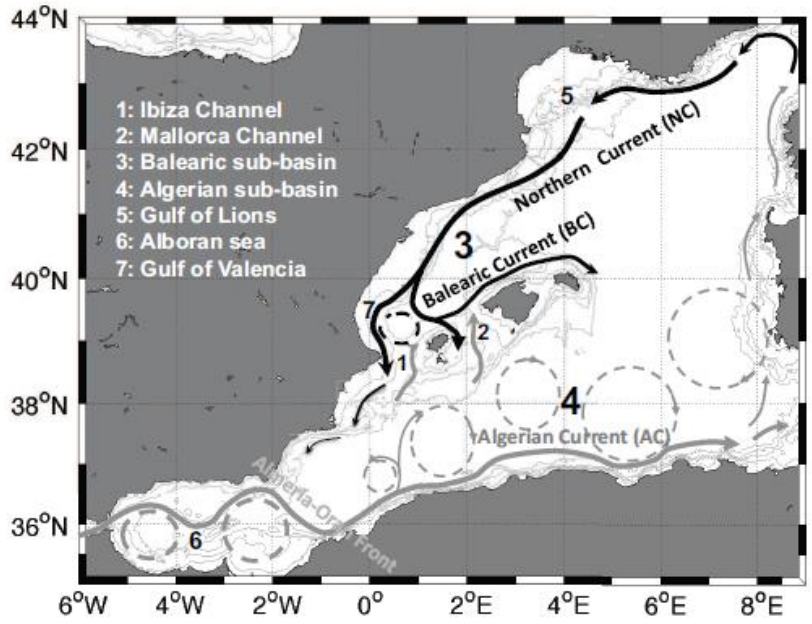


Fig. 2 The major currents characterizing the regional circulation around the Balearic Islands (From López-Jurado *et al.*, 2008).

associated with the stratification of the waters and the evolution of particular eddies and water fronts (Tintoré *et al.*, 1995). To understand the mesoscale water circulation in the study area in major detail, it is necessary to introduce some its geographic characteristics. The Balearic Islands (39° 30' N 3° 00' E / 39°50' N 3° 00' E) create a natural barrier between the Algerian and Balearic basins. Then, the continental shelf divides the Balearic basin in two parts; (1) the shelf that extents from the larger Mallorca-Menorca axis to the east, covering an extension ca. 10 times wider on the southern side, and (2) the smaller Ibiza-Formentera shelf to the west, which is over 10 times wider in the west. The slope on the western and southern sides is gentle (6º average inclination), whereas the shelf breaks abruptly (16º average inclination) towards the North and East, with a pronounced slope that ranges from 200 to 800 m in its shallowest part, to more than 2000 m at its base (Acosta *et al.*, 2003).

The circulation of the water masses in the Algerian and Balearic basins (Fig. 2) is determined by seasonal variations of the cyclonic gyre in the northern part of Mallorca (Pinot *et al.*, 2002). Both basins receive an input of superficial Atlantic Water (AW) that have different salinity and times of residence partly determining the dynamics of the water fronts; the AW input to the Algerian basin is more recent and the water-driving forces are controlled by density gradients, whilst the Balearic basin has saltier, cooler and older AW (resident waters) and its dynamics are basically regulated by atmospheric influences. The water exchange and circulation between the two basins takes place through the Ibiza and Mallorca channels. The recent AW that penetrates the Balearic channels have salinities lower than 37.5, and part of it transforms into the Balearic Current (BC), which flows upwards following the northern side of the islands, constituting a separate density front from the resident waters (Salat and Font, 1987; Salat, 1995). Thus, the Balearic Front separates old AW, present in the middle of the basin, from the less dense water transported by the BC (Salat,

1995). The southern region of the islands is influenced by gyres situated where the transitional Atlantic flows towards the Cabrera Island.

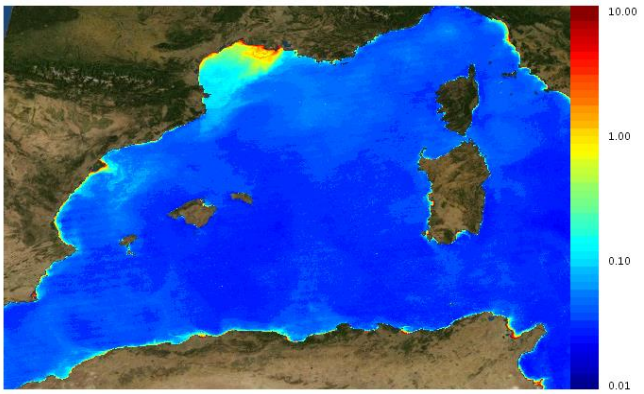
At intermediate depths appear two different water masses: 1) the Levantine Intermediate Water (LIW) originated in the eastern Mediterranean Sea and located immediately above the Deep Waters, and 2) the Winter Intermediate Water (WIW) that is originated from the gradually local cooling of the AW. The former water mass (LIW) is observed during all year-round and have a maximum relative of salinity and temperature, and the latter WIW are intermittent waters with an absolute minimum of temperature of 13 ºC. Finally, the Deep Waters are originated in the Gulf of Lion and Liguria Sea occurring in the deepest stratum of the water column. The seasonal and gradual formation of the WIW along with the local topography and influence of the AW generates mesoscale disruptive processes that alter the circulation in the Ibiza Channel. In contrast, the southern region is less affected by these processes, but is influenced by mesoscale gyres from the Algerian current that change the AW flow towards Cabrera and Menorca (López-Jurado *et al.*, 2008).

The basin hydrodynamics has thus a great repercussion on the characteristics of the Mediterranean ecosystems. As already described, the Strait of Gibraltar acts as a channel for an exchange of water of different substance concentrations: on one hand, enriched deep water outside towards the Atlantic, and on the other hand, poor superficial water gets into the MS, causing an eventual nutrient impoverishment. Additionally, during the spring, there is a major-scale shift of AW into the MS that sinks offshore in particular zones in the north of the basin (Armi and Farmer, 1988), and consequently nutrients depletion is enhanced with respect the southern part of Mallorca (Fernández de Puelles *et al.*, 2003). This has been the general explanation that scientists attributed for the oligotrophic profile of the MS. However, Huertas *et al.* (2012) elucidated that the inflow of nutrient-depleted waters is, concurrently, richer in phosphate than resident

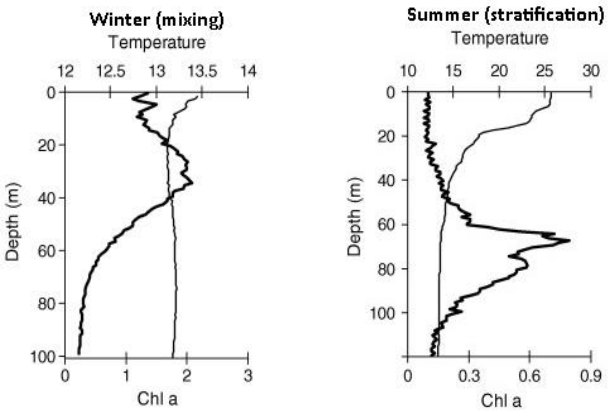
waters, and consequently the impoverishment of the MS might not come only from the AW. Minor-scale dynamics in the western Mediterranean are quite complex and still a matter of discussion (Pinardi and Masetti, 2000). The pelagic waters surrounding the Balearic Islands show a more pronounced nutrient depletion than the adjacent waters off the Iberian coast and the Gulf of Lion, as the latter receive a nutrient surplus from land runoff (Estrada, 1996; Bosc *et al.*,

Fig. 2

Above: Concentration of Chlorophyll a in the surface of the western Mediterranean Sea registered in August 2013. Units on the right: mg m^{-3}, 1 km resolution.
Source: *SeaWIFS satellite (www.myocean.u)*



Below: Temperature (ºC) and Chlorophyll a (µg/l) vertical profiles during winter and summer in the Catalan Sea (Source: Sabatés *et al.*, 2007).



2004). Frontal mesoscale events can act as an external mechanism of fertilization of the pelagic realm off the Balearic Islands (Pinot *et al.*, 1995).

The western Mediterranean dynamics are also strongly influenced by common seasonality in mid latitudes. The seasonal hydrodynamic cycle starts in winter with a vertical mixing of the water column, as a consequence of the balancing of the temperature, that permits the arrival of nutrients-enriched water to the

phytoplankton, recording the maximum of chlorophyll production at the beginning of February (Fig. 2 and 3) and an intense bloom in spring. The phytoplankton production is continuously devoured by the herbivorous plankton, thus in this sense, an apparently low primary production holds an important biomass of zooplankton. During summer the strong thermocline establishes in the first 25 m and the difference of temperature between the surface layer and the water mass underneath rounds 10º C at the end of July (Flos, 1985) (Fig. 2). This vertical stratification in the temperature gradient is notable in the upper layers during the summer (fig. 2), however the temperature will remain homogeneous under the epipelagic stratum, from ca. 200 m to the sea bottom (Würtz, 2010). The oligotrophy of the western Mediterranean increases with vertical water stratification in summer, and consequent nutrient depletion occurs in the surface layers just like the maximum depth reached in the chlorophyll profile. Therefore, winter is the productive season when the water masses of the epipelagic zone mix vertically and the maximum depth of chlorophyll a occurs near the surface to ca. 25-30 m depth, and the zooplankton biomass reaches the maximum values (Marty *et al.*, 2002).

Another issue to deal with in this introductory section refers to the pelagic domain in which the study develops. The pelagic domain of the Mediterranean basin bears a high diversity of species and endemicity. More than 8500 species of macroscopic fauna dwell in the Mediterranean Sea (Williams *et al.*, 2001), of

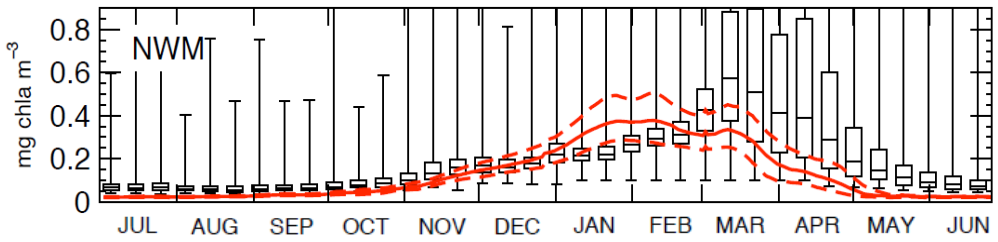


Fig. 3 Surface chlorophyll seasonal cycle (mg chl-a m^{-3}) in the North-Western Mediterranean basin (1999–2004) simulated by OPATM-BFM model (median: solid red; 25 and 75 percentile: dashed lines) and from SeaWIFs (box-plot) (Source: Lazzari *et al.*, 2012).

which ca. 30% can be considered pelagic organisms (Fredj *et al.*, 1992). In the small patch of the overall ocean extension that the Mediterranean represents, it houses about 5% of the marine fish species worldwide (Bianchi and Morri, 2000). In general, the animal biomass in the open ocean where depths are beyond 1000 m has very low values under 5% of the numerical biomass reported between 0 and 200 m (Rodríguez, 1982). The farther from the surface layer, the lower that biomass values become.

From the historical aspect, the biodiversity in the Mediterranean is linked to several serial events of colonization in different times its life. The evolutionary radiation of marine species in the MS is related to the succession of rapid geological events across the Tertiary, with the origins of some endemic components in the Pliocene, along with the spectacular entry of diversity in the post-Pliocene from the Atlantic Ocean. A precursor of the current Strait of Gibraltar closed tight and an evaporitic drawdown and subsequent desiccation of the Mediterranean basins occurred during the Messinian salinity crisis. Within a millennium the Mediterranean basin nearly completely dried out, until the Strait of Gibraltar finally reopened with the Zanclean flood. Sucesive hydrographic processes leaded eventually to an oligotrophic condition (Azov, 1991).

The poor primary productivity characteristic of this Mediterranean and other pelagic domains does not yet mean a reduction of the ecological links among their components. On the contrary, environmental factors define an intricate community structure, where interactions from the planktonic organisms to the megafauna (Etnoyer *et al.*, 2004) regulate the ecosystem balance. Low productivity is not that low comparing with the raw productivity worldwide, and it has been highlighted the relatively high productivity of this sea for a nutrient-empoverished ecosystem (Sournia, 1973).

Organic fluxes in the mesopelagic zone

The open sea or pelagic domain is vertically divided into five horizontal layers from the epipelagic zone, where basically occurs all the primary production, to the deepest hadalpelagic zone (from about 6000 m; Fig. 4). In the western Mediterranean the pelagic domain does not reach such depths and the deepest layer corresponds to the bathypelagic zone (1000-2000 m), where the only light comes from the bioluminescent organisms. The mesopelagial embraces the environment that underlies the epipelagic zone (ca. 200-1000 m depth), holding zooplankton populations and large pelagic organisms commonly known as nekton. Despite some light penetrates in the mesopelagic stratum, it is insufficient for photosynthetic processes at the same time that becomes gradually depleted of oxygen towards deeper waters. In temperate latitudes, as already stated, pelagic organisms are affected by the seasonal cycle of mixing and stratification of the water column. Seasonal fluctuations become less intense gradually in the mesopelagic zone than in surface layers, especially when the thermocline stablishes. A shallower and a deeper subzone can be typically

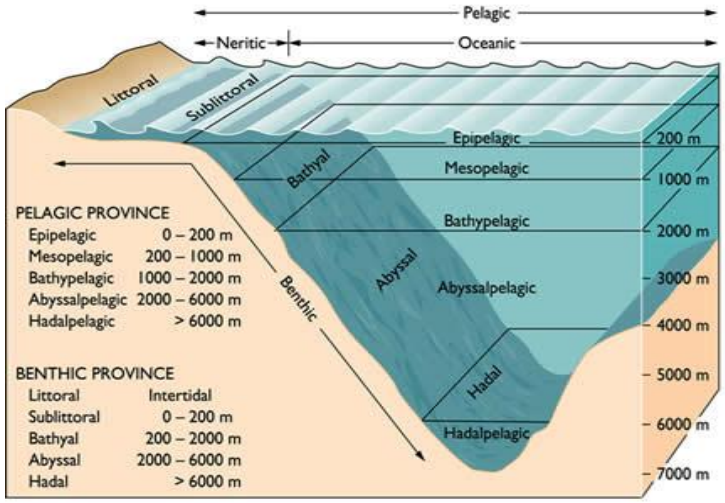


Fig. 4 Typical zonation and vertical strata in the oceans.

identified within the mesopelagic zone on the basis of the distributional patterns of characteristic fish and crustaceans (*shallow*, reflective-sided fish and transparent/partially red-coloured crustaceans; *deep*, non-reflective sided fish and entirely red crustaceans). It has been usually reported that most migrants from the *deep* sub-zone do not get further up the thermocline, and those from the *shallow* one move upwards into the epipelagic layers (Angel, 1997).

An explanation before considering the vertical displacements performed by some groups of organisms throughout the water column, concerns to the importance of the mesopelagic zone in the fluxes of organic matter. Traditionally, the biological pump has been uniquely addressed to the carbon transformation by the phytoplankton, in the upper ocean, into particulate organic carbon (POC) that is partially transported as sinking particles to the deep sea (Steinberg *et al.*, 2000). Additionally, bacterial processes in the upper mesopelagic zone have an important role in the long term storage of organic matter in the ocean and activating an alternative course for the organic carbon throughout the microbial loop. This carbon can be assimilated into the microbial loop or sink by diffusion processes as dissolved organic carbon (DOC). Larvacean and copepod faecal pellets, crustacean moults and other particulate and organic matter start aggregating in their fall to the depths with the sinking waters (Lampitt *et al.*, 1990). This passive sinking of detrital and living particles so-called gravitational flux has been commonly thought to be the dominant vertical transport process and, for that reason, most current research about the carbon transport has been focused on this passive path. However, carbon transport downwards to the depths occurs by three ways: 1) through the stated passive sinking of POM (particulate organic matter), 2) by diffusion and advection of DOM (dissolved organic matter), and 3) actively by the vertical migratory routines of zooplankton and micronekton across the water column (Hidaka *et al.*, 2001). Active flux has gained importance in the most recent

investigations as it is a major component of the biological pump whose study is generally lacking in most oceans (Hernández-León *et al.*, 2010). The transport of the planktonic organic matter consumed in the upper layers to the mesopelagic zones constitutes the active flux of the biological pump, including the carbon from the faecal releases, respiration (Longhurst *et al.*, 1990) and mortality (Zhang and Dam, 1997). Assuming that this transport to the mesopelagic zone is performed by vertical diel migrants, it has been estimated that its magnitude might be comparable to the gravitational flux (Hernández-León *et al.*, 2010).

Relevance of the vertical migrations in the food web

The hypothesis launched by Vinogradov (1970) about the existence of a chain of daily vertical migrations from the surface to depths is, nowadays, celebrated as an essential mechanism in the dynamic fluxes of organic matter through food webs. The major shift of matter from the euphotic zone to the bottom is produced by means of mesopelagic organisms, such as copepods, krill and small fishes that perform diel vertical migrations (DVM) (Lampitt *et al.*, 1990). Once in deeper waters, they may become potential prey for other demersal and benthopelagic species. Great part of the energy output flowing in the deep sea is generated by way of epi- and mesopelagic webs (Pepin, 2013). Recognized species of commercial pelagic fishes, e.g. anchovy or sardine, are known to perform a top-down control of the energy fluxes, but also myctophids (lanternfishes) and stomiiforms (lightfishes), from which we can find patchy information worldwide, may exert a significant constraint to these fluxes.

In the photic layers, light modulate migrant behaviours in zooplankton, defining circadian rhythms and ontogenetic changes. Upwards and downwards migrations happen if the light intensity thresholds are overtaken for both negative and positive geotaxis (Cohen and Forward, 2009). Moreover, food

depletion in temperate waters during the summertime might disturb the circadian displacements of some mesopelagic organisms until food concentrations increase again to return the energetic cost of migratory behaviours (Huntley and Brooks, 1982). Zooplanktonic DVMs may be secondarily activated by chemical routes as a phenotypic response to high predator pressure.

Midwater migratory fishes are the principal consumers of zooplankton across the water column. They are able to perform extensive vertical migrations through a day period, usually ascending at night to epipelagic zone, where food is more concentrated. Thus, the following of prey comes up as a reasonable explanation for these nocturnal displacements to the surface. Alternative explanatory hypotheses to their return into depths are, primarily, that they shelter from larger predators in deeper layers (Childress, 1995), and secondly, that descending to colder waters results in less energy consumption (Enright, 1977; Marshall, 1979). The extent of vertical migrations varies depending on the

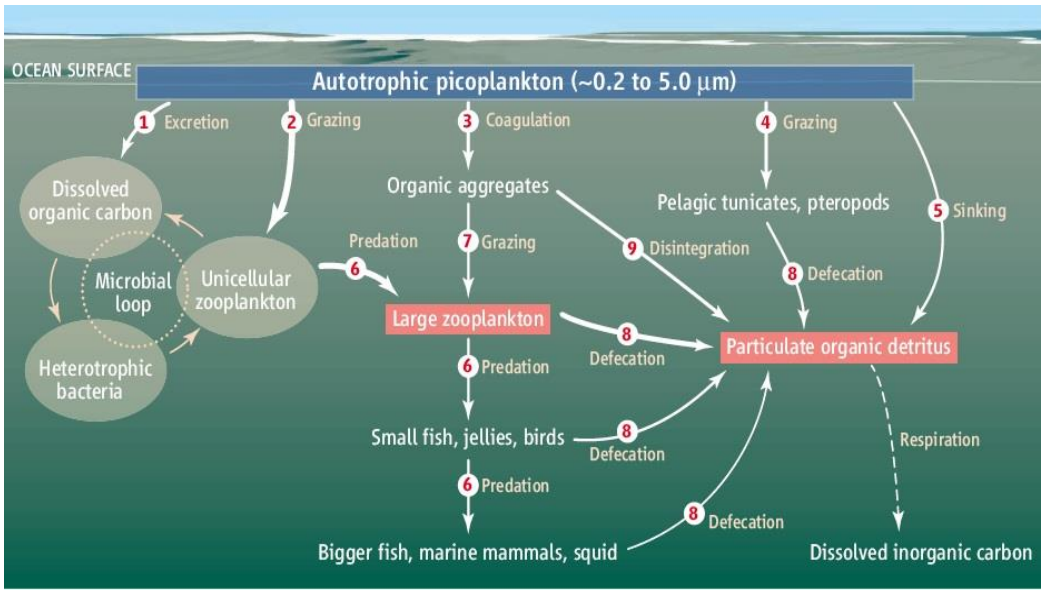


Fig. 5 Food web showing different paths of organic carbon flux, and the microbial loop on the left. Source: Barber (2007).

species, developmental stages and seasonal variation of the oxygen minimum layers (Parker-Stetter and Horne, 2009). Nonetheless, mesopelagic migratory fishes are essential components for the transfer of matter and energy from the richer upper layers to the deep bottom environments.

Mesopelagic fishes of the western Mediterranean

The main issue of this thesis embraces several aspects of the life cycle of the most abundant mesopelagic fishes in the western Mediterranean Sea, particularly the vertical distribution, biodiversity, and trophic ecology of species belonging to the orders Myctophiformes and Stomiiformes. Mesopelagic fishes include those species mainly inhabiting the portion of the water column from 200 to 1000 m (Gartner *et al.*, 1997), although many species are capable of performing extensive vertical displacement in the water column, reaching the surface layers. These mesopelagic fishes are abundant components in terms of biomass in the oceanic waters of all temperate and tropical regions, and probably the most abundant vertebrates worldwide (Jahn and Backus, 1976; Gjøsaeter and Kawaguchi, 1980). Taxonomically, it consists of a specious group fairly different from the well-known and commercially exploited small pelagic species (e.g. anchovy, sardine and mackerel) associated to the epipelagic region. In the MS, the biodiversity of mesopelagic fish species is low compared to that in other oceans (Olivar *et al.*, 2012). Lanternfishes (Myctophidae), silver hatchetfishes (Sternoptychidae) and gonostomatids constitute the main representatives (Gjøsaeter and Kawaguchi, 1980; Hulley, 1994; Sassa *et al.*, 2002; Acosta *et al.*, 2003) of the mesopelagic domain and share some particularities, such a fairly small body size (a few centimetres), dark colour, development of light organs commonly known as photophores, and presence of relatively large swim-bladders with respect their body size. Most of these mesopelagic fish species usually constitute aggregations at specific depths and during the DVMs (although not all species are involved in this activity),

emitting an acoustic signal at the scattering layers (Koslow, 2009; Olivar *et al.*, 2012).

It is essential to understand the life history of mesopelagic fishes, attending to all ontogenetic stages from larvae to adulthood. Larvae and adults of mesopelagic fishes usually occupy unlike trophic niches and show changes in the vertical distribution that they adopt in response to their different requirements. Diverse environmental drivers affect the distribution and habits of the ichthyoplankton of these mesopelagic fishes (e.g. Sabatés, 1990; Sabatés *et al.*, 2003; Sabatés, 2004; Alemany *et al.*, 2006; Mafalda *et al.*, 2008; Olivar *et al.*, 2010), which occurs in high biomass concentrations over the edge of the continental shelf and the epipelagic zone (upper 200 m). On the shelf, the number of species and larval abundance vary seasonally in relation with the spawning events of the mature individuals, usually in spring and summer (Sabatés *et al.*, 2007). Conversely, larvae of oceanic species do not experience large differences in abundance or diversity with seasons. Most myctophiforms and stomiiforms reproduce in the spring period but their larvae coexist during most of the year (Sabatés and Olivar, 1989). Their distribution might respond better to water currents than to trophic cues (Olivar *et al.*, 2010). The ichthyoplankton ecology has been better reported than that belonging to adult fishes in the deep sea, primarily due to the easier collection of data. This is particularly true for the MS, where information on feeding ecology of 6 coexisting fish larval species has been studies by Sabatés and Saiz (2000), whereas before the present project only two studies reported the feeding ecology of adult stages of 3 species of this mesopelagic group. Further information on juveniles and adults of lantern- and lightfishes was needed to extent the biological knowledge to the most abundant and common species, as adult stages of midwater fishes respond not the same way than larvae to the ecological pressures.

Myctophids exert a notorious feeding pressure in the pelagic ecosystem, and they have been reported to remove up to 10% of the zooplankton biomass per night (Watanabe *et al.*, 2002). In oligotrophic regions, sporadic concentration or scarcity of food resources might determine, consequently, feeding selective strategies by predators (Macpherson, 1981). Feeding selectivity can be revealed in two forms, related to prey size or species-specific (Haedrich, 1997). Nonetheless, Auster *et al.* (1992) places the occurrence of myctophids in aggregations of other pelagic fauna for to possible fulfilments; either reduce contact with predators or take advantage of the grouping to search prey.

Zooplanktonic groups as prey for mesopelagic fishes

Zooplankton plays an essential role in the energy transfer through marine food webs from phytoplankton to mesopelagic fishes, and occupies different trophic positions in the food chains leading to trophic cascades.

The distribution of zooplankton depends broadly on the seasonal variations and circulation of the water masses. The zooplankton composition in the Balearic Sea shows high levels of biodiversity and low numerical abundance compared with other pelagic regions (Fernández de Puelles *et al.*, 2003; Fernández de Puelles *et al.*, 2007; Siokou-Frangou and Mazzocchi, 2010). Moreover, the hydrodynamics of the Balearic water masses with different characteristics at the northwest and southeast of the islands influences the biological pelagic communities and, concurrently, the trophic web structures are also different at both sides in the Balearic and Algerian basins. Demersal communities in these basins are mainly sustained by the plankton biomass rather than the benthic production (Maynou and Cartes, 2000; Cartes *et al.*, 2001). Additionally, oscillations in the distributions of the major zooplankton prey of mesopelagic fishes might influence at the same time fish population structure, especially

when the individuals are more susceptible, e.g. during recruitment processes and in early developmental stages.

Copepods are important contributors to the zooplankton biomass (Razouls, 1977; Calbet, 2001) and generally constitute the alimentary basis of myctophids. Copepod diversity reaches more than 450 species in the Mediterranean (Razouls and Durand, 1991), although only a few oceanic species accounted the major proportion of total copepods (Fernández de Puelles *et al.*, 2007). Vertical distribution of plankton depicts its maximum of abundance accumulated at the deep chlorophyll maximum level in the epipelagic zone (Sabatés *et al.*, 2007) , conditioning the feeding behavior of many mesopelagic fish species. Moreover, some species of copepods (e.g. *Pleuromamma gracilis, Euchaeta marina, Candacia armata*) (Vives, 1978), ostracods (Riandey *et al.*, 2005) and euphausiids (*Meganyctiphanes norvegica, Euphasia krohni, Nematoscelis megalops*) (Banse, 1964; Mauchline, 1980; Barange *et al.*, 1991) perform circadian displacements to the surface at night, constituting an available food supply for myctophids at different levels of the water column. Myctophids and stomiiforms are not strictly zooplanktivores and might have feeding habits suitable from other dietary guilds, preying on micronekton with high caloric content that can constitute a surplus associated to dietary shifts (Gjøsæter, 1973; Hopkins and Baird, 1973; Gorelova, 1977).

The study of stomach contents

Stomach content examination is the most common method to provide an insight in the trophic ecology of fishes, although is very timing consuming and relies on the analysis of a great number of specimens in order to properly reflect a large array of feeding situations. It requires valuable taxonomic skills of the investigators to identify large sets of organisms in different degrees of degradation. Additionally, stomach content analysis provides detailed

information on the faunistic composition of the pelagic environment, and where the potential prey organisms are distributed. This information is difficult to extract when it comes to migratory predators that displace throughout the entire water column. Thus, the knowledge of the predator vertical distributions helps enhancing the understanding of predator-prey interactions. However, as each methodology, it has limitations and can bias prey composition by underestimating soft prey when food in the stomachs is in advance stage of digestion (Hyslop, 1980). In order to avoid this, stomach contents are often combined with alternative trophic techniques like stable isotope analyses. The use of a combined approach leads to a better understanding on how trophic interactions take place in the depths by determining specific prey taxa, their mass contribution and the integration of these items in the predator organisms.

* * *

The high diversity of the Mediterranean ecosystem brings up the need of taking actions for the protection of specific areas, leading scientist to design functional models of the ecosystems. Modelling of the pelagic domain has eventually been very inaccurate to define dynamics of their communities (Würtz, 2010). One of the major obstacles for its implementation is the absence of satisfactory data on their biotic and abiotic components. Thus, marine experts are continuously seeking to obtain accurate data for the construction of those models that are supplied with lots of records. Currently, there is little understanding of the comprehensive mesopelagic and Deep-Sea systems, at the time that we are slightly conscious of how quick environmental changes induced by physical and human factors are affecting coastal and offshore regions. The study of the ecological traits of key organisms is mandatory to evaluate feasible impacts on the whole mesopelagic system. Therefore, the distributional patterns and predator prey interactions of the marine organisms constitute pillars in conservation and fisheries management.

Project framework and justification

This thesis is framed within the IDEADOS Project (*Structure and dynamics of the benthopelagic slope ecosystem in two oligotrophic zones of the western Mediterranean: a multidisciplinary approach at different temporal scales in the Balearic Islands*) carried out by the *Instituto Español de Oceanografía* (IEO), the *Consejo Superior de Investigaciones Científicas* (CSIC) and the *Universitat de les Illes Balears* (UIB) that aimed to determine the coupling of the trophic relationships between the demersal fauna and the meso- and bathypelagic communities from an oligotrophic system located between the Algerian and Balearic basins. In order to assess the influence of seasonal hydrodynamic changeability, with a repeated annual sequence of mixing and stratification events, on the biota, the research team agreed performing two oceanographic cruises.

A foregoing multidisciplinary project (IDEA) on the dynamics of deep water exploited ecosystems of the Balearic archipelago, set a starting point to launch hypothesis on how distinct hydrodynamic scenarios of the traditional fishing grounds in the north and south of the islands (Sóller and Cabrera) affect the nekton-benthonic assemblages. Moreover, the IDEADOS project is the first in the Mediterranean using acoustic techniques of detection of the Sound Scatter Layers (SSL) in order to have a more accurate estimation of the fish abundances than with the solely data from net captures. As part of an integrated approach, this thesis alludes to the mesopelagic fish community, focusing on the principal species of myctophids and stomiiforms (Sternoptychidae and Gonostomatidae) that dwell the western Mediterranean. We have studied diverse biological aspects of these species to attempt discerning their ecological role in a complex system, whose inhabitants navigate across all the spatial dimensions.

The understanding of the pelagic environment is still scarce as a result of the difficulty accomplishing the topography of the depths and from the use of indirect methods of observation, whilst the technologic procedures that allow environmental introspection have a costly starting-up and a recent development in the 20th century. In addition, new data collections of the pelagic fauna are needed in high numbers and detail for the implementation of models that explain interspecific relationships in the ocean.

In the early 1900 an extensive research performed across the Mediterranean basins provided original information on the mesopelagic fish distributions and abundances, (Tåning, 1918; Jespersen and Tåning, 1926; Goodyear *et al.*, 1972b), including data on their vertical distributions. After that, most of the research on mesopelagic species was focussed on the distribution of early stages (Olivar and Sabatés, 1997; Sabatés *et al.*, 2007). Investigations on adult stages were restricted to the gonostomatid *Cyclothone* spp. (Andersen and Sardou, 1992; Andersen *et al.*, 1998), or the myctophid *Lampanyctus crocodilus* (Cartes and Stefanescu, 1992). Adult mesopelagic populations have been less studied in WM in comparison with other taxonomic groups, such as zooplankton or micronekton, with a comprehensive material available on their vertical motions (Andersen and Sardou, 1992; Andersen *et al.*, 1998), or commercial fish species.

In the last decades, the growing bulk of studies on trophic ecology of diverse organisms in the Mediterranean food webs have not been addressed to the most abundant group of midwater fishes. It means that the western basin is still lacking of detailed knowledge on the dietary habits of "one" essential piece of the marine food webs. There are several classic reports on the feeding routines of midwater fishes from productive upwelling areas to oligotrophic systems (e.g. Badcock, 1970; Hopkins and Baird, 1977; Kinzer, 1977; Hopkins and Baird, 1985). In the last decades new references are being added to the ecological

knowledge of midwater fishes since Gjøsaeter and Kawaguchi (1980) underlined their importance in food webs. Hardly a few studies on the feeding habits of their larvae have been published in the western Mediterranean, whilst less has been the interest on the adult stages. The present thesis was aimed to fill these *gaps*, to define a more integrated vision of the mesopelagic community in the Balearic Sea.

Aims and structure of the thesis

The Mediterranean is often considered to reflect processes that take place in the largest oceanic waters functioning as a model in which some of these phenomena can be described in a relatively small extension. For this reason its characterization deserves special interest. Much effort has been addressed in the characterization of the life patterns of commercial fishes in this basin, although the management and assessment of this diverse sea cannot be satisfied without the knowledge of the role that lanternfishes and lightfishes play in this region, especially within the trophic food webs of the pelagic and deep-sea ecosystems. In this regard, several aspects of their biology in the western Mediterranean that remained unexplored were considered for the development of this thesis, setting the following general hypotheses:

a) H_0: *Most mesopelagic fishes ascend to the epipelagic layers at night in the western Mediterranean, as it has been generally stated for myctophids from other regions*.

H_1: *Most mesopelagic fishes do not follow the common pattern of night-ascension to the surface in the western Mediterranean*.

b) H_0: *Mesopelagic fish species have different diets compositions, with low overlapping of food resources among intraspecific developmental stages and at interspecific level, as it is expected from oligotrophic regions, giving advantage to selective strategies on particular prey items.*

H_1: *Mesopelagic fish species have similar diet compositions that widely overlap, with low selectivity on prey items*.

The general objective that emerges from the main thesis approach is the analysis of the trophic ecology of the most important mesopelagic fish species from a

relative oligotrophic region, the western Mediterranean, during two contrasting seasonal periods (summer and autumn). The diet compositions will be analysed mainly considering the vertical distributions of these fishes, their ontogeny and predator abilities, and the changeability of the environmental conditions in the pelagic ecosystem with seasons.

Prior to the main analyses in this thesis, some useful biological aspects of the objective fish species are encompassed in **Chapter 1**, which includes two different works. (1) The lack of molecular evidence between the identity of larvae and adults of mesopelagic species, which hold an extremely dissimilar appearance, was decisive to reserve a preliminary section for the DNA barcoding of several larval stages. Moreover, this chapter introduces the main phylogenetic characteristics of both orders Myctophiformes and Stomiiformes, and determines a genetic support for the clades constituted by congeners in the western Mediterranean Sea. (2) Additionally, the length-weight relationships of the same species were provided in this section as background information for subsequent studies on body condition.

The **Chapter 2** was focussed on the community structure of the mesopelagic organisms across the water column, attending to *i)* the vertical distribution and abundance of the mesopelagic fish species, and *ii*) the abundance of the micronecktonic organisms responsible for the SSL in the western Mediterranean. The specific objective was to supply information on the specific abundance, spatial and seasonal distribution, and assemblages of the Mediterranean mesopelagic fishes.

The Chapters 3, 4 and 5 constitute the fundamental theme of the thesis, i.e. feeding ecology of mesopelagic fishes. The specific objectives were *i)* to describe the trophic ecology of the main mesopelagic species in the western Mediterranean through the analysis of stomach contents (**Chapter 4**) and

contrasting with stable isotope results (**Annex I**); *ii)* to approach the spatial, temporal and ontogenetic variation of the diet composition for each mesopelagic fish species. Predator-prey relationships were explored using regression relationships between prey dimensions and predator standard length (SL) and determining prey selection (**Chapter 4**). Studies about changes in feeding associated to ontogenetic factors were absent in the Mediterranean. In this thesis, shifts in the feeding patterns through the entire life cycle were analysed in a particular species, *Lampanyctus pusillus* (Myctophidae) (**Chapter 3**).

The **Chapter 5** was destined to the general discussion, where the total results of the investigations are consistently related and summarized.

The general conclusions are presented in **Chapter 6**.

The Part I of the thesis comprises the general introduction, the project framework, and objectives. The results of the thesis are presented in the Part II, in chapters 1-4 corresponding with the following published and submitted scientific works.

- **Bernal, A.**, Olivar, M. P., Viñas, J. 2014. Genetic support for the classic morphological classification of the larval and adult stages of lightfish and lanternfish. *Scientia Marina* (*accepted*).
- 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 Research Part I* 62: 53-69.
- **Bernal, A.**, Olivar, M. P., Fernández de Puelles, M. L. 2013. Feeding patterns of *Lampanyctus pusillus* (Pisces: Myctophidae) throughout its ontogenetic development. *Marine Biology* 160 (1): 81-95.
- Olivar, M. P., Molí, B., **Bernal, A.** 2013. Length-weight relationships of mesopelagic fishes in the north-western Mediterranean. *Rapport du 40th Congres de la Commission Internationale pour l'Exploration*

Scientifique de la Mer Mediterranée (CIESM), Vol. 40. Marseille, France.

- Olivar, M. P., Abelló, P., Quetglas, A., Castellón, A., **Bernal, A.**, Molí, B., Sabatés, A., Iglesias, M., Simao, D., Massutí, E. 2013. Micronekton groups contributing to the night scattering layers in the western Mediterranean. *Rapport du 40th Congres de la Commission Internationale pour l'Exploration Scientifique de la Mer Mediterranée (CIESM)*, Vol. 40. Marseille, France.

- **Bernal, A.**, Olivar, M. P., Maynou, F., Fernández de Puelles, M. L. 2014. Diet and feeding strategy of mesopelagic fishes in the western Mediterranean. *Progress in Oceanography* (*submitted*).

Additionally, the author includes in the **Annex I** — before the General Discussion — part of the work comprised in the following article, as a co-author contribution, to allow comparisons between the analysis of the diet compositions and stable isotope results. For this work on isotope analysis, the author of this thesis has assisted in suggesting hypothesis and the interpretation of results and discussion.

- Valls, M., Olivar, M. P. Fernández de Puelles, M. L., Molí; B., **Bernal, A.**, Sweeting, C. J. 2014. Trophic structure of mesopelagic fishes in the western Mediterranean based on stable isotopes of carbon and nitrogen. *Journal of Marine Systems* 138: 160-170.

The author also participated in a complementary study -not included in this memory- that provides information on the reproduction of a Mediterranean myctophid, *Benthosema glaciale*:

- García-Seoane, E., **Bernal, A.**, Saborido-Rey, F. 2013. Reproductive ecology of the glacier lanternfish *Benthosema glaciale*. *Hydrobiology* 727: 137-149.

Part II

Chapter 1

OBJECTIVE SPECIES

1. Genetic support for the morphological identification of larvae of Myctophidae, Gonostomatidae, Sternoptychidae and Phosichthyidae (Pisces) from the western Mediterranean

Abstract

Mesopelagic fishes experience an extreme body transformation from larvae to adults. The identification of the larval stages of fishes from the two orders Myctophiformes and Stomiiformes is currently based on the comparison of morphological, pigmentation and meristic characteristics of different developmental stages. Nevertheless, no molecular evidence to confirm the identity of the larvae of these mesopelagic species was available so far. Since DNA barcoding emerged as an accurate procedure for species discrimination and larval identification, we have used the cytochrome c oxidase 1 or the mitochondrial 12S ribosomal DNA regions to identify larvae and adults of the most frequent and abundant species of myctophiforms (Family Myctophidae) and stomiiforms (Families Gonostomatidae, Sternoptychidae and Phosichthyidae) from the Mediterranean Sea. The comparisons of sequences from larval and adult stages corroborated the value of the morphological characters that were used for taxonomic classification. The combination of the sequences obtained in this study and those of related species from GenBank were used to discuss the consistency of monophyletic clades for different genera. Pairwise nucleotide distances were notably higher inter- than intraspecifically, and were useful to discern between congeners such as *Cyclothone braueri* and *C. pygmaea*, *Hygophum benoiti* and *H. hygomii*, *Lampanyctus crocodilus* and *L. pusillus*, and *Notoscopelus bolini* and *N. elongatus*.

1.1 Introduction

Ecological interactions of fish assemblages in the pelagic environment can be partially determined by their larval distributions and recruitment to adult populations. The identification of early life stages, such as larvae and transforming, is essential for current studies on the distribution and reproductive strategies of pelagic fishes (Takeyama *et al.*, 2001; Moura *et al.*, 2008; Valdez-Moreno *et al.*, 2010). Thus, the assessment of biodiversity and its implication in the management of vulnerable marine ecosystems requires an accurate taxonomic assignment of fish larvae. Without this knowledge, the abundance of cryptic or unknown species might be under- or overestimated.

The identification of fish larvae has been an important morphological issue in marine ecology due to the dramatic transformations most species undergo from early larval stages to adulthood (Burton, 1996). Some ambiguity also arises when attempting to identify larval stages of closely related species with slight morphological and pigmentation differences (Blaxter, 1984). Recently, a few studies on pelagic fishes have used molecular markers to determine unidentified larvae or those larvae suspected of misidentification (e.g. Takeyama *et al.*, 2001; Kochzius *et al.*, 2010; Ko *et al.*, 2013) due to errors in the fishes' morphological identification. One such study showed that various fishes assigned to three families with widely differing morphologies were actually male, female and larvae of a single family (Johnson *et al.*, 2009).

The effective discrimination of species through mitochondrial DNA (mtDNA) analyses has been stated in earlier fish studies (e.g. Hare *et al.*, 1994; Takeyama *et al.*, 2001; Viñas and Tudela, 2009). An international interest in fisheries sparked a launch of the project "Barcode of Life Project (iBOL)" (Hebert *et al.*, 2003), which determined that mtDNA cytochrome c oxidase 1 (CO1) was a

suitable gene marker for fish species identification due to the fast evolution of the mtDNA, its maternal inheritance and haploid condition (Moore, 1995). Sequencing this gene allows the amplification of large and low-variable sequences (Hebert *et al.*, 2004; Steinke *et al.*, 2009). Hebert *et al*. (2004) suggested a 10X-threshold of intraspecific genetic divergence, known as the barcoding gap, to discriminate at species level. This threshold establishes a quantifiable limit between intra- and interspecific variability, and determines when the DNA sequences share a monophyletic origin. Miya and Nishida (2000) reported the validity of CO1 within a group of protein-coding genes as appropriate markers for the recovery of the expected phylogeny of teleosteans. In general, species identification applies the Forensically Informative Nucleotide Sequencing (FINS) methodology, which involves the establishment of a robust phylogeny followed by a subsequent species identification of query individuals based on the previous phylogeny (Bartlett and Davidson, 1992).

Originally, barcoding was only applied to fish species of commercial interest that were often mislabeled. Less focus has been placed on species with non-commercial value, such as the mesopelagic fishes. These fishes are mostly included within the orders Myctophiformes and Stomiiformes, documented amongst the most common and abundant vertebrates in the world (Gjøsæter and Kawaguchi, 1980). Both orders include relatively small, deep-water species with distinct luminous organs (photophores), commonly known as lanternfishes and lightfishes. The location of groups of photophores, together with osteological characteristics and the number of gill rakers are features typically used for species identification and to construct their evolutionary history (Paxton, 1972; Hulley, 1981; Fink, 1985). These features are less developed during the larval stages, leaving body shape and pigmentation patterns as the best descriptors (Jespersen and Tåning, 1926; Moser *et al.*, 1984; Olivar *et al.*, 1999). The morphotype and conspicuous specializations of myctophiform (e.g. Moser and

Ahlstrom, 1974; Olivar *et al.*, 1999) and stomiiform (e.g. Jespersen and Tåning, 1926; Ahlstrom, 1974; Richards, 2006) larval stages are highly diverse and used in the systematics of genera and subfamilies (Moser and Ahlstrom, 1974; Moser *et al.*, 1984). For example, eye morphology in myctophid larvae discerns the two existing subfamilies Lampanyctinae (round eyes) and Myctophinae (narrow eyes) (Moser and Ahlstrom, 1974).

In the Mediterranean Sea, the number of myctophiform and stomiiform species (Goodyear *et al.*, 1972b; Olivar *et al.*, 2012) is lower in comparison to the Atlantic, Pacific or Indian Oceans (Nafpaktitis *et al.*, 1977; Badcock, 1984; Hulley, 1984). Compared with the adjacent northeastern Atlantic, the western Mediterranean only harbors 17 of the 57 myctophid, 3 of the 17 gonostomatid, 2 of the 11 sternoptychid, and 3 of the 6 phosichthyid species (Badcock, 1984; Hulley, 1984). Furthermore, some of the Mediterranean species are endemic, such as *Notoscopelus elongatus* (Costa, 1844), and *Cyclothone pygmaea* Jespersen and Tåning 1926. The larvae of all these mesopelagic fishes are well known based on descriptions of specimens collected in the Mediterranean region (Tåning, 1918; Jespersen and Tåning, 1926) (Appendix 1.1).

The use of genetic markers for larval identification of myctophiforms and stomiiforms has, so far, been limited to the larvae of the *Hygophum* spp. (Myctophidae) (e.g.Yamaguchi *et al.*, 2000). The genetic evidence to construct the evolutionary history of lanternfishes and lightfishes that inhabit the Mediterranean Sea is lacking. Phylogenetic relationships within Myctophidae had been unresolved by genetic methods until a recent study by Poulsen *et al*. (2013) combined sequencing of mitogenomes, coding and non-coding regions, and gene order rearrangement. The results of their study supported the classical morphological phylogeny recognized for myctophids (Paxton, 1972; Paxton *et al.*, 1984).

The objective of the present work is to assess the validity of morphological larval identifications using two mitochondrial markers, CO1 or 12S rRNA, to accurately associate larvae and adults, of the same species, for the most abundant and frequent mesopelagic fishes of the western Mediterranean. Additionally, it was of interest to determine the similarity of congeneric taxa and infer the most external relationships under the resolution threshold of one mtDNA marker. New sequences were uploaded to GenBank, of which sequences of seven species were included for the first time (Appendix 1.2).

1.2 Materials and methods

1.2.1 Sample collection

Genetic analyses were conducted on the larvae and adults of 18 species, across 14 genera of mesopelagic fishes. The adult collection consisted of the most abundant and frequent mesopelagic fishes in the region, of the orders Myctophiformes and Stomiiformes. For myctophiforms, 7 of 9 species of the subfamily Lampanyctinae and all species from Myctophinae (6), known to be present in the western Mediterranean, were analyzed (Table 1.1 and Appendix 1.1). Stomiiforms included *Argyropelecus hemigymnus* (Cocco, 1829) and *Maurolicus muelleri* (Gmelin, 1789) (family Sternoptychidae), *Cyclothone braueri* (Jespersen and Tåning, 1926) and *C. pygmaea* (family Gonostomatidae), and *Vinciguerria attenuata* (Cocco, 1838) (family Phosichthyidae).

Samples were collected on board the vessel R/V *Sarmiento de Gamboa* during July 2010, offshore of the Balearic Islands (39° N, 2° E). Sampling procedures are described elsewhere (Olivar *et al.*, 2012). The specimens used in this study were identified on board and preserved in 96% ethanol prior to genetic analyses. In addition, the larvae of 12 species were sorted on board. The larvae of *A. hemigymnus* and *Notoscopelus* spp., which were not available in July 2010,

Table 1.1 List of the genetic markers used to identify each species, stage of development, body size range, number of sequenced individuals, total number of individuals of each species in the sampling collection, and sampling location and depth. Abbreviations: No. Number, NL / SL: Notochord Length / Standard Length; L: Larva; T: Transforming; A: Adult.

Species	Genetic marker	Stage (NL / SL, mm)	No. sequenced individuals	No. L in collection	Depth range, m	Longitude range, °E	Latitude range, °N
Argyropelecus hemigymnus	CO1	A (26-28)	1		909-925	2.15-2.41	39.01-39.80
Argyropelecus hemigymnus	CO1	L	0	407	150-245	2.22-2.70	39.09-39.67
Benthosema glaciale	CO1	A (28)	3		909-988	2.14-2.41	39.01-39.82
Benthosema glaciale	CO1	L (9.5-19.9)	2	589	216-587	2.06-2.18	39.65
Ceratoscopelus maderensis	CO1	A (52-54)	3		958-970	2.11-2.18	39.82
Ceratoscopelus maderensis	CO1	L (5.1-11.4)	4	1792	156-264	2.69-2.72	39.04-39.05
Diaphus holti	CO1	A (38-44)	3		422	2.17	39.70
Diaphus holti	CO1	L (7.2)	1	21	641	2.08	39.69
Electrona risso	CO1	T (9)	1		245	2.70	39.09
Hygophum benoiti	CO1	A (41-43)	3		909-977	2.11-2.41	39.01-39.82
Hygophum benoiti	CO1	L (8.7-12.9)	3	4834	230-938	2.15-270	39.07-39.81
Hygophum hygomii	CO1	A (47)	3		958-968	2.11-2.15	39.81-39.82
Hygophum hygomii	CO1	L	0	106	150-914	2.09-2.72	38.99-39.76
Lampanyctus crocodilus	CO1	A (69)	2		603-988	2.07-2.14	39.67-39.82
Lampanyctus crocodilus	CO1	L (5.4-12.2)	2	443	245-300	2.18-2.28	39.65-39.80
Lampanyctus pusillus	CO1	A (36)	2		626-980	2.07-2.14	39.67-39.82
Lampanyctus pusillus	CO1	L (5.3-6.0)	3	218	225-248	2.23-2.70	39.03-39.70
Lobianchia dofleini	CO1	A (37.0-37.4)	3		940-958	2.11-2.15	39.80-39.82
Lobianchia dofleini	CO1	L (6.0-7-2)	2	63	225-340	2.21-2.28	39.68-39.72
Maurolicus muelleri	CO1	A (31-45)	3		226-422	2.16-2.18	39.64-39.70
Maurolicus muelleri	CO1	L (9.5-10)	3	129	225-245	2.22-2.70	39.07-39.70
Myctophum punctatum	CO1	A (29-39)	3		156-988	2.11-2.73	39.07-39.82
Myctophum punctatum	CO1	L (9.9-13.9)	3	127	274-814	2.49-2.69	39.06-39.08
Notoscopelus bolini	CO1	A (90)	1		235	2.28	39.71
Notoscopelus elongatus	CO1	A (41-42)	2		226-245	2.18-2.70	39.09-39.65
Symbolophorus veranyi	CO1	A (56)	2		958-977	2.11-2.15	39.81-39.82
Symbolophorus veranyi	CO1	L (4.08-9.0*)	3*	199	222-248	2.18-2.70	39.03-39.68
Vinciguerria attenuata	CO1	T, A (12-37)	7		891-988	2.11-2.46	38.94-39.84
Cyclothone braueri	12S rRNA	A (15)	1		305-935	2.15-2.70	39.02-39.81
Cyclothone braueri	12S rRNA	L (7-7.7)	3	3164	222-245	2.18-2.71	39.03-39.65
Cyclothone pygmaea	12S rRNA	A (21-23)	2		900-938	2.15-2.43	38.98-39.81
Cyclothone pygmaea	12S rRNA	L (4.5-5.5)	0	68	893	2.45	39.01

* The sequences of *S. veranyi* larvae showed high nucleotide variation. They constituted an isolated cluster, far apart from other myctophids and stomiiforms. For this reason they were not included for evolutionary methods.

where taken from a preceding cruise (December 2009, IDEADOS project) in the same area, and were stored in 5% formalin for less than one year. Adult myctophids were identified following the descriptions of Hulley (1984), while those of Jespersen and Tåning (1926) and Badcock (1984) were used to identify the stomiiforms. The larvae chosen for DNA analyses were identified on board by the third author on the basis of detailed descriptions provided in the literature listed in Appendix 1.1. Their main distinctive characters are cited in Appendix 3. Photographs of each ethanol-preserved larva were taken prior to DNA extraction to ensure that they were identical to the voucher specimens in the ichthyoplankton collection of the Institut of Ciències del Mar (Spain).

1.2.2 DNA barcoding

The DNA extraction was accomplished for adults by obtaining a portion of excised musculature, while the whole body was used for larvae. The DNA was isolated from 250 mg of tissue using the commercial kit Real Pure Spin (Durviz, Valencia, Spain) according to the manufacturer's instructions. The DNA was re-suspended in 100 µl of deionized water. When larval specimens stored in ethanol were not available, we attempted to isolate the DNA from samples of larvae preserved in 5% buffered formalin using the Chelex and Phenol/Chloroform protocols. Unfortunately, these applications were not successful.

Species identification was achieved using one of two different markers for mtDNA: cytochrome c oxidase 1 or 12S ribosomal RNA (mtDNA 12S rRNA). The amplification of mtDNA CO1 fragments was performed using FishF1 (5'TCAACCAACCACAAAGACATTGGCAC3') and FishR1 (5'TAGACTTCTGGGTGGCCAAAGAATCA3'), a combination of primers previously described by Ward et al. (2005). The pair FishF1 / R1 was selected because of previous recommendations (Pegg *et al.*, 2006), relying on the

production of the longest and clearest amplicons that could be obtained for most species. Minimum within-species variation was expected for the CO1 sequences. Alternatively, for the samples that failed to amplify with this pair, two new sets of primers, LCO1Myc1 / LCO1Myc2 (5'CTTCGGTGCCTGAGCCGGCATAG3', 5'CCGCCGGCGGGGTCGAAGAA3') and L-Cyc_CO1 / R-Cyc_CO1 (5'ATGGTCGGCACAGCCTTA3', 5'AGGGTCGAAAAAGGAGGTGT3') were designed using Primer 3 (Rozen and Skaletsky, 2000) in order to yield more optimized sets. To design these primer sets, an alignment of the sequences was performed, using the primers reported in Ward *et al*. (2005). A fragment of at least 18 nucleotides, with the lowest possible variability among the sequences, was selected from the alignment. The 18-nucleotide fragment was input in Primer 3 to get an amplification length of at least 600 nucleotides.

The CO1 amplification, using the cited sets of primers, failed with most of the stomiiform specimens from *C. braueri* and *C. pygmaea*. Therefore, the mtDNA 12S rRNA region was selected for these species. DNA fragments were amplified using the primer combination L1085 / L1478 (5'TAAACCAGGATTAGATACCC3'; 5'GAGAGTGACGGGCGATGTGT3'), previously described by Miya and Nishida (2000).

The PCR reactions were performed in 25-µl or 12.5-µl reaction volumes using approximately 50 ng (0.5 µl) of the isolated DNA as a template. Each PCR reaction contained 1X Taq DNA polymerase buffer, 1.5–2 mM of $MgCl_2$, 200 mM of each dNTP, 10 pMols of each primer, and 0.5 U of Taq DNA polymerase: Amplitaq DNA polymerase (Applied Biosystems, Foster City, CA, USA) or Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA).

Negative controls were included in all of the PCR runs to ensure against cross-contamination. Thermal cycles involved an initial denaturation at 95 °C for 5

min, followed by 30 cycles of denaturing at 95 °C for 30 s, annealing at 50 °C for 45 s and primer extension at 72 °C for 1 min.

PCR products were purified using 0.6 U of Exonuclease I (Fermentas, Sankt Leon-Rot, Germany) and 0.3 U of Shrimp Alkaline phosphatase (Fermentas) at 37 °C for 1 h, followed by an inactivation step at 85 °C for 15 min. The nucleotide sequences of the PCR products were then cycle-sequenced using the BigDye terminator Cycle Sequencing kit 3.0 (Applied Biosystems) with the forward and reverse primers used for amplification, according to the manufacturer's recommendations. Sequences were read using an ABI Prism 310 Genetic Analyzer (Applied Biosystems).

1.2.3 Sequence editing and analysis

Sequence alignments were edited using BioEdit 7.0.9.0 (Hall, 1999), and aligned using Clustal W (Thompson et al. 1994) with a final optimization by eye. Homologous sequences were downloaded from GenBank (accession numbers listed in Appendix 1.2) to test the consistency of the groups at species-level obtained in this study. Additionally, sequences from GenBank for species that belong to the same genera, from regions other than the Mediterranean, were downloaded when available (Appendix 1.2).

Evolutionary analyses of the CO1 haplotypes were performed using the Maximum Likelihood (ML) procedure run on Mega 6.0 (Tamura *et al.*, 2013). Alternatively, the Bayesian inference (BI) was performed using MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). The most appropriate model of molecular evolution was identified using Mega 6.0. The ML analysis was conducted using the HKY+G+Y model, which was selected for its lowest BIC score, using a discrete gamma distribution *(G=0.95)*, with 5 rate categories and a number of invariant sites estimated from the data *(I=0.49)*. Evaluation of the statistical

confidence in the tree nodes was based on 10000 non-parametric bootstrap replicates. For the Bayesian analysis, the General Time Reversible (GTR) substitution model was implemented with a gamma-distributed rate variation across sites, and across a proportion of invariable sites. The standard deviation of the split frequencies fell to 0.14 after 500000 generations. With the mtDNA 12S rRNA marker, the K2P G+I model was the best to fit ML analysis and BI (mean split frequency=0.0089; ngen=1000000).

The evolutionary interpretation was cautiously restricted to the most external branches due to the problems derived from using one gene. The species *Bathylagus euryops* (Goode and Bean, 1896) was chosen as the outgroup taxa of early teleost to root both evolutionary trees.

1.3 Results

All of the individuals that were used in the genetic analysis were previously identified based on the morphological and pigmentation patterns (Appendix 1.3). Adults were characterized by complete squamation over the body and complete photophore and osteological development. The larval specimens selected for the study ranged in size from 4.1-13.9 mm standard length, and had completely different body shapes to the adults, with no photophore or osteological development.

A summary of the specimens analyzed, body size range, stage of development, the employed genetic marker and number of sequenced individuals is displayed in Table 1.1. New sequences were submitted to the GenBank database. Moreover, this study contributed to the GenBank database with unique CO1 and mtDNA 12S rRNA sequences of the exclusive Mediterranean species, such as *N. elongatus* and *C. pygmaea*, and more widely distributed fishes *Lampanyctus crocodilus* (Risso, 1810), *Lampanyctus pusillus* (Johnson, 1890), *Lobianchia*

dofleini (Zugmayer, 1911), *Diaphus holti* Tåning 1918 and *V. attenuata*. Their accession numbers along with the number of nucleotides of each sequence are listed in Appendix 1.2.

1.3.1 Analyses using CO1

At least 565 nucleotides of the mtDNA CO1 region were sequenced in 41 adult specimens (Appendix 1.2). For most species, at least 2 representative adults and 2 larvae were analyzed. However, the stomiiform *A. hemigymnus* was represented by only one adult because of technical problems during PCR amplification. The sample collection had only one adult specimen of *Notoscopelus bolini* Nafpaktitis 1975, *N. elongatus* and *Electrona risso* (Cocco, 1829). A single sequence of an adult of *Symbolophorus veranyi* (Moreau, 1888) (SvA3) was amplified using the different sets of primers, after re-sequencing several fragments from the larval and adult specimens without success. Some sequences from the larvae and adults, not reported here, were deleted from the alignment after an extensive span of ambiguous nucleotides.

A selection of 25 larval individuals (listed in Table 1.1) was amplified using FishF1 / R1 and LCO1Myc1 / LCO1Myc2, and sequencing was achieved for 22 of them. Three larvae of *S. veranyi* were the only myctophid specimens for which sequencing was not effective using the reported primers. No stop codons, insertions or deletions were found in any of the amplified sequences, indicating that these sequences constituted functional mitochondrial CO1 sequences. Negligible genetic variability was due to PCR errors (3.4×10^{-5}).

Sequence comparisons among the 70 adult sequences in Appendix 1.2, identified 331 conserved sites and 239 variable sites, with an overall mean distance of 0.201 (SD=0.024). Of these sites, 235 were phylogenetically informative. Of the 570 bp of unambiguously aligned sequences within the

myctophids (51 sequences), 347 were conserved sites (60.9%), and 223 were variable sites (nucleotide diversity, π=0.189). The sequences of the stomiiforms (n=19) contained 197 variable sites (π=0.164), therefore, 373 (65.4%) of the sites were invariant. The overall nucleotide frequencies were T (28%), C (31.6%), A (22.8%) and G (17.6%).

The mean sequence distances between the Mediterranean species was nearly 10-fold higher than the within-species mean distances. Estimates of net evolutionary divergence between species-groups of sequences are presented in Appendix 1.4. The maximum average divergence within species over sequence pairs was for *D. holti* (Table 2a). The maximum interspecific difference (number of base substitutions per site based on the estimation of the net average between species groups) was between *V. attenuata* and *L. pusillus* (29.7%), and the minimum distance was between *N. elongatus* and *N. bolini* (6.2%).

The overall mean distance computed when the 25 larval sequences were incorporated increased to 0.22 (SD=0.0136). The intraspecific variation ranged from 0.05% to 2.12% for the Mediterranean species.

ML and BI methods were employed to yield two alternative phylogenetic trees (Figs. 1.1 and 1.2, respectively), with *B. euryops* as the outgroup taxon (Protacanthopterygii), as well as the sister-group of the Neoteleostei, which includes the orders Myctophiformes and Stomiiformes. The tree topology was congruent between phylogenetic methods except for some intermediate nodes, although there was consensus for the congeneric clades and most tribes. A monophyletic group was considered significant when the bootstrap value for that clade reached 95% and was defined by more than two exemplars from the same species. Accordingly, the orders Myctophiformes and Stomiiformes are consistently monophyletic in both phylogenies (ML and BI).

Table 1.2
Within-species (a) and within-genus (b) genetic distances among Mediterranean individuals (larvae and adults). Number of base substitutions per site from averaging the overall sequence pairs within each group is shown in the second column along with the corresponding standard error estimates (S. E.). Analyses were conducted using the Maximum Composite Likelihood model and the rate of variation among sites was modeled using gamma distribution (shape parameter=5). The included codon positions were $1^{st}+2^{nd}+3^{rd}$+Noncoding. All ambiguous positions were removed for each sequence pair.

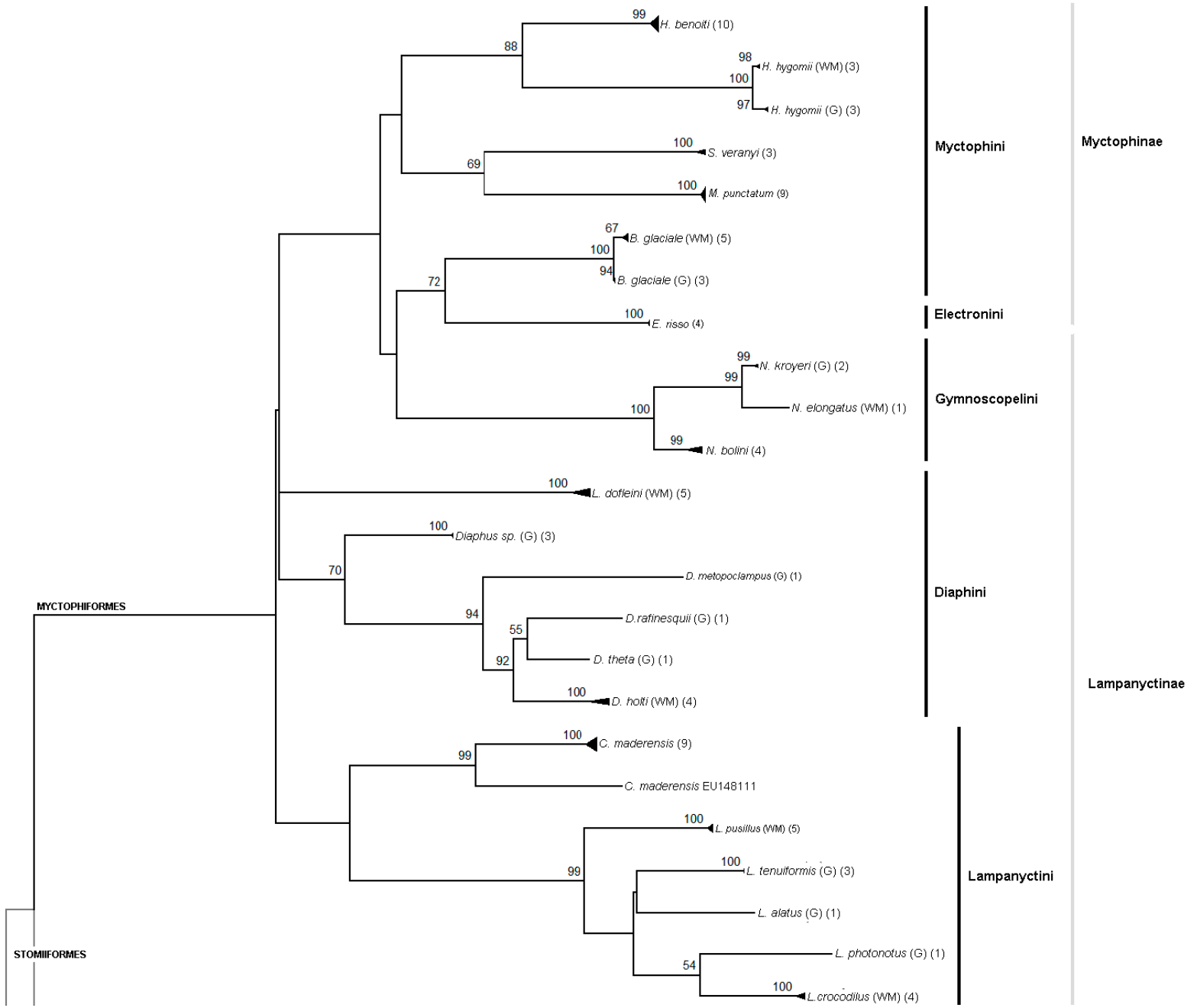
a)

Species	Distance	S. E.
Benthosema glaciale	0.0077	0.0024
Ceratoscopelus maderensis	0.0135	0.0031
Diaphus holti	0.0212	0.0042
Hygophum benoiti	0.0032	0.0014
Hygophum hygomii	0.0077	0.0030
Lampanyctus crocodilus	0.0059	0.0025
Lobianchia dofleini	0.0170	0.0029
Lampanyctus pusillus	0.0028	0.0014
Maurolicus muelleri	0.0028	0.0014
Myctophum punctatum	0.0040	0.0015
Vinciguerria attenuata	0.0005	0.0005

b)

Genus	Distance	S. E.
Hygophum	0.0741	0.0092
Lampanyctus	0.1003	0.0101
Diaphus	0.1077	0.0100
Notoscopelus	0.0538	0.0072
Vinciguerria	0.1146	0.0101

Several features were conserved in both trees. Within Lampanyctinae, *Lampanyctus* spp and *Ceratoscopelus maderensis* (Lowe, 1839), were sister groups and within Diaphini, *L. dofleini* and *D. holti* were clustered together. GenBank sequences of *Diaphus* spp. were also clustered with our sequences of *D. holti* (ML: 70% bootstrapping; BI: 100% posterior probability), which constituted a different unit that diverged from other individuals of the same genus. The tribe Diaphini (*L. dofleini* + *Diaphus* spp.) was only recovered via ML. The *Notoscopelus* spp. appeared mixed with the Myctophinae species via


H. benoiti (10)
H. hygomii (WM) (3)
H. hygomii (G) (3)
S. veranyi (3)
M. punctatum (9)
B. glaciale (WM) (5)
B. glaciale (G) (3)
E. risso (4)
N. kroyeri (G) (2)
N. elongatus (WM) (1)
N. bolini (4)
L. dofleini (WM) (5)
Diaphus sp. (G) (3)
D. metopoclampus (G) (1)
D.rafinesquii (G) (1)
D. theta (G) (1)
D. holti (WM) (4)
C. maderensis (9)
C. maderensis EU148111
L. pusillus (WM) (5)
L. tenuiformis (G) (3)
L. alatus (G) (1)
L. photonotus (G) (1)
L.crocodilus (WM) (4)
Myctophini
Electronini
Gymnoscopelini
Diaphini
Lampanyctini
Myctophinae
Lampanyctinae
MYCTOPHIFORMES
STOMIIFORMES

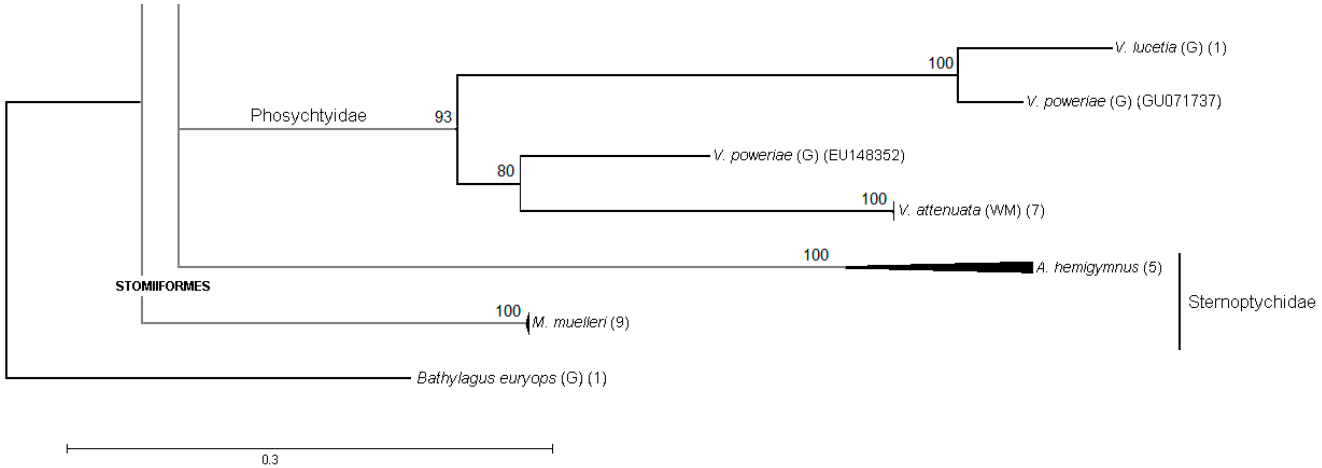


Fig. 1.1 Maximum Likelihood tree using CO1 sequences of different stages of species of Myctophiformes and Stomiiformes. Species were sequenced and downloaded from GenBank and inferred using the method based on the Hasegawa-Kishino-Yano model with the highest log likelihood (-6992.6923). A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories; +G, parameter=0.9685). The rate variation model allowed for some sites to be evolutionarily invariable (+I, 49.5529% sites). The analysis involved 111 nucleotide sequences. Clades represented by various individuals of the same species were contracted. Branches with the (WM) nomenclature correspond uniquely to sequences from the western Mediterranean (this study) and those with the (G) nomenclature were from GenBank. The rest of the branches depict a mixture of this study and GenBank sequences. Numbers in brackets represent the number of sequences for a clade. Bootstrap values are shown as percentages above the branches, and those values below 50 were not included.

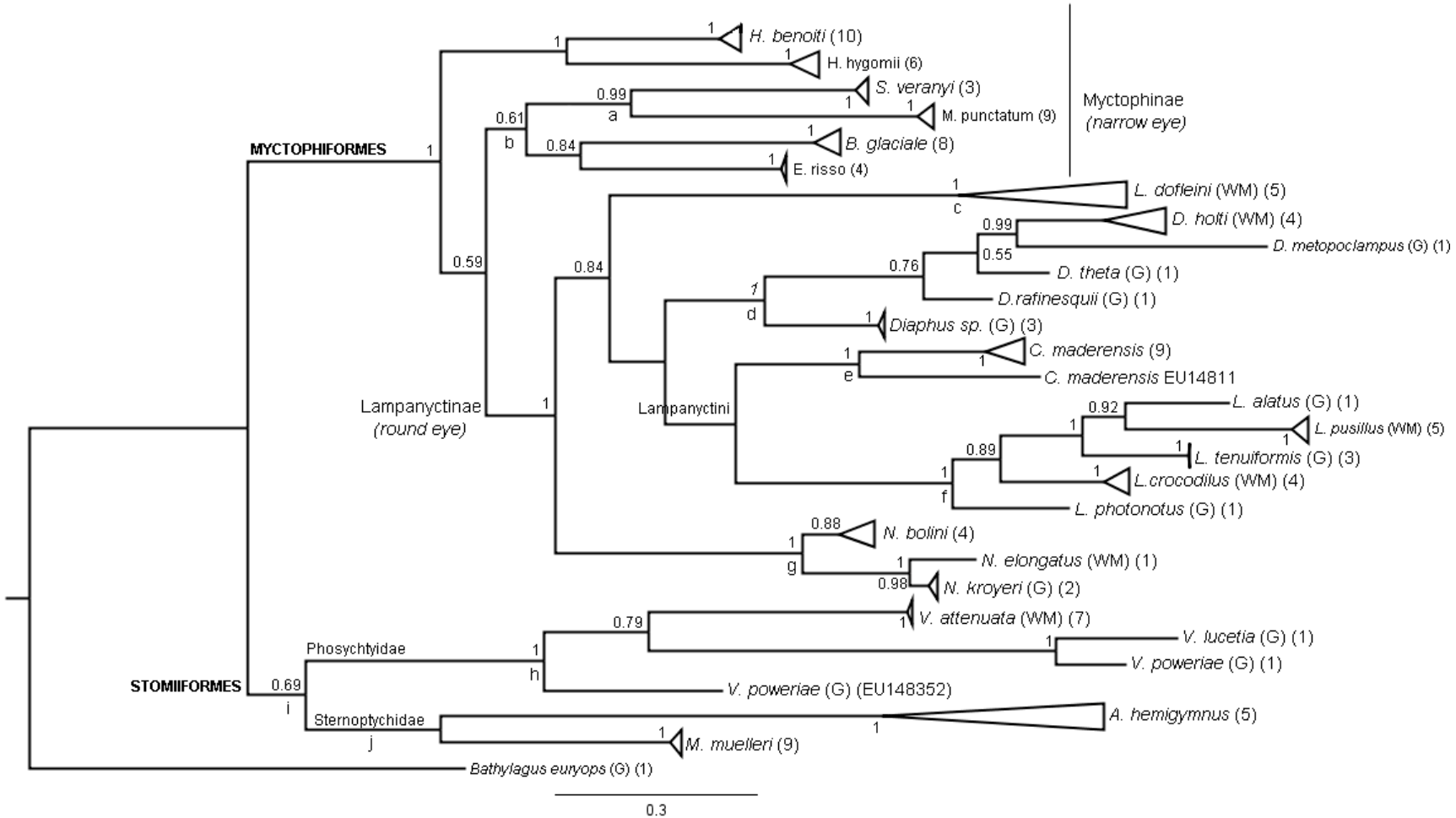


Fig. 1.2 Bayesian tree (split frequency=0.14; 500000 generations) involving the same 111 nucleotide sequences of larvae and adults as in the ML cladogram. Clades represented by various individuals of the same species were contracted. Branches with the (WM) nomenclature correspond uniquely to sequences from the western Mediterranean (this study) and those with the (G) nomenclature were from GenBank. The rest of branches depict a mixture of this study and GenBank sequences. Numbers in brackets represent the number of sequences for a clade. Letters in lower case close to some nodes present main morphological characters of larvae: a. pectoral fin moderately large and stalked eyes; b. slender body and gut length up to the mid-point of the body; c. conspicuous and very large pectoral fin; d. early photophore development; e. slender and slightly sigmoid gut; f. body moderately deep and S-shaped gut; g. pigment above brain and gas bladder; h. gut length c.a. the 75% of the body length; i. slender body and scant / lacking pigment; j. no pigmentation. Values of posterior probability are shown besides the nodes. The branches depict the proportional mean evolutionary distances among clades.

ML, but placed within the Lampanyctinae using BI, which was in agreement with the morphological classification. Within Myctophinae, *S. veranyi* and *Myctophum punctatum* Rafinesque 1810 formed a sister group with a support of 69% bootstrapping and 0.99 posterior probability using ML and BI, respectively.

The congeners *Hygophum benoiti* (Cocco, 1838) and *Hygophum hygomii* (Lütken, 1892) were clustered with bootstrap values over 80%; *L. pusillus* and *L. crocodilus* had values over 99%; and *N. bolini* and *N. elongatus* also had 99%, which underlies the monophyly of these genera. These nodes had 100% values using BI. The clades produced within the species accounted for values of 99-100% bootstrapping and 100% probability for the respective procedures. Some discrepancies between both evolutionary trees for the deeper nodes were evident and remained consistent at the genus and species levels.

In accordance with the main objective of this study, it can be demonstrated from both genealogies that the larvae identified using detailed morphological features corresponded with the adult specimens in the 11 species for which larvae were successfully sequenced. All specimens were clearly assigned to each species at 100% bootstrapping and posterior probability values. Therefore, the adult associations were well structured and could be used as a basis for FINS of the larvae, followed by phylogenetic reconstruction.

1.3.2 Analyses using mtDNA 12S rRNA

In the present work, all the species were sequenced with CO1, although some stomiiforms (*Cyclothone* spp.) did not render proper chromatograms. In these instances, the mtDNA 12S rRNA region was used. However, the disparity is unclear, as specimens were collected and stored in the same conditions as the rest of samples.

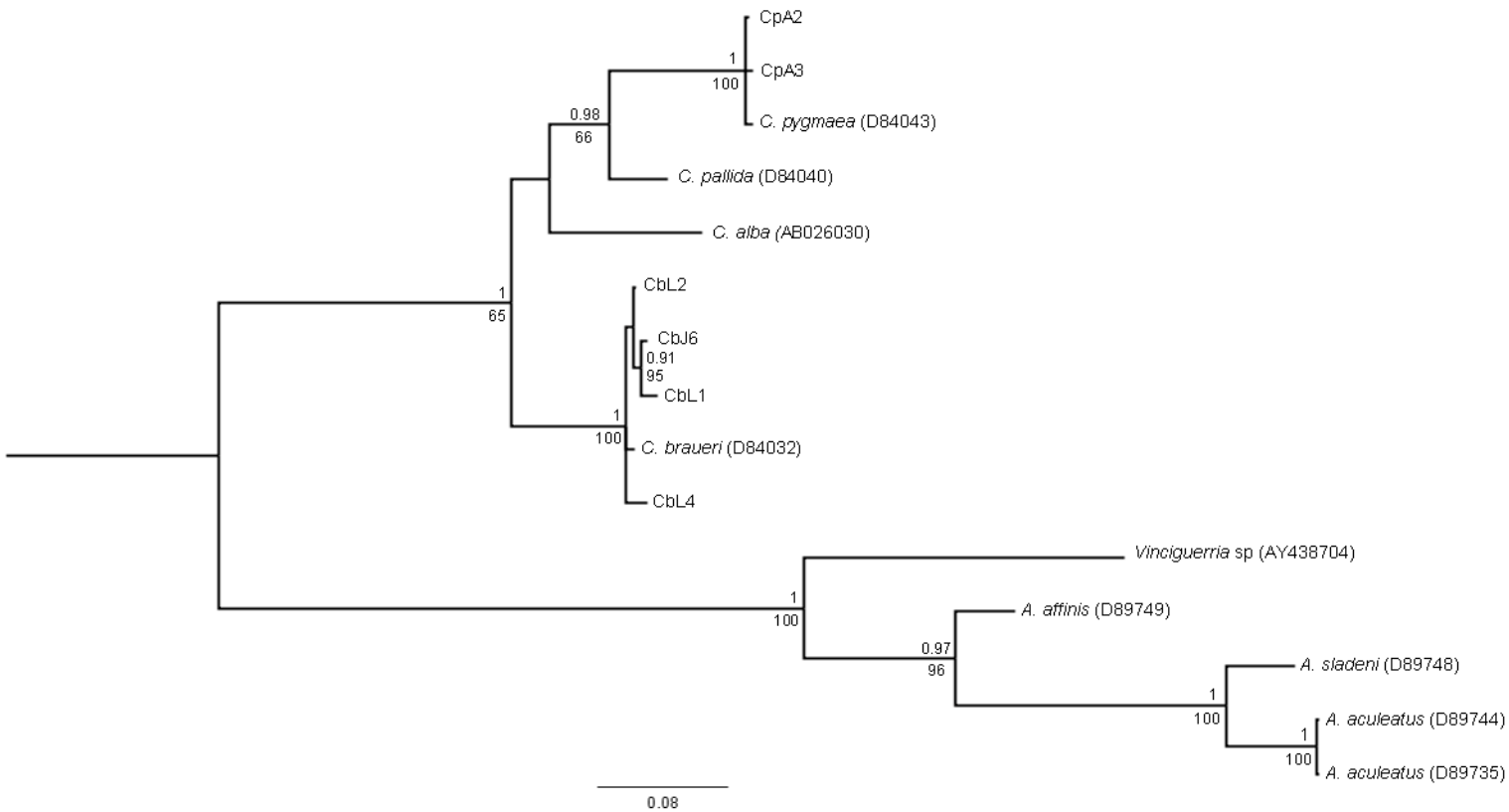


Fig. 1.3 Bayesian tree (in consensus with the ML and MP procedures) based on an alignment of 417 bp of 12S-rRNA mtDNA sequences. The branches depict the proportional mean evolutionary distances among clades. The posterior probability values of each node >0.9 are depicted above the branches, and the bootstrap values below the branches.

The validity of the mtDNA 12S rRNA sequences was briefly analyzed by depicting a tree of the *C. braueri* and *C. pygmaea* sequences from the Mediterranean in combination with GenBank sequences of other *Cyclothone* spp. and stomiiforms. The consensus trees obtained from BI and ML produced identical groupings (Fig. 1.3).

Six fragments of 375 nucleotides from three larvae and one adult *C. braueri*, and 2 adults of *C. pygmaea* were sequenced. Fifty-seven variable sites involving the 6 nucleotide sequences with low diversity (π=9.17·10^{-2}) were found. The 2 species of *Cyclothone* were strongly discriminated with bootstrap values of 100% and 100% posterior probability.

The species of *Vinciguerria* formed an assembly with 85% bootstrapping (*V. attenuata* was grouped with a value of 99%) and was joined to the sternoptychid species with a support of 61% by BI. The species *A. hemigymnus* and *M. muelleri* were clustered with values of 99% and 100% via ML and BI, respectively. Both species formed the sternoptychid cluster with a value of 99% probability using BI.

1.4 Discussion

Due to its rapid evolutionary rate, mtDNA has been used to discern between adults of closely related species, and is becoming a common tool for the identification of early developmental stages of fishes (Hare *et al.*, 1994; Pegg *et al.*, 2006; Ko *et al.*, 2013). The consistent genetic profiles between larvae and adults of all the analyzed mesopelagic fishes have proved the usefulness of this methodology for larval identification, and the accuracy of previous larval descriptions based on morphology and pigmentation patterns. The low species richness of mesopelagic fishes in the western Mediterranean, with just one or

two species per genus, makes it a good scenario for unequivocal identification when it comes to elusive characters such as those of larval stages.

The divergence values between nucleotide sequences give a perspective of the specific diversity of the sampling region (Steinke *et al.*, 2009). High sequence divergence between species compared with intraspecific variation indicates good barcode matching. For instance, the difficulties derived in the morphological identification of congeners within *Cyclothone*, *Hygophum, Lampanyctus* and *Notoscopelus* were solved through barcoding. The availability of large datasets of nucleotide sequences would be useful to design primers for particular species) in cases where morphological determination is difficult (Webb *et al.*, 2006; Ko *et al.*, 2013). As a result, the hybridization of unknown nucleotide sequences of mtDNA with fragments designed specifically, make possible an accurate species determination.

The significance of this study lies in the lack of previous larval identification by genetic methods to corroborate morphological classifications. Additionally, the study aimed to highlight the need for morphological identification in combination with DNA barcoding to detect the biodiversity of a region. As Barber and Boyce (2006) suggested, the synergy between taxonomists and geneticists advances our understanding of groups with high intraspecific genetic variation that could actually represent cryptic species.

Genetic analysis based on mtDNA has been useful in establishing phylogenetic relationships among mesopelagic and other deep-sea fishes (Miya and Nishida, 1998; Miya *et al.*, 2001; Poulsen *et al.*, 2013). The results presented here identified two wide clusters corresponding to the recognized monophyletic orders Myctophiformes and Stomiiformes (e.g. Paxton, 1972; Fink, 1984; Poulsen *et al.*, 2013) usign ML and BI methods (Fig. 1.1 and Fig. 1.2). The Bayesian tree was also able to recover the families Myctophidae, Phosycthyidae

and Sternoptychidae, and the subfamily Lampanyctinae. Nevertheless, the phylogenetic branches defined by morphological characteristics (Paxton, 1972; Paxton *et al.*, 1984) could be only identified in this study at a lower taxonomic level than tribe with well-structured congeneric groups. It is recognized that the use of concatenated, protein-coding genes or complementary mitochondrial regions is necessary to significantly categorize the higher ranks of taxonomy. It should be noted that to redefine the precise phylogenetic relationships of myctophiforms and stomiiforms was out of the main objective of the present study. However, some of the associations found within the subfamilies and sister groups deserve some comment.

The subfamily Lampanyctinae was represented by *Lampanyctus* spp. and *C. maderensis* within the tribe Lampanyctini, and *L. dofleini* and *Diaphus* spp. within the tribe Diaphini in agreement with the morphological classification by Paxton (1972) and Paxton *et al*. (1984). Paxton (op. cit.) stated lower divergence between *Lobianchia* and *Diaphus* than between *Lampanyctus* and *Ceratoscopelus*. The two former genera constituted a sister group that was characterized by the exclusive apomorphic state of having a wide pubic plate. However, the evolutionary distance within Diaphini (0.186, Appendix 1.4) was slightly lower than that within Lampanyctini (0.189).

Diaphus and *Lampanyctus* are greatly diversified genera with low morphological variation (Hulley, 1981). The observed average within-genus nucleotide diversity of *Diaphus* (4 species) and *Lampanyctus* (5 species) (10.7 and 10%, respectively) was nearly two times greater than that within *Notoscopelus* (3 species) (5.3%). Thus, it might reflect a more recent divergence of the genus *Notoscopelus.* This genus is represented in the Mediterranean by two species: *N. elongatus*, which is restricted to the Mediterranean Sea, and its congener *N. bolini,* which has also been captured in the Atlantic Ocean. *N.*

elongatus and *N. bolini* showed the minimum interspecific genetic distance between the Mediterranean species (6.22%), similar to other values reported for congeneric species in previous analyses of marine fishes (Ward *et al.*, 2005; Steinke *et al.*, 2009). Both congeners constitute separate species in the Mediterranean that are barely distinguished by the number of gill rakers and fin ray counts in adults (Hulley, 1984), and pigmentation features in larvae (Palomera, 1983). The low degree of morphological divergence was consistent with this study, suggesting a relatively recent separation of this genus in the Mediterranean. On the contrary, relatively high intraspecific divergence (2.1%) was found in *D. holti*, which could be masking cryptic species or reflecting an underlying population structure. Ideally, this could be overcome by an extensive within-species variation that embraces all of the species diversity (Viñas and Tudela, 2009).

In the subfamily Myctophinae, the sister groups *M. punctatum* and *S. veranyi* and *H. hygomii* and *H. benoiti* coincided with the morphological phylogeny (Paxton, 1972; Paxton *et al.*, 1984). *M. punctatum* and *S. veranyi* (tribe Myctophini) shared a slender larval morphotype, with a fan-shaped pectoral fin base and slightly stalked eyes (Appendix 1.3). Our molecular data produced high significance values (BI) to support the monophyly of *Hygophum*. The two species of *Hygophum* of the Mediterranean region were consistent with the morphological classification and with the genetic results reported by Yamaguchi *et al*. (2000).

Genetic divergence of the Mediterranean specimens with those from GenBank, which represent remote populations in some cases Atlantic or Pacific populations, revealed that some genera showed larger divergence than others. For instance, the intraspecific distance in *Benthosema glaciale* (Reinhardt, 1837) and *N. elongatus* from the Mediterranean *sensu stricto* was smaller than that

including individuals from other regions (i.e. GenBank sequences), which coincides with the assumption of different populations in the Mediterranean and North Atlantic.

Analysis of mtDNA 12S rRNA sequences for the Mediterranean *Cyclothone*, along with other GenBank sequences of stomiiform, produced clusters (BI, ML) in agreement with the cladograms of Weitzman (1974) and Fink (1984) using morphological characters. The species of the family Sternoptychidae and Phosichthyidae appeared closer than to the gonostomatid *Cyclothone* spp. The sternoptychids *M. muelleri* and *A. hemigymnus* were also clustered together using CO1 sequences.

The genus *Maurolicus* deserves special mention as it has been challenging in terms of species identification. Whether it is composed by a single species, *M. muelleri*, with wide distribution in the Atlantic, Indian and Pacific Oceans, or includes up to fifteen species as stated by Parin and Kobyliansky (1996), remains unclear. Mediterranean specimens of *M. muelleri* presented similar haplotypes with low genetic variation and were grouped with GenBank sequences from Atlantic specimens, revealing they originated from the same species.

In summary, the results of the present study highlighted the validity of DNA barcoding to differentiate the Mediterranean mesopelagic fish at the species level, even amongst those with high morphological resemblance. The good fit of genetic sequences between larvae and adults of each species proved the accuracy of earlier larval descriptions based on morphology and pigmentation characters. Phylogenetic relationships of myctophiforms and stomiiform species still require additional sequencing of mitochondrial or nuclear *loci* to be further resolved. Nevertheless, the use of long CO1 sequences allowed similar grouping of some tribes accepted in the current phylogeny.

Appendix 1.1
Literature containing information on larvae and adult identification characteristics of the mesopelagic fishes analyzed in this study.

	Larvae	Adults
O. Myctophiformes		
F. Myctophidae		
SF. Lampanyctinae		
Ceratoscopelus maderensis	Tåning, 1918	Lowe, 1839; Tåning, 1918; Hulley, 1984
Diaphus holti	Tåning, 1918	Tåning, 1918; Hulley, 1984
Lampanyctus crocodilus	Tåning, 1918	Risso, 1810; Tåning, 1918; Hulley, 1984
Lampanyctus pusillus	Tåning, 1918, Olivar *et al.* 1999	Johnson, 1890; Tåning, 1918; Hulley, 1984
Lobianchia dofleini	Tåning, 1918; Berdar and Cavaliere, 1975	Zugmayer, 1911;Tåning, 1918; Hulley, 1984
Notoscopelus bolini	Palomera, 1983	Nafpaktitis, 1975; Hulley, 1984
Notoscopelus elongatus	Tåning, 1918; Palomera, 1983	Costa, 1844; Tåning, 1918; Hulley, 1984
SF. Myctophinae		
Benthosema glaciale	Tåning, 1918	Reinhardt, 1837; Tåning, 1918; Hulley, 1984
Electrona risso	Tåning, 1918	Cocco, 1829; Tåning, 1918; Hulley, 1984
Hygophum benoiti	Tåning, 1918; Olivar and Palomera, 1994	Tåning, 1918; Cocco, 1838; Hulley, 1984
Hygophum hygomii	Tåning, 1918; Olivar and Palomera, 1994	Lütken, 1892; Tåning, 1918; Hulley, 1984
Myctophum punctatum	Tåning, 1918	Rafinesque, 1810; Tåning, 1918; Hulley, 1984
Symbolophorus veranyi	Tåning, 1918	Moreau, 1888; Tåning, 1918; Hulley, 1984
O. Stomiiformes		
F. Sternoptychidae		
Argyropelecus hemigymnus	Jespersen and Tåning, 1926	Cocco, 1829; Badcock, 1984
Maurolicus muelleri	Jespersen and Tåning, 1926, Sanzo, 1931	Gmelin, 1788; Jespersen and Tåning, 1926, Sanzo, 1931
F. Gonostomatidae		
Cyclothone braueri	Jespersen and Tåning, 1926	Jespersen and Tåning, 1926
Cyclothone pygmaea	Jespersen and Tåning, 1926	Jespersen and Tåning, 1926
F. Phosichthyidae		
Vinciguerria attenuata	Jespersen and Tåning, 1926, Sanzo, 1931	Cocco, 1838; Jespersen and Tåning, 1926, Sanzo, 1931

Appendix 1.2

List of sequences of myctophids and stomiiforms used in the present study. Codes for the sequences from our lab are shown on the right part of the table. Apart from our results, the sequences that were used in this study and selected from GenBank (http://www.ncbi.nlm.nih.gov/genbank) are listed with the corresponding accession number and source. The outgroup species *Bathylagus euryops* was included.

Family	Species / Genus	No. Nucleotides	Genetic marker	Source Identifier	Accesion number	Abbreviation
Sternoptychidae	*Argyropelecus hemigymnus*	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616410	AhA2
	Argyropelecus hemigymnus	650	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148085	
	Argyropelecus hemigymnus	651	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148086	
	Argyropelecus hemigymnus	651	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148087	
	Argyropelecus hemigymnus	652	cytochrome oxidase subunit I (COI) gene	Hastings, P. and Burton, R., 2010	GU440233	
	Argyropelecus affinis	391	mitochondrial gene for subunit 12S rRNA	Miya, M. and Nishida, M., 1998	D89749	
	Argyropelecus sladeni	391	mitochondrial gene for subunit 12S rRNA	Miya, M. and Nishida, M., 1998	D89748	
	Argyropelecus aculeatus	391	mitochondrial gene for subunit 12S rRNA	Miya, M. and Nishida, M., 1998	D89744	
	Argyropelecus aculeatus	391	mitochondrial gene for subunit 12S rRNA	Miya, M. and Nishida, M., 1998	D89735	
Myctophidae	*Benthosema glaciale*	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616365	BgL1
	Benthosema glaciale	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616366	BgA2
	Benthosema glaciale	566	cytochrome oxidase subunit I (COI) gene	"This study"	KC616367	BgT3
	Benthosema glaciale	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616368	BgA4
	Benthosema glaciale	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616369	BgA5
	Benthosema glaciale	648	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148097	
	Benthosema glaciale	648	cytochrome oxidase subunit I (COI) gene	Keskin,E., 2010	HQ167646	
	Benthosema glaciale	624	cytochrome oxidase subunit I (COI) gene	Sweetman, C. J., 2009	EU148098	
Gonostomatidae	*Cyclothone braueri*	348	mitochondrial gene for subunit 12S rRNA	Miya, M. and Nishida, M., 1996	D84032	
	Cyclothone braueri	372	mitochondrial gene for subunit 12S rRNA	"This study"	KC616355	CbL1
	Cyclothone braueri	372	mitochondrial gene for subunit 12S rRNA	"This study"	KC616353	CbL2
	Cyclothone braueri	372	mitochondrial gene for subunit 12S rRNA	"This study"	KC616356	CbL4
	Cyclothone braueri	372	mitochondrial gene for subunit 12S rRNA	"This study"	KC616354	CbJ6
	Cyclothone pygmea	372	mitochondrial gene for subunit 12S rRNA	"This study"	KC616357	CpA2
	Cyclothone pygmea	372	mitochondrial gene for subunit 12S rRNA	"This study"	KC616358	CpA3
	Cyclothone pallida	349	mitochondrial gene for subunit 12S rRNA	Miya, M. and Nishida, M., 1996	D84040	
	Cyclothone alba	329	mitochondrial gene for subunit 12S rRNA	Miya, M. and Nishida, M., 1996	AB026030	
	Cyclothone pygmea	349	mitochondrial gene for subunit 12S rRNA	Miya, M. and Nishida, M., 1996	D84043	
Myctophidae	*Ceratoscopelus maderensis*	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616359	CmL1
	Ceratoscopelus maderensis	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616360	CmL2
	Ceratoscopelus maderensis	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616361	CmL3
	Ceratoscopelus maderensis	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616362	CmL4
	Ceratoscopelus maderensis	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616363	CmA5
	Ceratoscopelus maderensis	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616364	CmA6
	Ceratoscopelus maderensis	650	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148109	
	Ceratoscopelus maderensis	630	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148110	
	Ceratoscopelus maderensis	494	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148111	
	Diaphus holtii	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616388	DhL1
	Diaphus holtii	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616389	DhA2
	Diaphus holtii	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616390	DhA5
	Diaphus holtii	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616391	DhA6
	Diaphus theta	652	cytochrome oxidase subunit I (COI) gene	Steinke, D., Zemlak, T. S., Gavin, H. and Hebert, P. D. N., 20	FJ164561	
	Diaphus rafinesquii	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148154	
	Diaphus metopoclampus	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148151	
	Electrona risso	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616411	ErT1

Family	Species / Genus	No. Nucleotides	Genetic marker	Source Identifier	Accesion number	Abbreviation
	Hygophum benoiti	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616370	HbL1
	Hygophum benoiti	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616371	HbL2
	Hygophum benoiti	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616372	HbT4
	Hygophum benoiti	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616373	HbA5
	Hygophum benoiti	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616374	HbA6
	Hygophum benoiti	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616375	HbA7
	Hygophum benoiti	648	cytochrome oxidase subunit I (COI) gene	Keskin, E., 2010	HQ167651	
	Hygophum benoiti	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148200	
	Hygophum benoiti	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148201	
	Hygophum benoiti	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148202	
	Hygophum hygomii	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616376	HhA1
	Hygophum hygomii	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616377	HhA2
	Hygophum hygomii	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616378	HhA3
	Hygophum hygomii	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148204	
	Hygophum hygomii	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148205	
	Hygophum hygomii	831	cytochrome oxidase subunit I (COI) gene	DeVaney, S. C., 2009	FJ918945	
	Lampanyctus alatus	594	cytochrome oxidase subunit I (COI) gene	Bucklin, A. *et al.* , 2009	GU071738	
	Lampanyctus festivus	613	cytochrome oxidase subunit I (COI) gene	Gleason,L.U., Walker,H.J., Hastings,P.A. and Burton,R.S., 2(	GU441539	
	Lampanyctus photonotus	623	cytochrome oxidase subunit I (COI) gene	Bucklin,A. et al.; 2009	GU071732	
	Lampanyctus tenuiformis	595	cytochrome oxidase subunit I (COI) gene	Gruenthal, K.M. *et al* ., 2008	EU489716	
	Lampanyctus tenuiformis	652	cytochrome oxidase subunit I (COI) gene	Hastings, P. and Burton, R., 2009	GU440365	
	Lampanyctus tenuiformis	652	cytochrome oxidase subunit I (COI) gene	Hastings, P. and Burton, R., 2009	GU440366	
	Lampanyctus crocodilus	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616379	LcL1
	Lampanyctus crocodilus	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616380	LcA2
	Lampanyctus crocodilus	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616381	LcA3
	Lampanyctus crocodilus	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616382	LcA5
	Lampanyctus pusillus	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616383	LpL1
	Lampanyctus pusillus	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616384	LpL2
	Lampanyctus pusillus	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616385	LpA3
	Lampanyctus pusillus	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616386	LpA4
	Lampanyctus pusillus	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616387	LpL6
	Lobianchia dofleini	564	cytochrome oxidase subunit I (COI) gene	"This study"	KF143896	LdL1
	Lobianchia dofleini	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616404	LdL2
	Lobianchia dofleini	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616405	LdA3
	Lobianchia dofleini	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616406	LdA4
	Lobianchia dofleini	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616407	LdA5
	Lobianchia sp --> *Diaphus dumerilii*	652	cytochrome oxidase subunit I (COI) gene	Valdez-Moreno, M. *et al.* , 2009	GU224892	
	Lobianchia sp --> *Diaphus dumerilii*	652	cytochrome oxidase subunit I (COI) gene	Valdez-Moreno, M. *et al.* , 2009	GU224894	
	Lobianchia sp --> *Diaphus dumerilii*	652	cytochrome oxidase subunit I (COI) gene	Valdez-Moreno, M. *et al.* , 2009	GU224895	
Sternoptychidae	*Maurolicus muelleri*	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616401	MmL1
	Maurolicus muelleri	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616398	MmA2
	Maurolicus muelleri	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616399	MmA3
	Maurolicus muelleri	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616402	MmL4
	Maurolicus muelleri	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616403	MmL5

Family	Species / Genus	No. Nucleotides	Genetic marker	Source Identifier	Accesion number	Abbreviation
	Maurolicus muelleri	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616400	MmA6
	Maurolicus muelleri	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148245	
	Maurolicus muelleri	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148246	
	Maurolicus muelleri	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148247	
Myctophidae	*Myctophum punctatum*	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616392	MpL1
	Myctophum punctatum	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616393	MpA2
	Myctophum punctatum	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616394	MpL3
	Myctophum punctatum	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616395	MpL4
	Myctophum punctatum	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616396	MpA5
	Myctophum punctatum	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616397	MpA6
	Myctophum punctatum	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148251	
	Myctophum punctatum	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148252	
	Myctophum punctatum	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148253	
	Notoscopelus bolini	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616408	NbA1
	Notoscopelus bolini	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148275	
	Notoscopelus bolini	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148276	
	Notoscopelus bolini	648	cytochrome oxidase subunit I (COI) gene	Keskin, E., 2010	HQ167653	
	Notoscopelus elongatus	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616409	NeA1
	Symbolophorus veranyi	565	cytochrome oxidase subunit I (COI) gene	"This study"	KF143897	SvA3
	Symbolophorus veranyi	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148338	
	Symbolophorus veranyi	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148339	
	Symbolophorus veranyi	652	cytochrome oxidase subunit I (COI) gene	Zhang, J., Byrkjedal, I. and Hanner, R., 2007	EU148340	
Phosichthyidae	*Vinciguerria attenuata*	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616412	VaA1
	Vinciguerria attenuata	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616413	VaA2
	Vinciguerria attenuata	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616414	VaA3
	Vinciguerria attenuata	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616415	VaA4
	Vinciguerria attenuata	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616416	VaA5
	Vinciguerria attenuata	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616417	VaA6
	Vinciguerria attenuata	565	cytochrome oxidase subunit I (COI) gene	"This study"	KC616418	VaA7
	Vinciguerria sp	307	mitochondrial gene for subunit 12S rRNA	Lopez, J. A., Chen, W. J. and Orti, G., 2004	AY438704	
Microstomatidae	*Bathylagus euryops*	652	cytochrome oxidase subunit I (COI) gene	Bentley, A. C. and Wiley, E. O., 2013	KF929651	

Appendix 1.3

Distinctive features of larvae of the most abundant and frequent mesopelagic species occurring in the western Mediterranean. Sources of larval descriptions referred in Appendix 1.1.

	EYES SHAPE		HEAD	BODY SHAPE		GUT LENGTH [1]		PECTORAL FIN
	Narrow	Ovalate-Round	Conspicuous morphology	Deep	Slender	Short	Moderately long	Conspicuous features
O. Myctophiformes								
F. Myctophidae								
SF. Lampanyctinae								
Ceratoscopelus maderensis		+			+		+	
Diaphus holti		+			+		+	
Lampanyctus crocodilus		+	moderately deep		+	+		
Lampanyctus pusillus		+	deep, snout blunt	+		+		
Lobianchia dofleini		+	broad with large snout	+		+		large , base wing-shaped with upper rays longer
Notoscopelus bolini		+	moderately deep	+		+		
Notoscopelus elongatus		+	moderately deep	+		+		
SF. Myctophictinae								
Benthosema glaciale	+		moderately deep		+	+		
Electrona risso	+		large, broad		+	+		
Hygophum benoiti	+				+		+	
Hygophum hygomii	+				+		+	
Myctophum punctatum	+		broad and flat		+		+	large
Symbolophorus veranyi	+		broad and flat		+		+	large , base wing-shaped
O. Stomiiformes								
F. Sternoptychidae								
Argyropelecus hemigymnus	+		moderately deep, snout blunt		+	+		
Maurolicus muelleri		+			+		+	
F.Gonostomatidae								
Cyclothone braueri		+			+		+	
Cyclothone pygmea		+			+		+	
F.Phosichthhyidae								
Vinciguerria attenuata	+		flat		+		+	

Appendix 1.3 (cont.)

	DISTINCTIVE PIGMENTATION LOCATION							
	Above head	Snout or Jaws	Gut	Ventral tail	Dorsal tail	Hypaxial	Caudal base	Pectoral fin
O. Myctophiformes								
F. Myctophidae								
SF. Lampanyctinae								
Ceratoscopelus maderensis	-	-	anus	series of spots	+	-	-	-
Diaphus holti	-	-	lateral, swim bladder, anus	series of spots (preflexion)	-		+	-
Lampanyctus crocodilus	+	-	anus	-	in postflexion	in postflexion	-	+
Lampanyctus pusillus	+	+	anus	-	+	+	-	+
Lobianchia dofleini	-	-	anus, dorsal surface	1 melanophore at anal fin	-		+	+
Notoscopelus bolini	+	+	anus, swin bladder	several spots	+	+	-	-
Notoscopelus elongatus	+	+	anus, swin bladder	one spot	+	-	-	-
SF. Myctophictinae								
Benthosema glaciale	+	+	anus	-	-		-	-
Electrona risso	-	+	-	-	-		-	+
Hygophum benoiti	-	+	lateral and anus	2-3 spots (preflexion)	-		+	-
Hygophum hygomii	-	+	lateral wall in preflexion, anus	1 conspicuous spot (preflexion)	-		-	+
Myctophum punctatum	+	+	ventral wall, anus	series of spots (preflexion)	+		+	+
Symbolophorus veranyi	+	+	lateral wall in preflexion, anus	series of spots (preflexion)	-		-	+
O. Stomiiformes								
F. Sternoptychidae								
Argyropelecus hemigymnus	-	-	-	-	-	-	-	-
Maurolicus muelleri	-	-	-	-	-	-	-	-
F.Gonostomatidae								
Cyclothone braueri	-	-	anus, swim bladder	11-12 melanophores	-	-	+	-
Cyclothone pygmea	-	-	anus, swim bladder	<8 melanophores	-	-	+	-
F.Phosichthhyidae								
Vinciguerria attenuata	-	-	-	-	+	-	+	-

Appendix 1.4

Estimates of the Net Evolutionary Divergence between species considering larval and adult sequences from our lab and GenBank. The number of base substitutions per site by estimating the net average between groups of sequences are shown. Standard error estimates are shown above the diagonal. Analyses were conducted using the Maximum Composite Likelihood model. The rate variation among sites was modeled using gamma distribution (shape parameter=5). The analysis involved 110 nucleotide sequences. The codon positions that were included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair, and 570 positions were included in the final dataset. Abbreviated names: AH, *Argyropelecus hemigymnus*; BG, *Benthosema glaciale*; CM, *Ceratoscopelus maderensis;* DH, *Diaphus holti*; ER, *Electrona risso*; VA, *Vinciguerria attenuata*; HB, *Hygophum benoiti*; HH, *Hygophum hygomii*; LA, *Lampanyctus alatus*; LC, *Lampanyctus crocodilus*; LD, *Lobianchia dofleini;* LP, *Lampanyctus pusillus*; Lph, *Lampanyctus photonotus*; LT, *Lampanyctus tenuiformis*; MM, *Maurolicus muelleri;* MP, *Myctophum punctatum;* NB, *Notoscopelus bolini*; NE, *Notoscopelus elongatus*; SV, *Symbolophorus veranyi*; VP, *Vinciguerria poweriae.*

	AH	BG	CM	DH	ER	VA	HB	HH	LA	LC	LD	LP	Lph	LT	MM	MP	NB	NE	SV	VP
AH		0.022	0.024	0.022	0.025	0.024	0.024	0.025	0.026	0.023	0.026	0.025	0.024	0.023	0.022	0.025	0.026	0.024	0.024	0.019
BG	0.216		0.021	0.020	0.018	0.027	0.021	0.021	0.024	0.023	0.024	0.023	0.024	0.023	0.024	0.021	0.020	0.020	0.020	0.020
CM	0.249	0.197		0.021	0.022	0.025	0.023	0.022	0.019	0.018	0.022	0.021	0.019	0.020	0.024	0.024	0.021	0.022	0.022	0.017
DH	0.209	0.195	0.197		0.020	0.027	0.020	0.022	0.023	0.022	0.020	0.022	0.021	0.023	0.024	0.022	0.022	0.020	0.021	0.017
ER	0.248	0.153	0.207	0.194		0.026	0.020	0.021	0.024	0.022	0.024	0.022	0.023	0.022	0.024	0.021	0.020	0.020	0.019	0.018
VA	0.257	0.269	0.254	0.281	0.266		0.028	0.027	0.029	0.029	0.026	0.029	0.028	0.028	0.025	0.027	0.025	0.025	0.026	0.015
HB	0.233	0.195	0.228	0.187	0.182	0.284		0.017	0.023	0.024	0.025	0.023	0.023	0.023	0.023	0.022	0.020	0.020	0.020	0.019
HH	0.257	0.205	0.218	0.215	0.193	0.280	0.137		0.025	0.024	0.021	0.022	0.025	0.024	0.024	0.021	0.022	0.022	0.021	0.018
LA	0.248	0.230	0.176	0.216	0.242	0.289	0.228	0.242		0.016	0.024	0.015	0.016	0.014	0.025	0.025	0.022	0.023	0.025	0.021
LC	0.227	0.218	0.170	0.201	0.203	0.285	0.232	0.235	0.127		0.024	0.016	0.014	0.014	0.026	0.024	0.022	0.022	0.023	0.019
LD	0.266	0.228	0.193	0.186	0.231	0.255	0.242	0.194	0.246	0.235		0.023	0.026	0.023	0.025	0.023	0.025	0.024	0.022	0.018
LP	0.240	0.221	0.189	0.206	0.208	0.297	0.222	0.213	0.111	0.135	0.232		0.018	0.016	0.026	0.023	0.022	0.023	0.023	0.019
Lph	0.247	0.222	0.174	0.195	0.218	0.281	0.220	0.249	0.127	0.108	0.251	0.148		0.015	0.027	0.024	0.021	0.021	0.023	0.019
LT	0.231	0.214	0.187	0.218	0.211	0.283	0.223	0.232	0.100	0.107	0.230	0.125	0.118		0.023	0.024	0.020	0.020	0.023	0.018
MM	0.204	0.244	0.236	0.233	0.234	0.255	0.221	0.235	0.234	0.253	0.232	0.253	0.268	0.219		0.025	0.025	0.024	0.024	0.018
MP	0.270	0.201	0.228	0.214	0.195	0.269	0.204	0.187	0.250	0.229	0.215	0.218	0.234	0.239	0.240		0.022	0.022	0.019	0.018
NB	0.251	0.173	0.196	0.206	0.187	0.246	0.182	0.219	0.189	0.206	0.231	0.209	0.184	0.177	0.247	0.211		0.010	0.023	0.018
NE	0.237	0.179	0.207	0.193	0.185	0.258	0.181	0.211	0.200	0.206	0.228	0.218	0.187	0.176	0.249	0.205	0.062		0.023	0.019
SV	0.234	0.184	0.207	0.201	0.173	0.267	0.188	0.196	0.241	0.222	0.208	0.220	0.213	0.219	0.233	0.170	0.213	0.220		0.017
VP	0.152	0.143	0.103	0.113	0.137	0.093	0.146	0.135	0.161	0.143	0.124	0.150	0.145	0.135	0.127	0.135	0.133	0.132	0.122	

1 b. Length-weight relationships of mesopelagic fishes in the north-western Mediterranean

Abstract

In the present study we analysed the length-weight relationships of the most common and abundant mesopelagic fishes of the northwestern Mediterranean: 11 Myctophidae, 1 Gonostomatidae, 1 Phosichthyidae and 1 Sternoptichydae. Data of fish length and weight were fitted to a power function, and estimations of the fitted equation parameters were given as background information for subsequent studies on body condition. The slope estimation was taken as indicative of the relative increase in weight in relation to growth in length. The small fish *Cyclothone braueri* has a lower allometric coefficient, with a significant negative value, than that corresponding to larger species such as myctophids (some of them showing positive allometric growth).

The largest biomasses of the open ocean have been attributed to mesopelagic and bathypelagic fishes that occur together with small crustaceans, cephalopods and a few other invertebrates. The most common and abundant mesopelagic fishes in the north-western Mediterranean are the lanternfish of the family Myctophidae and bristelmouth of family Gonostomatidae (Goodyear *et al.*, 1972a; Olivar *et al.*, 2012). The diversity of these groups of species in the Mediterranean Sea is lower than in open oceans and the maximum sizes tend to be smaller than those reported for the same species in the Atlantic. They constitute a key component of the food web in oceanic waters, being commonly cited as prey for larger marine inhabitants (e.g. commercial pelagic fishes, cetaceans, marine birds).

In the present study, the body length- weight relationships of the most common and frequent mesopelagic fishes in the north-western Mediterranean were analysed. Information regarding to fish allometry has been indicated as an important issue to approach body condition (Safran, 1992; Petrakis and Stergiou, 1995). However, data to allow allometric scaling in fishes is not always complete and lack for many species, particularly for those inhabiting the water column, which are less frequently sampled in routine surveys. In this aspect, information has been already presented for some meso- and bathypelagic species of the central Mediterranean (Battaglia *et al.*, 2010). The mesopelagic fishes studied here were collected during autumn (December 2009) and summer (July 2010) off Mallorca Island by means of different midwater trawls towed in the epipelagic layers (up to 80 m), the 400 m deep scattering layer (DSL) and benthic boundary layer (50 m above the bottom, BBL).

After collection and identification to species level on board, fishes were preserved in formalin 5%. Measures of fish length and weight were taken in the laboratory (length to the nearest mm and weight to the nearest mg). Length and

weight parameters were fitted to a potential equation $W=aSL^b$, where W is the weight in mg, SL is the standard length in mm, *a* is the intercept and *b* the allometric coefficient. Fitting was performed after the logarithmic transformation of the data, and results are given as 95% confidence intervals (95% CI). The values for coefficient *b* that were significantly different from 3 indicated that fishes growth relatively faster in weight than in length (positive allometry $b>3$) or, on the contrary, more slowly (negative allometry $b<3$). Mathematically, the values of the intercept, *a*, indicated the expected weight at SL=1 mm.

As generally found in many other fish species, the obtained length-weight relationships for mesopelagic fishes in the present study as well, as in those from the central Mediterranean (Battaglia *et al.*, 2010) fit a potential function with a coefficient of nearly 3 and correlation coefficients always close to 1 (Table 1 b). Significantly positive allometric relationships were observed for several myctophid species, i.e. *Benthosema glaciale, Ceratoscopelus maderensis, Diaphus holti, Lampanyctus crocodilus, L. pusillus, Lobianchia dofleini, Notoscopelus elongatus* and *Symbolophorus veranyi*. Comparisons of autumn and summer cruises (if available data allow it) (i.e. for *B. glaciale, C. maderensis, L. crocodilus* and *L. pusillus*) gave the same allometric patterns for both periods. Significant negative allometry was only observed for the gonostomatid *Cyclothone braueri,* indicating more slowly growth in weight than in length throughout development. Therefore, *C. braueri* has a pronounced slender body shape in contrast to those species with positive allometry, such as myctophids. Differences in body shape could be related to the different behaviour of myctophids, characterized by performing extensive diel migrations from near the bottom to the epipelagic layers, whereas *Cyclothone braueri* is a non-migratory species (Goodyear *et al.*, 1972a; Olivar *et al.*, 2012). Having a robust body with a relatively high muscular and osteological development must

act as an adaptive contribution to the vertical migration, while the more attenuated shape of *Cyclothone braueri* reflects a less energetic demanding behaviour.

Table 1 b

Parameters of the allometric relationship between body length (SL mm) and weight (mg) for the most common and abundant mesopelagic fishes occurring in the upper 400 m of the water column in the western Mediterranean. *a*: intercept, *b*: slope (allometric coefficient), 95% CI: 95% confidence interval, n: number of measured individuals, r: correlation coefficient. Significant positive or negative allometry is denoted by an asterisk (*).

	a	***b***	**95% CI**	**n**	**r**	**SL range**
F. Gonostomatidae						
Cyclothone braueri	0.008	-2.769*	0.173	113	0.9488	12-27
F. Sternoptychidae						
Argyropelecus hemigymnus	0.018	3.032	0.179	63	0.9744	13-34
F. Phosichthyidae						
Vinciguerria attenuata	0.010	2.942	0.190	26	0.9884	14-35
F. Myctophidae						
Benthosema glaciale	0.008	3.093*	0.088	249	0.9751	14-47
Ceratoscopelus maderensis	0.005	3.191*	0.048	188	0.9947	16-64
Diaphus holti	0.004	3.360*	0.207	32	0.9866	25-53
Hygophum benoiti	0.015	2.938	0.133	34	0.9921	13-48
Hygophum hygomii	0.010	3.136	0.326	40	0.9533	39-58
Lampanyctus crocodilus	0.002	3.345*	0.089	117	0.9898	22-128
Lampanyctus pusillus	0.004	3.232*	0.059	238	0.9902	14-43
Lobianchia dofleini	0.005	3.338*	0.279	53	0.9587	21-43
Myctophum punctatum	0.009	3.052	0.175	37	0.9864	19-60
Notoscopelus elongatus	0.004	3.248*	0.069	209	0.9883	30-107
Symbolophorus veranyi	0.005	3.181*	0.100	25	0.9974	23-90

Chapter 2

VERTICAL DISTRIBUTION

2. Vertical distribution, diversity and assemblages of mesopelagic fishes in the western Mediterranean

Abstract

The mesopelagic fish community of the western Mediterranean was studied during two cruises carried out in December 2009 and July 2010 in the shelf and slope zones around the Balearic Islands. Much of what was previously known about this deep water group of fishes in the Mediterranean Sea came from studies performed using planktonic and small midwater nets. This study was the first attempt to use large pelagic trawls and small nets combined with information about the main sound scattering layers to analyse mesopelagic fish composition, diversity and species assemblages. This community is characterised by a relatively low diversity compared to other oceanic regions of the world, with Myctophiformes and Stomiiformes being the main contributors. Bathymetry and the level of the water column were the most important factors structuring the investigated fish assemblages, and similar vertical patterns were observed for the different species collected during the two study periods. A shelf assemblage composed of a few species of myctophids, with *Notoscopelus elongatus* being the main contributor, was distinguished. The slope assemblage included both Myctophiformes and Stomiiformes that showed differences in their day and night main location along the water column. In terms of species behaviour, two important groups were detected. The first was non-migrant or weakly migrant species, with the paradigmatic example being the gonostomatid *Cyclothone braueri*, which occurred at a depth of 400-600 m; this species is partly responsible for the permanent acoustic (38 kHz) response at this depth. The second group, near-surface migrants at night, was represented by most of the juvenile and adult myctophids, exemplified by *Ceratoscopelus maderensis*, with the exception of just a few of the largest size classes of some species, such as *Lampanyctus crocodilus* and *Notoscopelus elongatus* that remain near the bottom.

2.1 Introduction

Mesopelagic fishes are numerically the most important fish component of the oceanic waters of all temperate and tropical regions in the world, with Myctophiformes and Stomiiformes species being the main representatives (Gjøsæter and Kawaguchi, 1980; Hulley, 1994; Sassa *et al.*, 2002). This group of small fishes constitutes an important component of the food web in these ecosystems, linking zooplankton (their main prey) to top predators, such as larger pelagic and benthic fishes and mammals (Williams *et al.*, 2001; Cherel *et al.*, 2008). In spite of their small size, extremely high biomasses have been reported for some of these fishes, such as for the myctophids *Benthosema pterotum* in the Arabian sea (Gjøsæter, 1981) and *Lampanyctodes hectoris* in the Benguela system, SE Atlantic (Hulley, 1986). Their abundance and diversity change depending on the region, from certain areas being dominated by a few species, such as the two previously mentioned or like *Benthosema glaciale* in Nova Scotia (NW Atlantic) (Sameoto, 1988), or the stomiiform *Cyclothone braueri* in the western central Atlantic (Badcock and Merret, 1976), to others, as in southern Australia, where the community is more diverse (Williams and Koslow, 1997).

A distinguishing feature of adult stages of many of the species in this group is their wide vertical distribution range in the water column, from near surface to depths >1000 m (Badcock, 1970; Hulley, 1981; Gartner *et al.*, 1987), although their larvae develop in the upper 200 m (Loeb, 1979; Sabatés, 2004; Sassa *et al.*, 2004; Olivar *et al.*, 2010). Classical studies on the systematics and distribution patterns of midwater fishes carried out during the second half of the 20th century in different geographical regions indicated that many species, particularly those belonging to the family Myctophidae, perform important vertical migrations

(Clarke, 1973; Badcock and Merret, 1976; Pearcy *et al.*, 1977; Hulley, 1984; Watanabe *et al.*, 1999; Hidaka *et al.*, 2003).

Many of the mesopelagic fishes with well-developed swimbladders have also been found to be the usual components of and are mostly responsible for sound scattering layers (SSLs) that can be detected with acoustic echosounders (Badcock, 1970; Opdal *et al.*, 2008; Godø *et al.*, 2009; Hazen and Johnston, 2010). The high biomasses of these species in open oceanic waters (Hulley, 1994), coupled with their migratory behaviour and the fact that they are an important component of the diet of numerous benthic dwelling species (Cartes *et al.*, 2009), imply that they play an important role in energy pathways through the water column (Longhurst and Harrison, 1989). The importance of the SSLs as a prey resource in oligotrophic seas has been addressed in several studies (Opdal *et al.*, 2008; Hazen and Johnston, 2010), but determining the main species responsible for the observed changes in vertical trends is an issue that still requires additional investigation (Hazen and Johnston, 2010).

The most complete research on mesopelagic fish species for the Mediterranean Sea was carried out in expeditions performed during the early 1900s that expanded from eastern to western sectors (Tåning, 1918; Jespersen and Tåning, 1926; Goodyear *et al.*, 1972a). These studies found that this community was characterised by a relatively lower number of families and species than that in other oceans, including some notable absences, such as for the family Bathylagidae, among others. More recent investigations in this area have dealt with species, such as the gonostomatid *Cyclothone* spp. (Andersen and Sardou, 1992; Andersen *et al.*, 1998), or the myctophid *Lampanyctus crocodilus* (Stefanescu and Cartes, 1992), but there is not sufficient information on other species, particularly because many of the available samples were collected near the sea bed using bottom trawls, in which the most common species was the

Myctophidae *Lampanyctus crocodilus* (Stefanescu and Cartes, 1992; Moranta *et al.*, 2008). Studies conducted using small plankton nets in the water column have been more numerous and have resulted in large collections of larvae of these species (Sabatés *et al.*, 2007; Olivar *et al.*, 2010) as well as juveniles and adults of *Cyclothone braueri* and *C. pygmea* (Andersen and Sardou, 1992; Cartes *et al.*, 2010). The presence of adults of other mesopelagic and bathypelagic species was only sporadically reported in these studies.

In the Mediterranean Sea, no detailed studies have been performed on mesopelagic fish assemblages or to identify the species responsible for the scattering layers detected with echosounders. To our knowledge, this study is the first that uses acoustics to analyse mesopelagic fish communities in this region. The few studies of this type, combining pelagic trawl and echosounder data, were targeted at epipelagic fish distributed over the continental shelf off the Iberian Peninsula and Gulf of Lions (Abad and Franco, 1995; Iglesias *et al.*, 2008). Much of what is known regarding the vertical location of many of these species comes from earlier investigations (Tåning, 1918; Jespersen and Tåning, 1926; Goodyear *et al.*, 1972b) and the studies of Andersen and Sardou (1992) and Andersen *et al*. (Andersen *et al.*, 1998), focusing on *Cyclothone* spp. Species distribution and abundance as well as vertical distribution patterns have been investigated for the central-eastern North Atlantic (CENA) (Badcock, 1970; Badcock and Merret, 1976; Roe and Badcock, 1984; Bordes *et al.*, 2009). Although all of the species known for the Mediterranean Sea are also common in the CENA, geographic, hydrographic and faunistic differences between the two regions (the CENA includes the Canary upwelling region, while the Mediterranean is an oligotrophic sea) indicate that the extrapolation of patterns from one area to the other is not straightforward.

The use of acoustic tools is becoming common in estimating biomasses of mesopelagic fishes, and there is a large body of literature comparing these techniques and net sampling (e.g. Cornejo and Koppelmann, 2006; Kloser *et al.*, 2009; Koslow, 2009). While there is general agreement regarding the difficulties involved in making biomass estimates from midwater trawls (Gartner, 1989; Koslow *et al.*, 1997), the identification of species of sound scatterers is also not a straightforward exercise, and some ground-truthing of species and size composition is always required to identify the different layers. In the present study, we analyse the diversity and vertical distribution patterns of mesopelagic fishes in the main scattering layers through the water column in an oligotrophic region of the western Mediterranean Sea off the Balearic Islands. To this end, we gathered data collected by means of large pelagic trawls and more conventionally used midwater trawls. Our main objective is to ascertain whether the night vertical displacements of mesopelagic fishes to near-surface layers reported for other oceanographic regions are also a general feature (both spatially and seasonally) of the species that compose the mesopelagic fish community in the western Mediterranean. Additionally, we seek to obtain a baseline regarding species assemblages and vertical distribution patterns for future studies on the pelagic community of this region.

2.2 Materials and methods

Sampling was conducted on board the R/V *Sarmiento de Gamboa* in late autumn (December 2009) and early summer (July 2010) off Mallorca (Balearic Islands, western Mediterranean). The Balearic Islands are the natural limit between two sub-basins of the western Mediterranean, the Algerian and the Balearic basins, where the Mallorca and Ibiza channels play an important role in regional circulation (Fig. 2.1) (López-Jurado *et al.*, 2008).

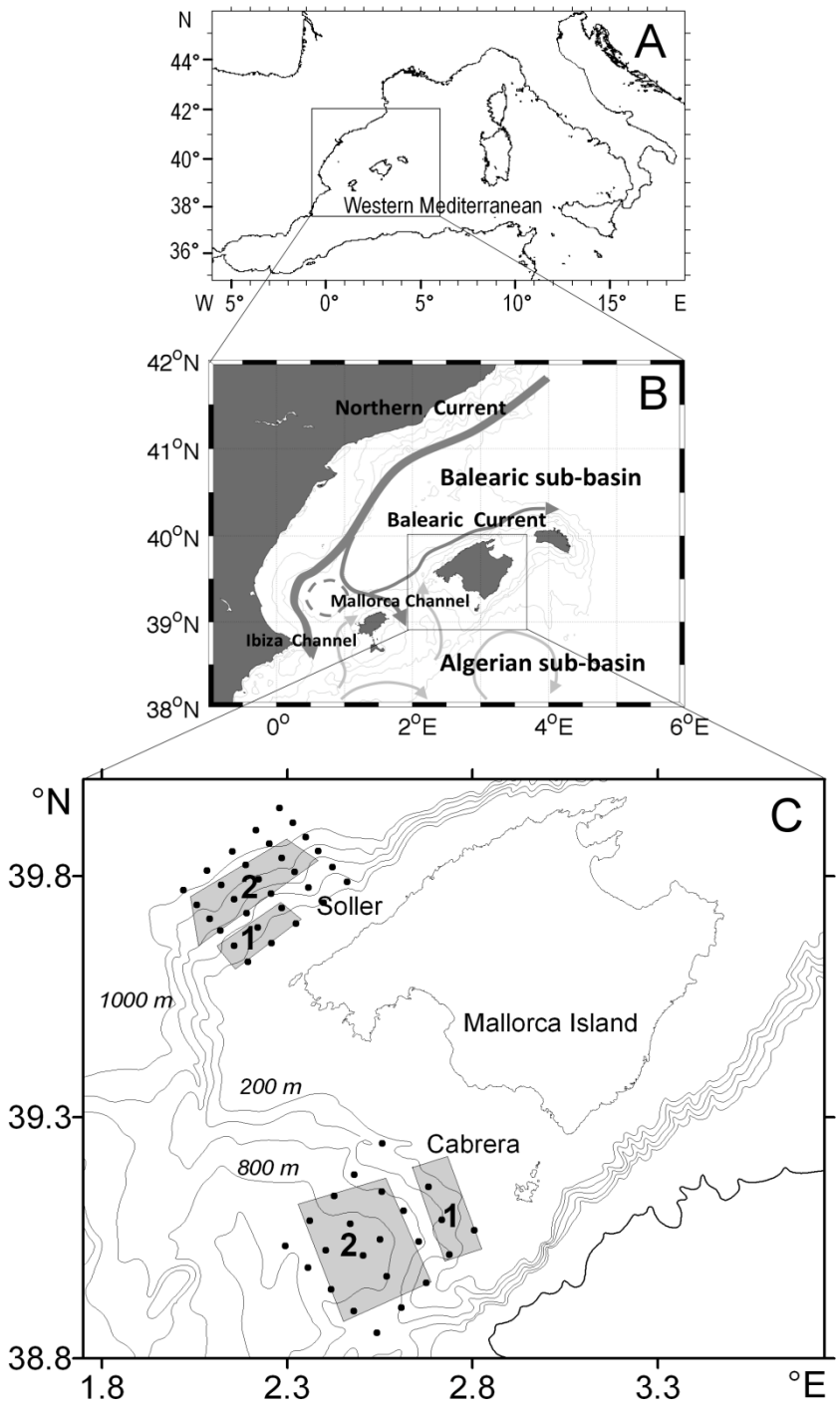


Fig. 2.1 (A) Western Mediterranean. (B) The Balearic Islands and the major currents characterising the regional circulation, showing the Mallorca and Ibiza channels, the Northern and Balearic currents in dark grey arrow-lines and the Algerian gyres in light grey arrow-lines. Light grey lines denote isobaths (100, 500, 1000 and 2000 m). (C) Study area, showing the two zones (Sóller and Cabrera) and bathymetric strata (shelf, 1 and slope, 2) studied during the December 2009 and July 2010 cruises.

Hydrographic data were collected using one CTD (SBE911). Samples were collected on the shelf (200 m depth, bathymetric stratum 1) and slope (600-900 m, bathymetric stratum 2) off the northwest and southeast of the Island (Sóller and Cabrera zones, respectively) (Fig. 2.1). In both zones, a relatively small area was repeatedly sampled throughout the day using a variety of nets. A double-warp modified commercial mid-water trawl, with standard pelagic trawl doors (otter boards) and graded-mesh netting to the cod-end (10 mm) was used. The main difference between the pelagic trawls (PTs) of the December and July cruises was the estimated mouth area (280 and 112 m^2, respectively). Several smaller nets were also used to collect smaller specimens: an Isaaks-Kidd Midwater Trawl of 3 m^2 with a final mesh size of 3 mm (IKMT) and two 1 m^2 multiple nets in which two of the nets had a 1.5-mm mesh size (a Rectangular midwater trawl, RMT, used in December, and a Multiple Opening and Closing Net and Environmental Sampling System, MOCNESS, used in July).

Hauls were carried out in the strongest and widest acoustic sound layers of the water column, which tended to be located at a depth of 400-600 m (both day and night) and near the surface during the night. Furthermore, during daylight hours, several samples were collected near surface to obtain evidence of the absence or low concentrations of fishes at those hours and levels. Acoustic backscatter was measured with a Simrad EK60 echosounder at 18, 38, 70, 120 and 200 kHz. Acoustic raw data were stored during the cruises and post-processed in the laboratory with Echoview software (Higginbottom *et al.*, 2008).

Depth for PTs, IKMT and RMT hauls was controlled by means of a SCANMAR system, which was also used for the estimation of PTs mouth area, while the MOCNESS used a depth sensor connected through the cable to the vessel. At the shelf bathymetric stratum, sampling was carried out at the near surface (SUR1) from 0-60 m, or in the benthic boundary layers (BBL1), less than 50 m

above the bottom. At the slope bathymetric stratum, sampling was performed at the near surface (SUR2) from 0-80 m depths and in the 400-600 m deep scattering layers (400 DSL). For comparative purposes, a few hauls were also performed near the bottom in this slope bathymetric stratum (BBL2) (Table 2.1). Throughout each haul, the ship was maintained following the same bathymetric depth.

Table 2.1
Summary of the sampling effort accomplished with different nets and in different levels of the water column by cruise (December 2009 and July 2010) and bathymetric strata (shelf: 1 and slope: 2).

	Pelagic trawls				IKMT				RMT		MOCNESS	
	December		July		December		July		December		July	
	N	*D*	*N*	*D*	*N*	*D*	*N*	*D*	*N*	*D*	*N*	*D*
SLOPE												
SUR2	4	0	2	3	3	0	4	0	3	3	0	0
400 DSL	2	0	3	5	4	2	2	6	2	3	4	6
BBL2	1	0	0	1	0	0	0	1	0	0	2	2
SHELF												
SUR1	2	0	1	1	3	0	6	7	7	4	5	6
BBL1	2	1	1	1	1	4	4	6	3	3	1	1

N, number of night samples; *D*, number of day samples; SUR, surface; 400 DSL, 400 m deep scattering layer; BBL, benthic boundary layer.

None of the PTs or the IKMT nets had an opening or closing mechanism. Reducing contamination during lowering or hoisting IKMT net was achieved by decreasing ship speed and increasing winch speed. The fishing speeds used were 4 (PTs) and 3 knots (IKMT). The effective tow duration was one hour for PTs and 30 minutes for IKMT. The RMT and MOCNESS nets were prepared to deploy two nets in every trawl operation. The first net sampled the near-surface layers while descending to the selected depth (40-80 m), where horizontal hauls were performed for 15 to 30 min, and the second net was then opened and lowered to the 400 DSL, BBL1 or BBL2, where the second horizontal haul was performed for 15 to 30 min. The ship speed was 2.5-3 knots. The abundance

values obtained from the PTs were presented as the number of individuals collected per hour, standardised to a similar mouth area of 100 m^2. The abundance of individuals collected with the smaller nets was standardised to 10000 m^3 of water filtered. Volumes were estimated for the IKMT and RMT nets according to mouth opening, the time of the hauls and ship speed, while those for the MOCNESS nets were estimated from the flowmeter readings.

Species identification was performed using relevant literature (Tåning, 1918; Jespersen and Tåning, 1926; Hulley, 1984). Identification and measurements of PTs material were mostly carried out on board using fresh material. Samples from the smaller nets were stored and fixed in buffered 5% formalin and identified and measured in the laboratory. All given measures correspond to standard lengths. The criteria to differentiate mature and immature stages were based on sizes, following Hulley (1984).

As migration times are associated with the mixing of layers, dawn or sunset samples were discarded for most of the analysis and for the summary of day/night vertical distributions and were considered only for comparative purposes. Day was considered to extend from one hour after sunrise to one hour before sunset, while night was from one hour after sunset to one hour before sunrise.

Due to the different dimensions of the nets and mesh sizes employed, which could affect their sampling efficiency with respect to species and sizes, data from the PTs and those from the smaller nets were analysed separately. Diversity was assessed based on the number of species, Shannon diversity and Pielou's evenness indices calculated for average values of species abundance for the same month, zone, bathymetric stratum, level and light conditions. Dominance plots were prepared, in which the species in each sample were

ranked in decreasing order of abundance, and their relative abundance in the sample was plotted against the increasing rank.

Fish assemblage structure was analysed through hierarchical agglomerative and unweighted arithmetic average clustering (CLUSTER procedure; Clarke and Gorley, 2006) by calculating Bray-Curtis similarity resemblance matrices. The significant groups were determined using the SIMPROF procedure (Clarke and Gorley, 2006). The SIMPER routine was applied to identify the species that characterise each group and those most responsible for the differences between groups. Individual analyses were initially performed for each particular survey and then for the combined data from the two surveys. Because groups obtained were analogous, only the pooled analysis was presented here. In order to reduce the weight of numerically dominant species, a fourth root transformation of the data was performed before building the similarity matrices. Furthermore, the differences in fish assemblages due to factors previously established (i.e. month (December and July), zone (Sóller and Cabrera), bathymetric stratum (shelf and slope), level of the water column (near-surface, deep scattering layers and benthic boundary layers) and light (day and night)) were tested using a nested PERMANOVA. All of these procedures were performed using PRIMER6+Permanova software (Clarke and Warwick, 2005; Anderson *et al.*, 2008).

Summaries of the night and day vertical distributions were presented as the mean abundance obtained with the PTs or smaller nets for each cruise, combining day (D) or night (N) samples collected near surface (SUR1) and near bottom (BBL1) at the shelf bathymetric stratum, or near surface (SUR2), 400 m (400 DSL) and near bottom (BBL2) for the slope.

2.3 Results

2.3.1 Vertical structure of the water column

During the two cruises, the study zones were under the influence of Atlantic waters. The vertical profiles of potential temperature and salinity (θ and S) during the autumn cruise were homogeneous for the first 60-80 m of the water column, and the highest gradient was located from there to 120 m. Summer stratification was evident in both θ and S, with a shallow upper mixing layer of just 10 m and a gradient zone from that point to a depth of 100 m (see Fig. 2.2). Below the thermocline layer, temperature was quasi-homogeneous at approximately 13 ºC down to the bottom, with a relative temperature maximum (13.3 ºC) being observed at the of Levantine Intermediate Waters (LIW) core

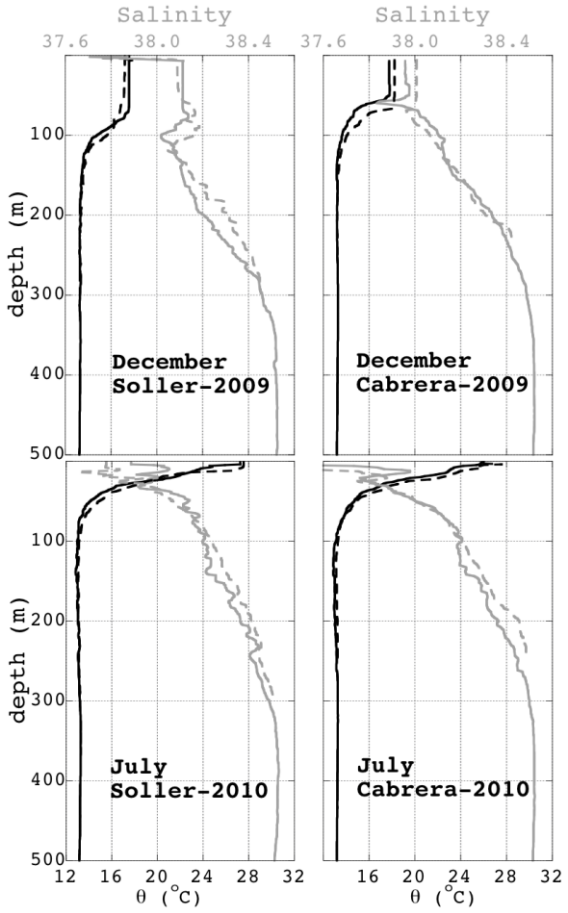


Fig. 2.2 Potential temperature (black) and salinity (grey) versus pressure in the two. Study zones of Cabrera and Sóller during the December 2009 and July 2010 cruises. Solid lines are slope stations (900m), and dashed lines are shelf stations (200 m).

depth. In contrast, salinity slowly increases up to 38.53, a value typical of LIW found at 400 m, where the core of this water mass is usually found in this area. This depth slightly oscillates seasonally from autumn to summer (350 to 450 m).

2.3.2 Acoustic layers

The main acoustic layers generally exhibited stronger scattering in the 18 and 38 kHz echograms, with myctophids being responsible for the 18 kHz layers (caught mainly with Pelagic Trawls nets), while a mixture of different small organisms produced the 38 kHz layers (caught mainly with small nets). In the slope region a conspicuous acoustic layer from 400 to 600 m (400 DSL) was detected with the 38 kHz echosounder. This was a permanent layer detected

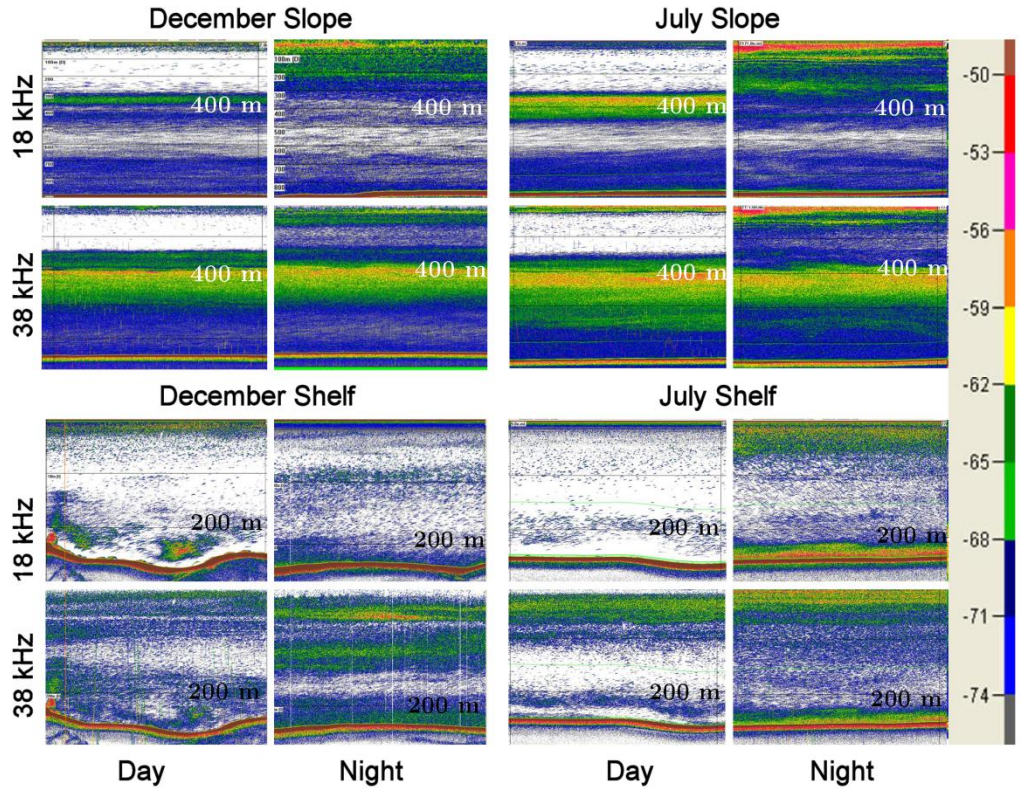


Fig. 2.3 Echograms illustrating some of the acoustic layers (18 and 38 kHz) encountered at the slope and shelf for the December 2009 and July 2010 surveys. The colour scale corresponds to decibel values from the starting at threshold (-80 dB). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

both day and night, during autumn and summer surveys (Fig. 3.2). Surface layers were mostly detected at night, with both the 18 and 38 kHz echosounders, during the two seasons. Shelf echograms (Fig. 3.2) were generally less conspicuous, showing the main concentration zones at the near surface and near bottom layers during both day and night. The results of day samples taken near surface both at the slope and shelf (with small nets in autumn and both PT and small nets in summer) showed that these surface layers were not due to mesopelagic fishes, and that most of the collections were medusae.

2.3.3 Species collections by nets

More than 99% of the fish appearing in both late autumn and summer belonged to meso- and bathypelagic species (>60000 individuals), and the only noticeable contribution of other fishes corresponded to several pelagic species, such as *Trachurus* spp. and a few juveniles of *Xiphias gladius.* The PTs collected mostly Myctophidae (93.2%) and Sternoptichydae (5%), and the small mesh-size nets, IKMT, RMT and MOCNESS, were more efficient at catching adults and juveniles of the small Gonostomatidae *Cyclothone* spp. (>80%), while adult myctophids were less abundant in these small nets (Table 2.2). Taking into consideration all of the nets, the species present in both periods (December and July) and zones (Sóller and Cabrera) were the same.

The species collected with the PTs were the same between cruises, but some differences in the relative abundance were apparent. In December, *Ceratoscopelus maderensis* was the most abundant (>50%) and frequent species (in >90% of the samples), followed by other species that each represented less than 10% of the abundance, including *Lampanyctus crocodilus, Notoscopelus elongatus, Maurolicus muelleri* and *Benthosema glaciale*. During the summer survey, there were more even contributions from several species, with *C. maderensis, B. glaciale*, *Hygophum benoiti* and *N. elongatus* being the most

Table 2.2
Relative species abundance (A) and frequency of occurrence (O) in percentage of the species collected in surveys (December 2009 and July 2010) with pelagic trawls and the small nets (IKMT, RMT and MOCNESS).

Order	Family	Species	Pelagic Trawls				Small nets			
			December		July		December		July	
			A (%)	O (%)	A (%)	O (%)	A (%)	O (%)	A (%)	O (%)
Stomiiformes	Gonostomatidae	*Cyclothone braueri* Jespersen & Tåning, 1926	1.3	25.0	0.6	16.7	77.7	39.5	90.7	31.6
	Gonostomatidae	*Cyclothone pygmaea* Jespersen & Tåning, 1926	0.0	0.0	0.0	0.0	0.0	0.0	2.5	7.9
	Sternoptychidae	*Argyropelecus hemigymnus* Cocco, 1829	0.2	25.0	2.5	50.0	0.7	16.3	2.3	11.8
	Sternoptychidae	*Maurolicus muelleri* (Gmelin, 1788)	8.1	25.0	0.0	0.0	0.0	0.0	0.3	6.6
	Phosichthyidae	*Ichthyococcus ovatus* Bonaparte, 1840	0.0	0.0	*	*	0.0	0.0	0.0	0.0
	Phosichthyidae	*Vinciguerria attenuata* (Cocco, 1838)	0.0	8.3	0.9	61.1	0.1	7.0	0.3	7.9
	Stomiidae	*Bathophilus nigerrimus* Giglioli, 1884	0.0	0.0	0.0	11.1	0.0	0.0	0.0	0.0
	Stomiidae	*Chauliodus sloani* Sneider, 1801	0.0	8.3	0.0	11.1	0.0	0.0	0.0	0.0
	Stomiidae	*Stomias boa* (Risso, 1810)	0.1	50.0	0.3	44.4	0.6	16.3	0.0	1.3
Aulopiformes	Evermannellidae	*Evermannella balbo* (Risso, 1820)	*	*	0.0	0.0	0.0	0.0	0.0	0.0
	Paralepididae	*Lestidiops jayakari* (Boulenger, 1889)	0.3	50.0	0.0	5.6	0.0	0.0	0.0	0.0
	Paralepididae	*Notolepis risso* (Bonaparte, 1840)	0.0	8.3	0.2	27.8	0.0	0.0	0.0	0.0
Myctophiformes	Myctophidae	*Benthosema glaciale* (Reinhardt, 1837)	5.6	58.3	15.3	50.0	7.5	30.2	2.0	14.5
	Myctophidae	*Electrona risso* (Cocco, 1829)	0.0	16.7	0.1	27.8	0.0	0.0	0.0	1.3
	Myctophidae	*Hygophum benoiti* (Cocco, 1838)	2.0	66.7	15.2	66.7	0.7	14.0	0.2	3.9
	Myctophidae	*Hygophum hygomii* (Lütken, 1892)	0.1	25.0	0.4	27.8	0.0	0.0	0.0	0.0
	Myctophidae	*Myctophum punctatum* Rafinesque, 1810	0.5	66.7	1.6	50.0	0.6	14.0	0.1	7.9
	Myctophidae	*Symbolophorus veranyi* (Moreau, 1888)	0.6	50.0	0.4	50.0	0.0	2.3	0.0	0.0
	Myctophidae	*Ceratoscopelus maderensis* (Lowe, 1839)	57.8	91.7	42.8	61.1	8.7	32.6	0.8	11.8
	Myctophidae	*Diaphus holti* Tåning, 1918	0.2	33.3	1.0	44.4	0.2	9.3	0.0	0.0
	Myctophidae	*Lampanyctus crocodilus* (Risso, 1810)	9.4	50.0	0.5	22.2	0.3	7.0	0.1	3.9
	Myctophidae	*Lampanyctus pusillus* (Johnson, 1890)	1.3	33.3	0.8	38.9	2.4	18.6	0.3	6.6
	Myctophidae	*Lobianchia dofleini* (Zugmayer, 1911)	4.0	66.7	5.1	61.1	0.6	16.3	0.0	5.3
	Myctophidae	*Notoscopelus bolini* Nafpaktitis, 1975	0.2	16.7	0.0	5.6	0.0	0.0	0.0	0.0
	Myctophidae	*Notoscopelus elongatus* (Costa, 1844)	8.5	75.0	12.0	66.7	0.0	2.3	0.4	11.8

Asterisk indicates a single specimen collected. Order and families sorted according to Nelson (2006).

abundant and frequent during the survey (Table 2.2). Smaller nets were dominated by *Cyclothone braueri* in both surveys, and the only relevant presence of myctophids in these nets corresponded to *C. maderensis* and *B. glaciale* in the summer (Table 2.3).

Table 2.3
Number of species (*S*), Shannon–Wiener diversity index (*H'*), Pielou's evenness index (*J'*) and mean number of individuals for each level of the water column, light condition, bathymetric stratum and month.

Pelagic trawls	***S***	***H'***	***J'***	**A**
400DSL_N_December	19	3.03	0.71	285
400DSL_D_July	18	3.17	0.76	334
400DSL_N_July	18	2.17	0.52	2754
BBL2_N_December	16	1.77	0.44	788
BBL2_D_July	15	2.91	0.74	616
SUR2_N_December	14	1.25	0.33	1323
SUR2_N_July	12	2.13	0.59	4635
BBL1_N_December	10	1.39	0.42	266
SUR1_N_December	7	1.03	0.37	429
BBL1_N_July	6	1.67	0.65	1096
SUR1_N_July	5	0.72	0.31	2819
BBL1_D_December	1	0.00	****	703
SUR2_D_July	0	0.00	****	0
SUR1_D_July	0	0.00	****	0
BBL1_D_July	0	0.00	****	0
Small nets	***S***	***H'***	***J'***	**n/10000 m^3**
SUR2_N_December	13	2.38	0.64	148
BBL2_D_July	11	2.55	0.74	107
400DSL_N_December	11	0.58	0.17	160
SUR2_N_July	10	2.19	0.66	23
400DSL_N_July	10	0.43	0.13	463
400DSL_D_July	7	0.30	0.11	537
400DSL_D_December	7	0.26	0.09	527
BBL1_N_July	5	1.52	0.65	6
BBL1_D_December	4	1.48	0.74	5
SUR1_N_December	3	1.26	0.80	2
SUR2_D_December	2	0.77	0.77	9
BBL2_N_July	2	0.54	0.54	71
BBL1_D_July	2	0.37	0.37	2
BBL1_N_December	1	0.00	****	1
SUR1_D_July	1	0.00	****	0
SUR1_D_December	0	0.00	****	0
SUR1_N_July	0	0.00	****	0

SUR, surface; 400 DSL, 400 m deep scattering layer; BBL, benthic boundary layer; N, night samples; D, day samples; 1, shelf bathymetric stratum; 2, slope bathymetric stratum; A, abundance per hour and 100 m^2 of mouth area.
**** indicates that this value cannot be calculated.

Noticeably, most of the hauls carried out during daylight hours at the near surface (SUR1 and SUR2) were devoid of mesopelagic fishes: none were obtained for the 4 PTs, and <10/10000 m^3 were collected in the 20 small nets hauls (Table 2.3). The day samples at BBL1 were also very poor, with a low number of specimens and low number of species, with just one PT haul catching a large quantity of individuals of a single species, *Maurolicus muelleri* (ca. 700 specimens/h/100 m^2).

The dominance plots for the PTs showed that the composition of most night near-surface samples (SUR1 and SUR2) was based on a single species (which represented from 60 to 90% of the total collection), while many of the 400 DSL samples taken with the PTs exhibited more even contributions of 2 or 3 species (Fig. 2.4). Although a relatively large number of species appeared at night at SUR2 (up to 14), the dominance of a single species results in a relatively low diversity at this level. The best combination with respect to a high number of species and high diversity for PTs corresponded to the 400 DSL (day and night) (Table 2.3). However, dominance plots using data from the small nets showed that the 400 DSL was dominated by a single species during night and day (Fig. 2.4). For these nets, the highest diversities, accompanied by a high number of species, were calculated for night at SUR2. In general, the small nets were associated with a lower number of species (17, with a maximum of 13 per sample), although in most of these nets, there were less than 5. It is noteworthy that the gonostomatid *C. pygmea* was collected in only some of the hauls performed with these nets and was absent from the PTs samples.

The size frequency distributions of all of the myctophids collected with the PTs showed a modal class larger than that of first maturity (in all layers); the smaller nets caught wider size ranges (from earlier stages), but were characterised by modal sizes classes corresponding to immature adults and juveniles. The RMT and MOCNESS also collected some larvae (not considered in this study);

however, it is worth mentioning that only a few specimens corresponded to transforming stages.

For the gonostomatid *Cyclothone braueri*, the small nets collected the whole size range of the population, from juveniles to adults, while the PTs collected only a few of the largest adult size classes >20 mm, and the rest were extruded through the meshes.

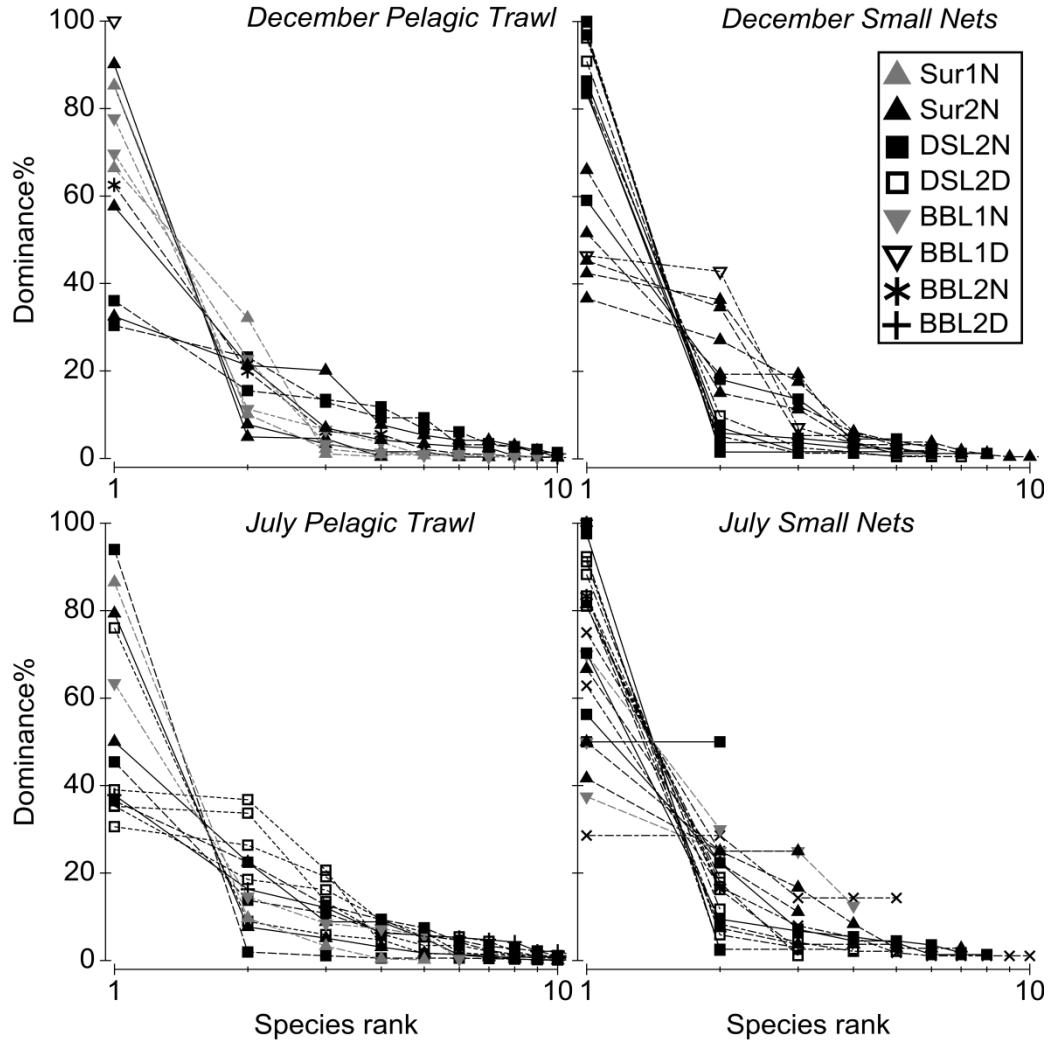


Fig 2.4 Dominance plots for the Pelagic Trawls (left) and small nets (right) for the December (top) and July (bottom) surveys. The lines represent the percentage of the total abundance in the sample plotted against the species rank for each station (x-axis on a log scale). Key symbols indicate the sample level: Sur, surface; DSL, 400 m deep scattering layer; BBL, benthic boundary layer; 1, shelf bathymetric stratum; 2, slope bathymetric stratum; D, day; N, night.

2.3.4 General overview of the species assemblages

In addition to the PTs samples with no fish (all of which were day samples), three other PTs samples were excluded from the multivariate analysis: (i) one day BBL1 sample from the shelf bathymetric stratum because it was composed exclusively of one species (*Maurolicus muelleri*); and (ii) the only two BBL2 samples for the slope bathymetric stratum (one during night in December and the other during day in July).

Table 2.4
Results of nested PERMANOVA used to test the effects of month (fixed factor, Mo), zone (Zo), bathymetric stratum (St), level of the water column (Le) and light (Li) on the structure of the entire mesopelagic fish assemblage for the pelagic trawls (top table) and small nets collections (bottom table).

Source	d*f*	SS	MS	Pseudo-*F*	*P* (perm)	perms
Pelagic trawl						
Mo	1	2957.8	2957.8	1.799	0.1841	9956
Zo(Mo)	2	3168.8	1584.4	0.47317	0.9173	9943
St(Zo(Mo))	3	9904.5	3301.5	3.4704	**0.0007**	9931
Le(St(Zo(Mo)))	7	6682.9	954.69	1.0787	0.4588	9930
Li(Le(St(Zo(Mo))))	2	1764.9	882.47	0.99244	0.4566	9939
Res	6	5335.2	889.2			
Total	21	35122				
Small nets						
Mo	1	7732.3	7732.3	2.1787	0.1388	9962
Zo(Mo)	2	7233.9	3617	0.69815	0.7542	9958
St(Zo(Mo))	2	9948.4	4974.2	1.0552	0.4243	9954
Le(St(Zo(Mo)))	5	41203	8240.5	7.3002	**0.0001**	9940
Li(Le(St(Zo(Mo))))	5	5666.4	1133.3	1.0193	0.4433	9910
Res	31	34466	1111.8			
Total	46	1.12E+05				

Significant differences are shown in bold.

A nested PERMANOVA to test for differences in the mesopelagic fish assemblages from PTs samples, taking month, zone (per month), bathymetric stratum (per zone), level (per bathymetric stratum) and light (per level) as factors, showed that the only significant differences ($p<0.0007$) were due to bathymetry (Table 2.4). Individual analyses for each cruise also indicated that

the only significant differences were due to bathymetry. Clustering classification of the PTs samples (Fig. 2.5) differentiated (at 40% similarity) two large groups of stations based on bathymetry (shelf and slope), plus one very dissimilar day sample at 400 DSL containing two stomiiforms (*Argyropelecus hemigymnus* and *Vinciguerria attenuata*) and one myctophid (*Diaphus holti*) (group a in Fig. 2.5). Within each of the bathymetric groups, samples from the two study periods (December and July) and the two zones (Sóller and Cabrera) appeared to be mixed. All shelf stations were included in a unique and fairly similar cluster (group b in Fig. 2.5) composed of just four myctophids: *Notoscopelus elongatus, Ceratoscopelus maderensis, Myctophum punctatum* and *Hygophum benoiti* (Table 2.5). The SIMPROF test also showed three other significant groups ($p<0.05$) at the slope stations: (i) group c, which included two night near-surface samples from the Cabrera zone from the December cruise composed of just three myctophids (*C. maderensis, Benthosema glaciale* and *H. benoiti*), with dominance of *C. maderensis* (53.46%); (ii) group d, including the rest of the samples collected at the near-surface level during the night (for the two zones and cruises), which were composed of a greater number of species (6, all of them myctophids), with *C. maderensis* again representing the main contributor, followed by *Lobianchia dofleini* and *Lampanyctus crocodilus*; and (iii) group e, which included all 400 DSL samples characterised by a relatively high number of species, including both Myctophidae and stomiiforms (10 species presenting a cumulative contribution of 85.08%). The main difference between the shelf and slope groups was due to the absence of the myctophid *B. glaciale* and the stomiiforms *A. hemigymnus* and *Cyclothone braueri* in the shelf samples as well as the relative abundance of *N. elongatus* for the shelf bathymetric stratum. The complementary species classification from the PTs samples allowed the identification of two large groups of species (Fig. 2.6): those that characterise the 400 DSL and migrant species associated with near-surface (SUR1 and

SUR2) night samples. Species with lower contributions were grouped at a higher dissimilarity with the rest.

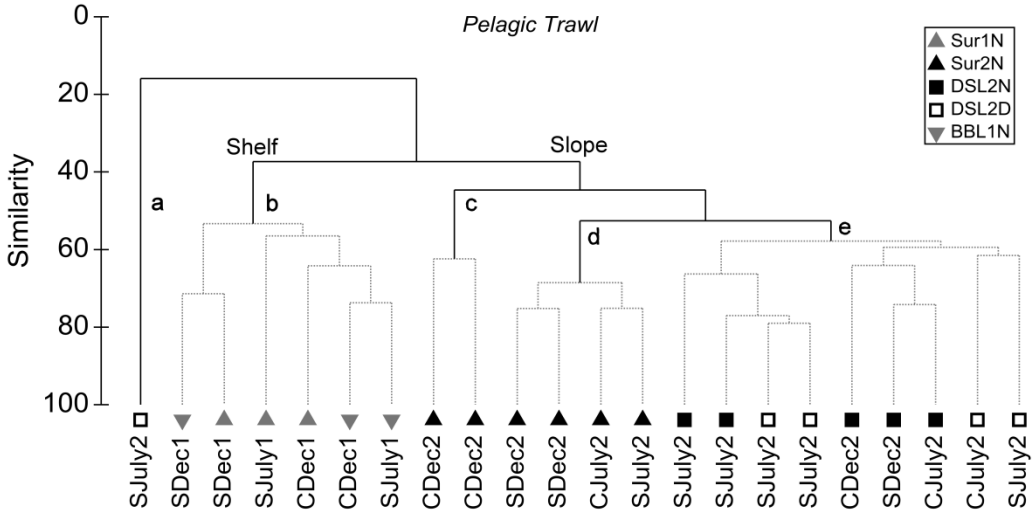


Fig. 2.5 Dendrogram obtained after cluster analysis applied on the Bray Curtis similarity matrix for Pelagic Trawl samples. The species abundance per sample was fourth root transformed. Significant groups ($p < 0.05$) of samples (a–e) were defined by the SIMPROF procedure. Key symbols indicate the sample level: Sur, surface; DSL, 400 m deep scattering layer; BBL, benthic boundary layer; 1, shelf bathymetric stratum; 2, slope bathymetric stratum; D, day; N, night. Each sample month and zone (S, Sóller and C, Cabrera) is also indicated.

Table 2.5
Similarity percentages within groups determined from cluster analysis for the Pelagic Trawls collections and percentage contribution per species (cut-off 90%).

Groups	b	c	d	e
Average similarity within group	57.99	62.4	70.77	61.38
Cyclothone braueri				3.7
Argyropelecus hemigymnus				12.4
Vinciguerria attenuatta				6.86
Stomias boa				2.81
Benthosema glaciale		29.4	10.55	7.59
Hygophum benoiti	5.77	17.14	6.21	12.75
Myctophum punctatum	19.61			3.64
Symbolophorus veranyi			10.03	3.84
Ceratoscopelus maderensis	28.55	53.46	18.94	8.65
Diaphus holti				6.93
Lampanyctus crocodilus			12.96	
Lampanyctus pusillus			10.92	
Lobianchia dofleini			13.76	12.82
Notoscopelus elongatus	38.55		9.52	9.54

Group a, comprising a single sample (see Fig. 2.5), is not included in the table.

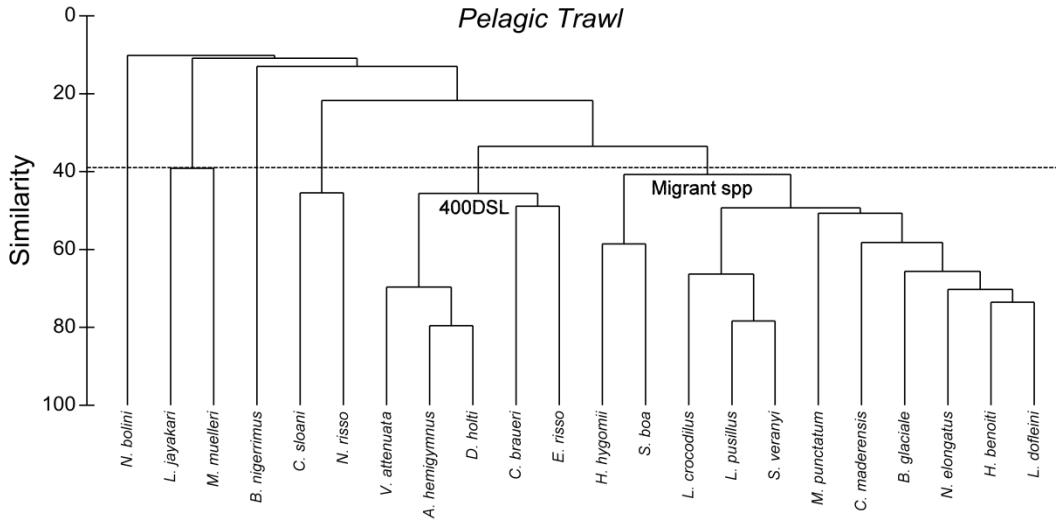


Fig. 2.6 Dendrogram obtained after cluster analysis applied on the Bray Curtis similarities calculated among variables (species) for the pelagic trawls samples. 400 DSL: indicates the group of species mainly found at the 400 m deep scattering layer.

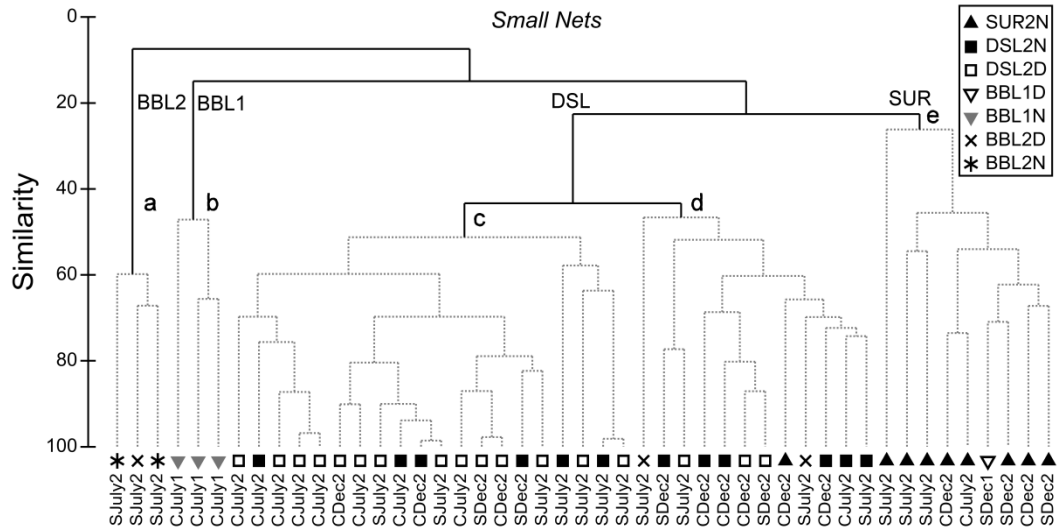


Fig. 2.7 Dendrogram obtained after cluster analysis applied on the Bray Curtis similarity matrix for the small net samples. The species abundance per sample was four root transformed. Significant groups ($p<0.05$) of samples (a–e) were defined by the SIMPROF procedure. Symbols as in Fig. 2.4.

For analysis of the mesopelagic fish assemblages from the small nets collections, samples with just one species represented by less than two

individuals were not considered (these include most of the day SUR1 and SUR2, night SUR1, and most of BBL1 samples). PERMANOVA showed that the only significant differences ($p<0.0001$) were due to the level in the water column (Table 2.4). Cluster analysis with the remaining samples identified five significant groups (Fig. 2.7 and Table 2.6) within which samples from the two periods (December and July) and zones (Sóller and Cabrera) appeared mixed, but differentiated: (i) group a, with three samples at BBL2 on the slope, where only individuals of the gonostomatid *Cyclothone pygmea* appeared; (ii) group b, with three samples from BBL1 of the shelf, where *Notoscopelus elongatus* was the main contributor (63%); (iii) groups c and d, including day and night 400 DSL samples, with the first group being composed exclusively of 400 DSL samples and characterised by an absolute dominance of *C. braueri* (94%); (iv) group d, including few samples at other levels with *C. braueri* as the main contributor (39%), but with other species also being important (Table 2.6); and finally, (v) group e, which consisted of night SUR2 samples (except for one day sample at BBL1), mostly comprised of the myctophids *Ceratoscopelus maderensis* and *Benthosema glaciale*. The complementary species classification dendrogram did not show a clear large species association. Most of the groups were linked with very low similarity values, probably due to the lower efficiency of these nets with respect to catching myctophids.

Table 2.6
Similarity percentages within groups determined from cluster analysis for the small net collections and percentage contribution per species (cut-off 90%).

Groups	a	b	c	d	e
Average similarity within group	62.29	53.31	62.93	58.71	47.34
Cyclothone braueri		15.86	93.83	39.43	
Cyclothone pygmaea	100				
Argyropelecus hemigymnus				15.45	
Maurolicus muelleri		21.55			
Vinciguerria attenuatta				3.17	
Stomias boa					6.87
Benthosema glaciale				14.24	26.94
Ceratoscopelus maderensis				12.92	40.05
Lampanyctus pusillus				3.3	12.51
Lobianchia dofleini				3.68	7.89
Notoscopelus elongatus		62.59			

2.3.5 Diel Vertical distribution by species and size

Despite the differences in the vertical structure of the water column between the two periods (see Fig. 2.2), the diel vertical patterns of distribution were similar for the different species collected, with some species never found to migrate to near-surface levels and others that travel to upper layers in both stratified (July) and mixing (December) periods. Of particular note, some species, such as the myctophids *Hygophum benoiti* and *Lampanyctus pusillus*, showed a stronger near-surface migration during the stratified period, as indicated by their shallower distribution in July (Tables 2.7 and 2.8).

The majority of the stomiiform species *Argyropelecus hemigymnus*, *Cyclothone braueri*, *Chauliodus sloani*, *Maurolicus muelleri* and *Vinciguerria attenuata* were absent from the near-surface layers, being found at 400 DSL (during day and night) and, to a lesser extent, in the near bottom layer of the slope (BBL2) (Tables 2.7 and 2.8). When they appeared at the shelf bathymetric stratum, they were located exclusively near the bottom. For *C. braueri*, no differences with respect to size range or modal size classes were evident between day and night samples (Fig. 2.8), and the few specimens caught near the bottom were of similar sizes to those captured at 400 DSL. In contrast, some differences were found between months, with clear bimodal distributions being detected in December (14 and 22 mm SL), which were not observed in July, with the bulk of specimens in the range of 16-22 mm SL. For the sternoptychid *A. hemigymnus*, no clear vertical size stratification from BBL2 and 400 DSL was apparent, but the size frequency distributions for day 400 DSL samples exhibited a mode that was larger than that of the night ones (19-25 and 14-16 mm SL, respectively). A different vertical pattern was shown by the few individuals of *Cyclothone pygmea* collected, which appeared almost exclusively close to the bottom, as well as by *Stomias boa*, which exhibited a wide vertical

Table 2.7
Mean abundance (per hour and 100 m^2 of mouth area) of the different mesopelagic fish species collected with the pelagic trawls for the same level of the water column (SUR, surface; 400 DSL, 400 m deep scattering layer; BBL, benthic boundary layer), bathymetric stratum (1, shelf and 2, slope), light (day and night) and month (December 2009 and July 2010). X: no samples.

	O. Stomiiformes							O. Aulopiformes	
	Cyclothone braueri	*Argyropelecus hemigymnus*	*Maurolicus muelleri*	*Vinciguerria attenuata*	*Bathophilus nigerrimus*	*Chauliodus sloani*	*Stomias boa*	*Lestidiops jayakari*	*Notolepis risso*
December									
Slope									
Night									
SUR2	0.0	0.0	0.0	0.0	0.0	0.0	1.6	4.6	0.0
400DSL	49.7	22.3	0.4	0.7	0.0	0.2	0.7	0.6	0.0
BBL2	16.5	3.6	0.0	0.0	0.0	0.0	1.4	0.4	1.8
Day									
SUR2	X	X	X	X	X	X	X	X	X
400DSL	X	X	X	X	X	X	X	X	X
BBL2	X	X	X	X	X	X	X	X	X
Shelf									
Night									
SUR1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0
BBL1	0.0	0.0	0.5	0.0	0.0	0.0	0.2	0.7	0.0
Day									
SUR1	X	X	X	X	X	X	X	X	X
BBL1	0.0	0.0	702.9	0.0	0.0	0.0	0.0	0.0	0.0
July									
Slope									
Night									
SUR2	0.0	0.0	0.0	4.2	0.0	0.0	16.8	0.0	0.0
400DSL	5.4	89.7	0.0	18.8	1.2	0.0	1.8	0.0	11.9
BBL2	X	X	X	X	X	X	X	X	X
Day									
SUR2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
400DSL	24.3	61.6	0.0	18.7	0.2	0.9	1.1	0.0	1.4
BBL2	0.0	25.9	0.0	3.2	0.0	0.0	32.4	0.0	2.7
Shelf									
Night									
SUR1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.3	0.0
BBL1	0.0	0.0	0.0	63.3	0.0	0.0	0.0	0.0	0.0
Day									
SUR1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
BBL1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2.7 (cont.)

	O. Myctophiformes												
	Benthosema glaciale	*Electrona risso*	*Hygophum benoiti*	*Hygophum hygomii*	*Myctophum punctatum*	*Symbolophorus veranyi*	*Ceratoscopelus maderensis*	*Diaphus holti*	*Lampanyctus crocodilus*	*Lampanyctus pusillus*	*Lobianchia dofleini*	*Notoscopelus bolini*	*Notoscopelus elongatus*
December													
Slope													
Night													
SUR2	65.1	0.0	15.0	1.1	1.9	10.4	1057.5	0.9	71.6	12.6	70.0	4.4	6.2
400DSL	35.0	0.7	30.5	0.0	1.5	1.4	83.2	4.4	21.1	25.0	16.5	0.2	5.1
BBL2	157.1	0.4	48.4	0.0	1.2	2.4	43.7	1.2	492.7	8.9	7.1	0.0	1.2
Day													
SUR2	X	X	X	X	X	X	X	X	X	X	X	X	X
400DSL	X	X	X	X	X	X	X	X	X	X	X	X	X
BBL2	X	X	X	X	X	X	X	X	X	X	X	X	X
Shelf													
Night													
SUR1	0.0	0.0	0.0	0.4	5.0	0.3	129.9	0.0	0.0	0.0	0.3	0.0	292.0
BBL1	0.0	0.0	1.7	0.2	9.5	0.5	176.9	0.0	0.0	0.0	13.9	0.0	61.7
Day													
SUR1	X	X	X	X	X	X	X	X	X	X	X	X	X
BBL1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
July													
Slope													
Night													
SUR2	1152.8	0.0	235.7	10.9	16.3	24.8	2159.6	0.0	45.9	61.0	232.6	0.0	674.4
400DSL	408.8	2.7	139.4	6.2	16.8	8.5	1585.9	46.8	11.3	17.3	147.2	0.0	234.5
BBL2	X	X	X	X	X	X	X	X	X	X	X	X	X
Day													
SUR2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
400DSL	6.4	2.1	33.4	7.3	0.5	2.0	72.6	19.8	0.0	0.4	53.5	0.0	27.5
BBL2	74.4	0.0	35.6	6.5	100.3	12.9	29.1	6.5	0.0	12.9	38.8	1.8	233.0
Shelf													
Night													
SUR1	0.0	0.0	2438.0	0.0	92.7	0.0	5.2	0.0	0.0	0.0	0.0	0.0	273.1
BBL1	0.0	0.0	80.2	0.0	92.8	0.0	695.0	0.0	0.0	0.0	4.2	0.0	160.4
Day													
SUR1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
BBL1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2.8

Mean abundance (number of specimens per 10,000 m3) of the different mesopelagic fish species collected with small nets for the same level of the water column (SUR, surface; 400 DSL: 400 m deep scattering layer; BBL, benthic boundary layer), bathymetric stratum (1, shelf and 2, slope), light (day and night) and month (December 2009 and July 2010) .X: no samples.

	O. Stomiiformes						O. Myctophiformes										
	Cyclothone braueri	*Cyclothone pygmaea*	*Argyropelecus hemigymnus*	*Maurolicus muelleri*	*Vinciguerria attenuata*	*Stomias boa*	*Benthosema glaciale*	*Electrona risso*	*Hygophum benoiti*	*Myctophum punctatum*	*Symbolophorus veranyi*	*Ceratoscopelus maderensis*	*Diaphus holti*	*Lampanyctus crocodilus*	*Lampanyctus pusillus*	*Lobianchia dofleini*	*Notoscopelus elongastus*
December																	
Slope																	
Night																	
SUR2	13.8	0	1.0	0	0.2	2.4	40.9	0	4.6	1.8	0.2	59.5	0.8	2.0	16.9	3.8	0
400DSL	148.4	0	2.0	0	0.8	0.2	3.7	0	0.8	0	0	2.7	0	0.2	0.8	0.6	0.2
BBL2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Shelf																	
Day																	
SUR2	10.4	0	0	0	0	3.0	0	0	0	0	0	0	0	0	0	0	0
400DSL	509.3	0	2.6	0	0	0	11.9	0	0	0.2	0	1.7	0.5	0	0	0.5	0
BBL2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Night																	
SUR1	0.8	0	0	0	0	0	0	0	0	1.0	0	0.1	0	0	0	0	0
BBL1	0	0	0	0	0	0	0	0	0	1.3	0	0	0	0	0	0	0
Day																	
SUR1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BBL1	0	0	0	0	0	0.2	2.0	0	0	0	0	2.2	0	0	0.3	0	0
July																	
Slope																	
Night																	
SUR2	0	0	0	0	0.6	0.3	11.0	0	0.3	0.6	0	5.9	0	0.9	1.8	0.9	0.3
400DSL	437.7	4.1	13.5	0	1.8	0	2.4	0	0	1.0	0	1.0	0	0.2	1.0	0	0.8
BBL2	0	62.2	0	0	0	0	0	0	0	0	0	8.9	0	0	0	0	0
Day																	
SUR2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
400DSL	516.1	0	8.6	0	1.0	0	8.1	0	0	0	0	1.9	0	0	0	0.1	0.8
BBL2	30.0	33.5	12.6	0	0.4	0	12.6	0	6.7	0.4	0	3.0	0	0.7	6.7	0	0.4
Shelf																	
Night																	
SUR1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BBL1	0.4	0	0	2.5	0	0	0	0.1	0	0.1	0	0	0	0	0	0	3.1
Day																	
SUR1	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0
BBL1	1.9	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0

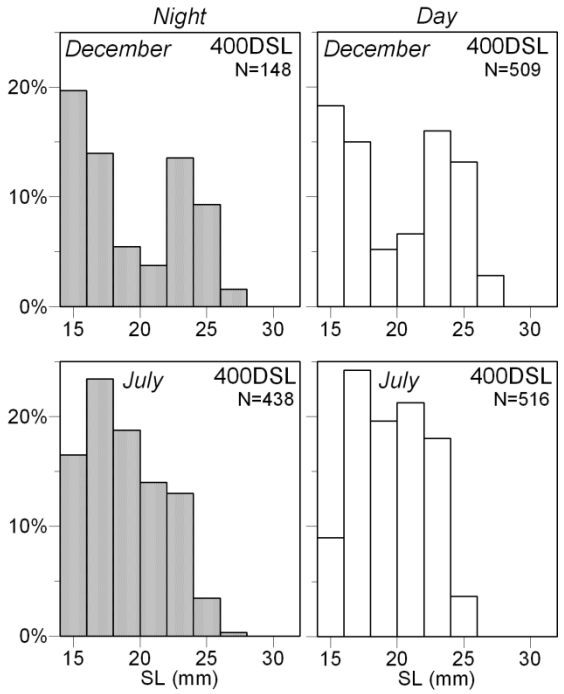


Fig. 2.8 Length frequency distributions of Cyclothone braueri collected with the small nets at the 400 m deep scattering layer (400 DSL) during night (left) and day (right, white bars) hauls in the December (top) and July (bottom) surveys. N: mean number of specimens per 10,000 m3. SL: standard length.

distribution along the water column, appearing from BBL2 to SUR2 (Tables 2.7 and 2.8).

Only two Myctophidae (*Diaphus holti* and *Electrona risso*) showed this pattern of day and night preference for 400 DSL and were absent from the surface. For all other myctophids, main collection were obtained at night near surface and, to a lesser extent, at 400 DSL, contrasting with an absence or much lower concentrations at these levels during the day (Tables 2.7 and 2.8). The species with higher concentrations near the surface compared to those at 400 DSL at night were the myctophids, *Benthosema glaciale*, *Ceratoscopelus maderensis*, *Hygophum hygomii*, *Lampanyctus crocodilus*, *Lobianchia dofleini*, *Notoscopelus elongatus*, *Symbolophorus veranyi* and the paralepidid, *Lestidiops jayakari* (in both periods) and *Lampanyctus pusillus* and *Hygophum benoiti* (in summer). The myctophid *Myctophum punctatum* was more evenly distributed in these two levels. Of particular note, in the single night PT sample at BBL2 (December), were the collections of *L. crocodilus*, *B. glaciale* and *H. benoiti*

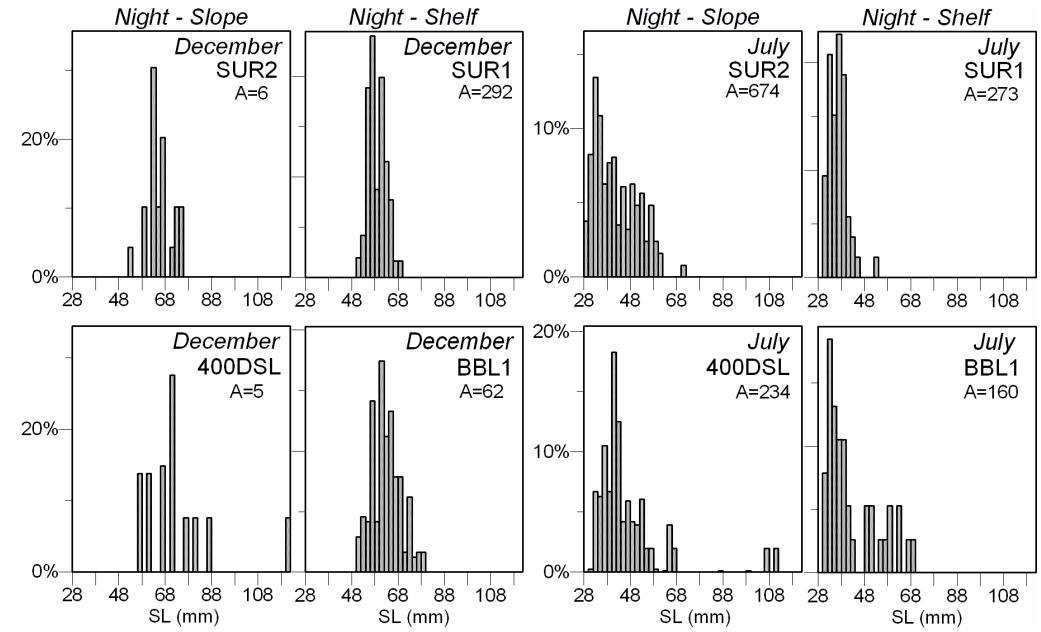


Fig. 2.9 Night length frequency distributions of *Notoscopelus elongatus* collected in the December (left panels) and July (right panels) surveys with the Pelagic Trawl at the different levels of the water column of the slope (2) and shelf (1) bathymetric strata. SUR: surface, 400 DSL: 400 m deep scattering layer, BBL: benthic boundary layer. (A) Mean number of specimens per hour and $100m^2$ of mouth area collected with the pelagic trawl. SL: standard length.

(found in higher concentrations than at SUR2 or 400 DSL), while in the single day PT sample at BBL2 (July), no particular abundance of any of these species or of other mesopelagic fishes were recorded. Of all the species appearing to be relatively abundant at the shelf bathymetric stratum, *N. elongatus* and *H. benoiti* were more abundant at the near-surface layer during the night, while *C. maderensis* and *L. dofleini* were mostly concentrated near the bottom.

For most myctophids, differences among layers for the night hauls were important in terms of abundance but were less important in terms of size ranges or modal size classes. However, for *Benthosema glaciale*, *Lampanyctus crocodilus* and *Notoscopelus elongatus*, some size stratification was apparent. Catches of *B. glaciale* detected at SUR2 and 400 DSL were composed of immature individuals and adults, while in the single PT sample at BBL2, no immature specimens (<30 mm SL) were found. For *L. crocodilus*, the largest

sizes (>74 mm SL) were collected only in near-bottom trawls and never occurred near the surface. Similarly, larger individuals of *N. elongatus* (>70 mm SL) were absent from the near-surface layers (both at slope and shelf bathymetric strata) (Fig. 2.9). This was the only relatively abundant myctophid in the single daylight PT sample at BBL2 (Table 2.7). It is also noteworthy that the size frequency distributions observed for *N. elongatus* were fairly different in December and July, with a predominance of larger individuals being found in December (modal size classes ca. 60 mm SL and an absence of specimens <48 mm SL), while July samples were dominated by immature individuals (Fig. 2.9).

2.4 Discussion

The western Mediterranean mesopelagic fish fauna is fairly similar to that of the central north-eastern Atlantic, from which it originates, but is represented by a lower number of species and families (Tåning, 1918; Jespersen and Tåning, 1926; Goodyear *et al.*, 1972a). Many of the publications summarising the species of this group of fishes present in the western Mediterranean (e.g. Hulley, 1984; Mercader *et al.*, 2001; Coll *et al.*, 2010) are based on collections performed for these three earlier studies, and as far as we know, no new studies have addressed the investigation of these fish assemblages using information from catches performed in the main scattering layers of the water column or using large pelagic nets. In the present study, 13 of the 18 myctophid species reported for the whole Mediterranean Sea and 9 of the 11 stomiiforms were relatively frequent; all of these are within the temperate or subtropical pattern (Hulley, 1984). At the stations sampled in the western Mediterranean during previous studies by Tåning (1918) and Goodyear *et al.* (1972), only a few additional species of myctophids or stomiiforms were reported, all of which were very infrequent, i.e., *Diaphus rafinesquei* (rare), *Diogenichthys atlanticus* (only one postlarva) and *Lobianchia gemellari* (rare and farther to the west),

Gonostoma denudatum and *Vinciguerria poweriae* (not common and farther to the west).

Compared to the species richness of midwater fishes in other regions of the world, the western Mediterranean is characterised by relatively lower values, which contrasts with the overall high species richness for all teleostean fishes, particularly shelf-dwelling and pelagic species (Abad and Franco, 1995; Mercader *et al.*, 2001; Coll *et al.*, 2010). The fact that the Mediterranean is a semi-closed sea, with limited contact with other oceanic regions (only Atlantic waters entering in near-surface layers through the Strait of Gibraltar and Indian waters through the Suez Canal), combined with its isolation from the ocean and repeated desiccations during the Messinian period, may have contributed to the paucity in this oceanic group.

The efficiency of the several nets used in this study was different depending on the size of each species and their developmental stages. Smaller nets collected immature stages of myctophids and sternoptychids, but were particularly appropriate for the collection of juveniles and adults of the thin and small *Cyclothone* spp. (which tend to be extruded from the PTs). The appropriateness of the large PTs to catch myctophids and sternoptychids is demonstrated by the total number of species found, as well as by the abundance and wide size ranges retained by the nets in the two periods. Nevertheless, the difficulty of assessing the exact volume of water filtered by the nets (due to the uncertainty concerning the effective mouth area and the filtration efficiency) constrains the ability to make straightforward abundance comparisons between surveys. Furthermore, it is not possible to calculate a catchability coefficient between the two PTs, as in other studies (Heino *et al.*, 2011) because our two nets were used in different cruises. Problems in obtaining accurate estimations of mesopelagic fishes are often argued to be the main reasons for the differences in total biomass

assessments from trawl data (lower values) and acoustic surveys (Koslow *et al.*, 1997).

In terms of relative abundance, previous studies in the western Mediterranean (using small nets) identified the species *Cyclothone braueri*, *C. pygmea*, *Ceratoscopelus maderensis, Benthosema glaciale* and *Lampanyctus pusillus* as the most abundant species (Tåning, 1918; Jespersen and Tåning, 1926; Andersen and Sardou, 1992). With the exception of the deep water species *C. pygmaea*, which was poorly sampled in our December survey, the rest of these species were also in the upper rank of abundance for our smaller nets. However, when considering catches performed with the large PTs, other species that were poorly or not represented in the small nets emerge as important, i.e., *Notoscopelus elongatus, Lampanyctus crocodilus* and *Hygophum benoiti.*

Regarding the study of species assemblages, the combined use of PTs catches obtained for the two surveys, in spite of the different mouth size of the gear, was consistent with the results of individual analysis, in which the main factor defining assemblages within each survey was also the bathymetric stratum. Furthermore, the fourth root transformation of species abundance also contributes to smooth any possible bias in the amount of fish that could be attributed to the different mouth size.

Dominance of mesopelagic fish assemblages by a few families, particularly Myctophidae and Gonostomatidae, and by a few species within them is a general pattern that has previously been pointed out in oceanic areas (Koslow *et al*., 1997 for SE Australia; Ross *et al*., 2010, for the Gulf of Mexico; Bordes *et al*., 2009, for CEN Atlantic). Notably, species of the genus *Cyclothone* tend to exhibit the highest number of individuals in many ecosystems (Ross *et al*., 2010, and references there; Opdal *et al*., 2008; Bordes *et al*., 2009). The influence of seasons on midwater fish assemblages does not appear to be important in the present study, where bathymetry and the level in the water column were found

to be the main factors related to the structure of the assemblages. Despite geographical separation and oceanographic differences among study sites, similarities in mesopelagic fish assemblages were also noted by Ross *et al*. (2010) in the Gulf of Mexico; these authors found that inshore samples showed the most notable differences, as in our study. No relevant seasonal differences were observed either between the species that appeared in the two studied months or in their diel vertical distribution patterns. The different vertical structure in the water column, from a mixed upper 100 m in autumn to a marked seasonal thermocline from 20 to 50 m in summer, does not seem to prevent vertical displacements of mesopelagic fishes towards the surface. The different selectivity of the nets demonstrates the importance of combining their results to draw conclusions regarding vertical diversity patterns. The high species richness at the near surface during night and at the DSL during day and night tends to be offset by the dominance of a few myctophids (as indicated by the PTs catches) or by *Cyclothone braueri* (as indicated by the small net catches), respectively, which makes the actual diversity at these levels relatively low. Therefore, both levels represent ecosystems with numerous species interactions, although the dominance of a few species and the dynamic nature of these assemblages (day night migrations and patchy distribution) do not contribute to the development of stable relationships among species.

The most commonly reported vertical movements of myctophids involve travelling up to near-surface layers at night (Badcock and Merret, 1976; Gartner *et al.*, 1987; Watanabe *et al.*, 1999; Sassa *et al.*, 2002; Yatsu *et al.*, 2005), as was observed for most of the myctophids in our study. However, as in many studies in other regions, a non-migratory portion of the population was found in deeper layers (Watanabe *et al.*, 1999; Pearre, 2003), forming persistent near bottom aggregations in some cases (Gartner *et al.*, 2008; Sutton *et al.*, 2008). Interestingly, some of the species that exhibited the most intense night vertical

migrations to near-surface layers in our study, including *Benthosema glaciale*, *Lampanyctus crocodilus* and *Notoscopelus elongatus*, were also present near the bottom at night. The size frequency distributions in the sample taken at BBL2 at night indicated that it is likely that not all of the population migrates every day and that young fish migrate to the near surface to a greater extent than older individuals. Whether this distribution is the result of an asynchronous migration of individuals (Pearre, 2003) or due to the fact that populations are formed by migrating and non-migrating individuals has been the subject of numerous discussions (Gartner, 1991; Stefanescu and Cartes, 1992) and has become a topic of recent studies of in situ behaviour (Kaartvedt *et al.*, 2009). However, the absence of individuals of larger sizes at the surface and their presence near the bottom - BBL in this study, and just above the bottom as in Stefanescu and Cartes (1992), Moranta *et al*. (2008) and our own data from concurrent bottom trawls (unpublished data) - allow the assumption to be made that at least for *L. crocodilus* and *N. elongatus*, as fish become older, their migration pattern ceases. As observed in other myctophids, this lack of vertical migration could be a consequence of a reduced or atrophied swimbladder (Butler and Pearcy, 1972). Vertical velocities have been suggested as possible causes of passive vertical transport of inactive individuals of *B. glaciale* and *M. muelleri* elsewhere (Kaartvedt *et al.*, 2008). A strong front was observed during the autumn survey in the Sóller area, which generated vertical velocities up to 6 m/day, approximately 10 nautical miles from the fishing stations (Balbín *et al.*, 2011). This effect could fertilize this area, although it does not necessarily induce extensive passive vertical migrations up to the surface layer. The vertical velocities were negligible at the Cabrera zone and during the summer cruise in this area. For this reason, active behaviour must be involved to explain the observed extensive vertical migrations up to the near surface.

As a general rule, the biomass of mesopelagic fish in the daytime near the surface is very low (Koslow *et al.*, 1997), as suggested in the present study by daylight echograms and by the low or null catches of the small nets in the autumn and summer surveys. Further evidence of this was obtained in the four daylight PTs of the summer survey, devoid of mesopelagic fishes and composed mostly by medusae. Additionally, the daylight concentrations of myctophids for the 400 DSL were never as high as those obtained for the night near-surface samples, which makes it difficult to ascertain where the majority of myctophids were located during these hours. One possibility is that they do not travel to deeper layers in a straight vertical direction but move farther offshore. Another possibility is that they are less aggregated at this time than during their upper migrating hours, which makes difficult the determination of their location by using acoustic equipment and performing collections through discrete hauls. In fact, as it has been previously pointed out for other groups of organisms, vertical movements to the near surface or to the bottom are likely not undertaken in a straight vertical dimension, but occur over a wider area that includes the horizontal onshore-offshore axis, as observed by Benoit-Bird and Au (2006) and McManus *et al*. (2008).

Light, food and temperature have been reported to be the main factors related to vertical migration (Badcock and Merret, 1976; Pearre, 2003); additionally, there is evidence that shows that a sharp temperature gradient may limit upward migration in some regions (Tont, 1976). In the western Mediterranean, the main vertical thermal gradients, of ca. 14 °C, were detected in July in the upper 100 m in front of just ca. 5 °C in December. In both periods, however, temperature below 150 m is similar, ca. 13 °C. If temperature was the determinant for vertical migration of the species in this study, the influence would be noticeable in the species that migrate to near-surface layers; however, for the most common species, no different diel-migrating pattern was observed between the two

periods. The effect of light seems to be reliable because of the similar day-night patterns detected for each species between months. Finally, the cause of these vertical upward displacements at night has often been explained as fishes chasing after zooplankton (food) and by their feeding at night at these upper layers. We infer that this pattern, observed in other oceanic regions, also fits the Mediterranean, where many species present full stomachs during night hours in these upper layers (A. Bernal, personal observations). The role that these migrating species may play as a possible mechanism of carbon transport to bottom layers is a subject that deserves further attention through the investigation of trophic interactions.

In contrast to the general upward night migration of most myctophids, the most abundant stomiiforms (*Cyclothone* spp., *Argyropelecus hemigymnus* and *Vinciguerria attenuata*) do not perform extensive diel migrations to the upper layers, appearing during both day and night at 400 DSL and at lower concentrations near the bottom layer. This pattern is common for most of the numerous species of the genus *Cyclothone* worldwide (Badcock and Merret, 1976; Ross *et al.*, 2010) and has been reported for *C. braueri* in the NW Mediterranean (Andersen and Sardou, 1992). Although it has been found that static scattering layers do not necessarily remain static through the year (Tont, 1976), in the present study, the 400 DSL appeared as a constant echotrace (at 38 kHz), in which the most abundant and frequent fish was the swimbladdered species *C. braueri* during both day and night in both December and July surveys. Another distinct species is the sternoptychid *Maurolicus muelleri*, which has been reported to appear near land over relatively shallow bottoms (Jespersen and Tåning, 1926), as was found in the present study; this finding confirms the pseudoceanic nature of the species in the Mediterranean, has been observed for its SE Atlantic congeneric species (Hulley and Prosch, 1987).

In summary, the combined use of small and large midwater trawls showed that the mesopelagic fish community in the western Mediterranean is characterised by relatively low diversity with a few species that are dominant in terms of the number of individuals, specifically *Cyclothone braueri* at the 400-600 m DSL (both day and night) and *Ceratoscopelus maderensis*, *Benthosema glaciale* and *Notoscopelus elongatus* at the near surface at night. The mesopelagic fish community is structured similarly in terms of space and time (regarding similarity between our two study sites and periods), with bathymetry and the level of the water column being the most important factors differentiating the fish assemblages. Two behaviour patterns were detected: species that do not migrate to the near surface, exemplified by *Cyclothone braueri* and *Argyropelecus hemigymnus*, which are partly responsible for the permanent acoustic response at ca. 400 m DSL; and near-surface migrants at night, represented by most of the juvenile and adult myctophids, with the exception of the largest size classes of some species, such as *Lampanyctus crocodilus* and *Notoscopelus elongatus*. The extent of these diel vertical displacements along the water column links pelagic demersal and benthic fauna and demonstrates the importance of this group of fishes with respect to energy and carbon transfer along the water column. In fact, the mesopelagic fishes *Maurolicus muelleri*, *Ceratoscopelus maderensis* and *Notoscopelus elongatus*, together with the euphausid crustacean *Meganyctiphanes norvegica*, are the main preys of *Merluccius merluccius* (Cartes *et al.*, 2009), one of the key species of the deep sea ecosystems in the study area (Moranta *et al.*, 2008). Within the framework of shelf-break and slope ecology, Cartes *et al*. (2009) have shown how the 'boundary' mesopelagic community inhabiting the middle slope sustains the trophic requirements of hake through an inverse energy transfer from deep- to shallow-water marine ecosystems. Similarly, the demersal sharks *Etmopterus spinax* and *Galeus melastomus* inhabiting the upper and middle slope regions of the Balearic Islands consume mesopelagic prey species of the Benthic Boundary

Layer, including myctophids, euphausiids and cephalopods, such as *Histioteuthis* spp. (Valls *et al.*, 2011), indicating the high dependence of slope demersal elasmobranchs on the pelagic ecosystem. In the slope ecosystems off the Balearic Islands there are more marked oligotrophic conditions than on the continental slope in other parts of the western Mediterranean, where submarine canyons play an important role in the transfer of matter (Buscail *et al.*, 1990; Puig *et al.*, 2000). This vertical transport is especially important on the insular slope, and the organisms of the benthos depend more directly on planktonic and nektonic prey along the water column (Maynou and Cartes, 2000; Cartes *et al.*, 2008).

In spite of the increasing trend in the sea surface temperature in the western Mediterranean, which has been proven to affect to some coastal species (Sabatés *et al.*, 2006), no changes in species composition and dominance with respect to the temporally distant study performed by Goodyear *et al.* (1972) were apparent. However, data on the actual abundance of the different species are not available to further analyse the climatic effects on the distribution and relative abundance of oceanic fishes, which have been demonstrated to have a significant influence on both abundance and onshore-offshore distributions in other regions (Hsieh *et al.*, 2009).

2 b. Micronekton groups contributing to the night scattering layers in the western Mediterranean

Abstract

The biomass and numerical contribution of micronekton organisms responsible for the scattering layers of the upper water column at night were investigated. The relative abundance in weight and number of individuals of different groups of micronekton collected using simultaneously midwater trawls and echosounders for the detection of the scattering layers are presented in this study.

Vertical nycthemeral migrations of the different mesopelagic fishes, decapods and cephalopods of the shelf-break and slope zone off the Mallorca Island (western Mediterranean) were already analysed in previous studies (Olivar *et al.*, 2012; Quetglas *et al.*, 2013; Simão *et al.*, 2013), showing upwards displacements to the upper layers of many species during the night, and very low concentrations in the upper layers during the day. We have investigated the overall relative contribution of the several micronekton groups to the epipelagic and Deep Scattering Layers (DSL) detected with acoustic methods. To this aim, micronekton samples from the slope region off Mallorca Island obtained during two surveys (December 2009 and July 2010) at night were analysed. Samples were collected by means of modified Pelagic Trawls (PT) with a mouth opening of 200 m^2 (December) and 100 m^2 (July) and a cod-end mesh size of 10 mm.

Complementary information on the presence of the smallest fishes was obtained with an Issaks-Kidd Midwater Trawl (IKMT) of 3 m^2 and mesh size of 3 mm to collect smaller specimens. The nets were placed at the denser scatter layers detected during night-time with the Simrad EK60 echosounder at 18, 38, 70, 120 and 200 kHz. Hauls were carried out in the epipelagic layers from 40 to 80 m and at the 400 m DSL.

Night samples collected with the PT showed that crustaceans and fishes were the most diverse groups contributing to the scattering layers; crustaceans were represented by 14 decapods, 5 amphipods, 3 euphausiids and 1 lophogastrid, and fishes were represented by 13 myctophiform species, 7 stomiiform species and 1 aulopiform species. Most of the biomass in the upper 400 m was formed by fishes and crustaceans in autumn, and fishes and gelatinous plankton (siphonophores and jellyfish) in summer (Fig. 2b.1). Molluscs and tunicates accounted for less than 20% of the overall weight collected in these midwater hauls. In autumn all groups showed higher biomasses at the epipelagic levels

than at the DSL, but in summer differences were less pronounced. The highest fish biomass was rendered by species of the family Myctophidae, particularly, the species *Ceratoscopelus maderensis*, and with a higher representation in summer. Most mesopelagic fishes reach the epipelagic layer during the nycthemeral migration, with the exception of *Argyropelecus hemigymnus*, *Cyclothone braueri* and *C. pygmaea*, which do not migrate to the near surface layers. The two latter species were seldom caught with PT, but their vertical location could be established through the IKMT hauls. Therefore, myctophiforms and stomiiforms contributed to the night DSL, and myctophiforms were the main fish responsible for the epipelagic scattering layers.

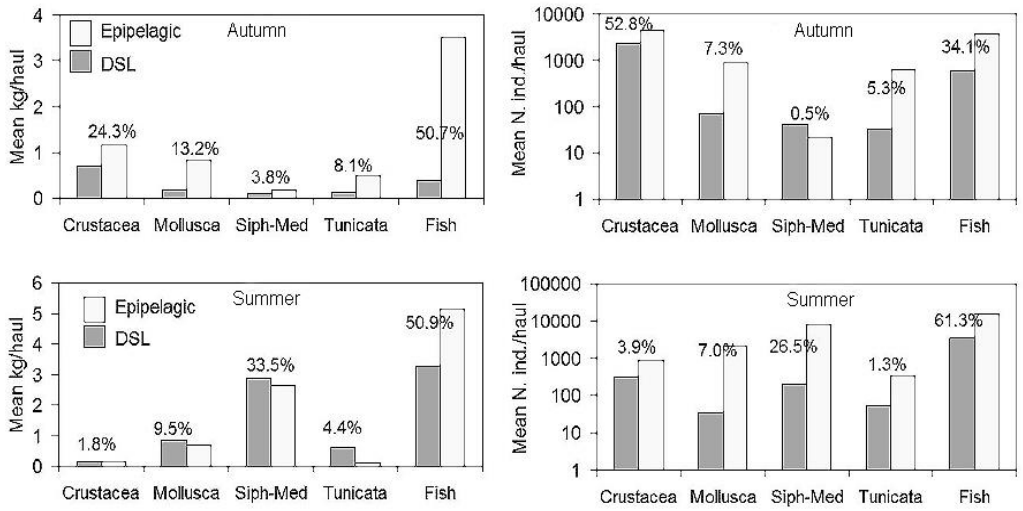


Fig. 2 b.1 Abundance of the different micronekton groups to the epipelagic and deep scattering layers at night. Left graphs show mean weight per haul and right graphs indicate mean number of individuals per haul in logarithmic scale.

The biomass of crustaceans was mainly represented by euphausiid and decapod concentrations; the euphausiid *Meganyctiphanes norvegica* was the principal contributor at both epipelagic and deep scattering layers, but with a higher abundance in epipelagic layers. The decapods *Sergestes arcticus* and *Pasiphaea multidentata* were the next species in terms of weight biomass of crustaceans.

Molluscs were mostly represented by cephalopods, predominantly *Todarodes sagittatus*, and the pterpod *Cymbulia peroni*, that accounted for most of the mollusc biomass in the autumn collections, particularly at the epipelagic layers. The tunicate *Pyrosoma atlanticum* showed higher concentrations in epipelagic layers, and also higher concentrations in autumn than summer. On the contrary, in summer, the great contribution of *Salpa maxima* in the DSL increased tunicate biomasses at the DSL.

Abundance patterns showed some differences when considering the number of organisms, which were mostly due to the number of euphausiids that enhanced the importance of crustaceans. This was particularly relevant in autumn, when large concentrations of *M. norvegica* were collected near the surface and in the DSL. Nevertheless, the individuals of *Cyclothone braueri* collected with the IKMT indicated that these fish species were, in numerical terms, the most abundant mesopelagic fish in hauls performed with this net at the DSL (Olivar *et al.*, 2012). Despite we are aware that the biomass values presented here are strongly dependent on the net and haul types, and therefore they cannot be taken as the most accurate biomass values. However, we think that the results provided above offer a clear insight into the main groups responsible for the upper column scattering layers detected with echosounds at night.

In summary, myctophids, euphausiids and certain invertebrate taxa, such as *Cymbulia peroni* and *Pyrosoma atlanticum* in autumn, and medusae in summer, are the most common and abundant (both in weight and number of individuals) organisms encountered in the epipelagic and deep scattering layers detected acoustically. Interestingly, other very abundant mesopelagic fishes, such as *Cyclothone* spp. and *Argyropelecus hemigymnus*, are also important contributors only for the DSL.

Chapter 3

TROPHIC ECOLOGY

3. Feeding patterns of *Lampanyctus pusillus* (Pisces: Myctophidae) throughout its ontogenetic development

Abstract

The trophic ecology of the lanternfish *Lampanyctus pusillus* was investigated using individuals captured off the Balearic Islands (39º N, 2º E) (western Mediterranean) in December 2009. Based on gut content analyses, the trophic niche breadth, diet composition and selectivity were determined for the entire life cycle of *L. pusillus*. The larval stages fed actively near the surface during the day, with a feeding incidence (FI) of approximately 71%. In contrast, the adults fed at night, both in near-surface depths and in the 400 m deep scattering layer, with a higher FI (83%). Diet analysis revealed a shift in the prey choice throughout ontogenetic development, from preflexion individuals, which selected nauplii and small oncaeids, to postflexion larvae, which consumed a variety of calanoids, mainly *Clausocalanus* spp., to the adults, which preyed on large organisms, exhibiting positive selectivity for *Pleuromamma* spp. and euphausiids. These results show that the vertical distribution of larvae and adults is partly conditioned by their respective feeding habits, with larvae feeding on small zooplankton in the upper layer and adults preferring to consume larger taxa that perform nycthemeral migrations.

3.1 Introduction

Myctophids (lanternfish) are meso- and bathypelagic fishes found abundantly in the open ocean. Lanternfish are important vectors for the vertical transport of organic matter from the ocean surface to the ocean floor because of their extensive migrations throughout the water column (Kozlov, 1995). They play an essential role as mediators of the trophic fluxes in the pelagic environment, constituting an important part of the diet of diverse predators, such as large fish, squids, marine birds and marine mammals (Pauly *et al.*, 1998; Williams *et al.*, 2001; Hunt *et al.*, 2005; Connan *et al.*, 2007), and in the top-down control of zooplankton (Certain *et al.*, 2011).

Current knowledge about the feeding behaviours of marine organisms is critical for understanding how these organisms interact with each other and the matter fluxes that they generate. By characterising the diet composition of the dominant mesopelagic fishes, trophic connections can be made between surface production and deep water dwellers. Studies addressing the feeding habits of myctophids in tropical and temperate waters have revealed common alimentary patterns of larvae and adults: the majority of the species are zooplanktivorous, generally feeding in the upper hundreds of metres at night, exhibiting similar preferences for large crustaceans, such as large copepods or euphausiids (e.g. Gorelova, 1977; Clarke, 1978; Scotto di Carlo *et al.*, 1982; Hopkins and Baird, 1985; Young and Blaber, 1986; Pakhomov *et al.*, 1996; Conley and Hopkins, 2004). Additionally, studies examining the feeding patterns of myctophid larvae have shown that they rely mostly on small zooplankton items in the upper 200 m of the water column (Sabatés and Saiz, 2000; Rodríguez-Graña *et al.*, 2005; Sassa, 2010). However, these studies have not examined whether there is a dietary shift from the onset of feeding in larvae to adulthood within each target species.

The Myctophidae family in the western Mediterranean is composed of sixteen species (Jespersen and Tåning, 1926; Hulley, 1984), among which *Ceratoscopelus maderensis* is generally the most numerous (Jespersen and Tåning, 1926; Goodyear *et al.*, 1972a; Olivar *et al.*, 2012). Although lanternfish are often recognised as food for many other fish species in the Mediterranean (Cartes *et al.*, 2001; Carrasón and Cartes, 2002; Cartes and Carrasón, 2004; Bozzano *et al.*, 2005), information on their trophic ecology is limited. The available data are restricted to data on the stomach contents of the most common lanternfish found in bottom trawls, that is, *Lampanyctus crocodilus* (Stefanescu and Cartes, 1992). The oldest adults of *L. crocodilus* no longer migrate, and their diet adapts to an epibenthic environment, differing from the mesopelagic diet of juveniles (Stefanescu and Cartes, 1992). No study has yet been performed on the trophic ecology of the other *Lampanyctus* species found in the Mediterranean Sea, that is, *Lampanyctus pusillus* (Johnson, 1890), which inhabits all temperate and subtropical oceans (Hulley, 1981). Although *L. pusillus* larvae have often been reported in ichthyoplankton analyses performed in the western Mediterranean (Sabatés, 1990; Olivar *et al.*, 2010), adults have rarely been included in fish collections carried out in the region since the early works of Jespersen and Tåning (1926) and Goodyear *et al.* (1972a), suggesting that this species is usually mistaken for *L. crocodilus* (see Tåning 1918). Recent analyses of the vertical distribution of mesopelagic fishes in the western Mediterranean have demonstrated that *L. pusillus* is a frequent, but not very abundant, species; its adults constituted <2% of the overall myctophid populations and were the 7th most abundant out of the 13 myctophid species (Olivar *et al.*, 2012).

This study examines *L. pusillus* off Mallorca Island (Balearic Sea, western Mediterranean). This region exhibits low primary production (Margalef, 1985), but its oligotrophic environment is dynamic: due to seasonal hydrological

forces, the seawater alternates between showing thermal stratification during the summer, with consequent nutrient depletion in surface layers, and a period of mixing during the autumn-winter (Estrada, 1996). The hydrodynamics of the Algerian and Balearic basins (north and south of Mallorca Island), where our samples were collected, are determined by intra-annual variations of the cyclonic gyre in the northern part of Mallorca (Pinot *et al.*, 2002). The southern region is influenced by gyres that carry transitional Atlantic waters toward Cabrera. Compared with other pelagic regions, the zooplankton in the Balearic Sea are characterised by low abundance and high diversity (Fernández de Puelles *et al.*, 2003; Fernández de Puelles *et al.*, 2007; Siokou-Frangou and Mazzocchi, 2010). The variations in the abundance and distribution of the zooplankton in the western Mediterranean are determined by these complex currents and water masses at intermediate layers that well up during the mixing period, making nutrients accessible to planktonic organisms and pelagic fish larvae at the ocean surface.

The purpose of this study is to determine the trophic ecology of *L. pusillus* from larval stages to adulthood, focusing on the diet preferences among the different ontogenetic stages via an analysis of their gut contents. The relationship between prey availability and diet was used to evaluate the biological factors determining prey selection by *L. pusillus*. Finally, comparisons with the available information on the diet of other myctophids are also discussed. The present work constitutes part of a large multidisciplinary study examining how small pelagic fish in the Balearic Islands favour trophodynamic fluxes within the water column.

3.2 Materials and methods

3.2.1 Study area and sampling procedure

The study was conducted in December 2009 over the shelf (200 m) and mid-continental slope (700-900 m) off the Balearic Islands (Fig. 3.1). Sampling was performed at fixed stations during day- and night-time using various types of nets to collect the different developmental stages of fishes and their potential prey species. The majority of adults collected in this survey came from night hauls. Due to this limitation, the few adults caught from a survey carried out in July 2010 were also dissected to allow inferences to be made about diel feeding habits.

Zooplankton samples were collected by means of vertical hauls of micro- (50-200 µm) and mesozooplankton (200-1000 µm) from a depth of 200 m to the surface using Calvet (53 µm) and 3-WP2 (200 µm) nets. Using each of these nets, 7 day and 7 night hauls were performed. Fish larvae, mesozooplankton and macrozooplankton were collected via depth-stratified oblique hauls performed with a Hydro-Bios 0.25 m^2 Multinet (300 µm) at five discrete depths in the water column from the surface to 20 m above the seabed (see Fig. 3.1). A total of 8 day and 8 night hauls were carried out with the Multinet. Postflexion larvae in surface layers (0-60 m) were also caught through oblique hauls employing a 1 m^2 RMT (Rectangular Midwater Trawl, with a 1.5 mm mesh size) in 6 day and 8 night hauls. The ship speed for the Multinet and RMT hauls was 2-2.5 knots.

Adult collections were performed in the water depths showing the highest acoustic response, where the major myctophid concentrations were found (Olivar *et al.*, 2012). Sampling was executed with a 280 m^2 pelagic trawl (with a 10 mm mesh size in the cod-end) and a IKMT-3 m^2 net (Isaacs-Kidd Midwater Trawl, with a 3 mm mesh size) for 60 and 30 min, respectively, in the layers that

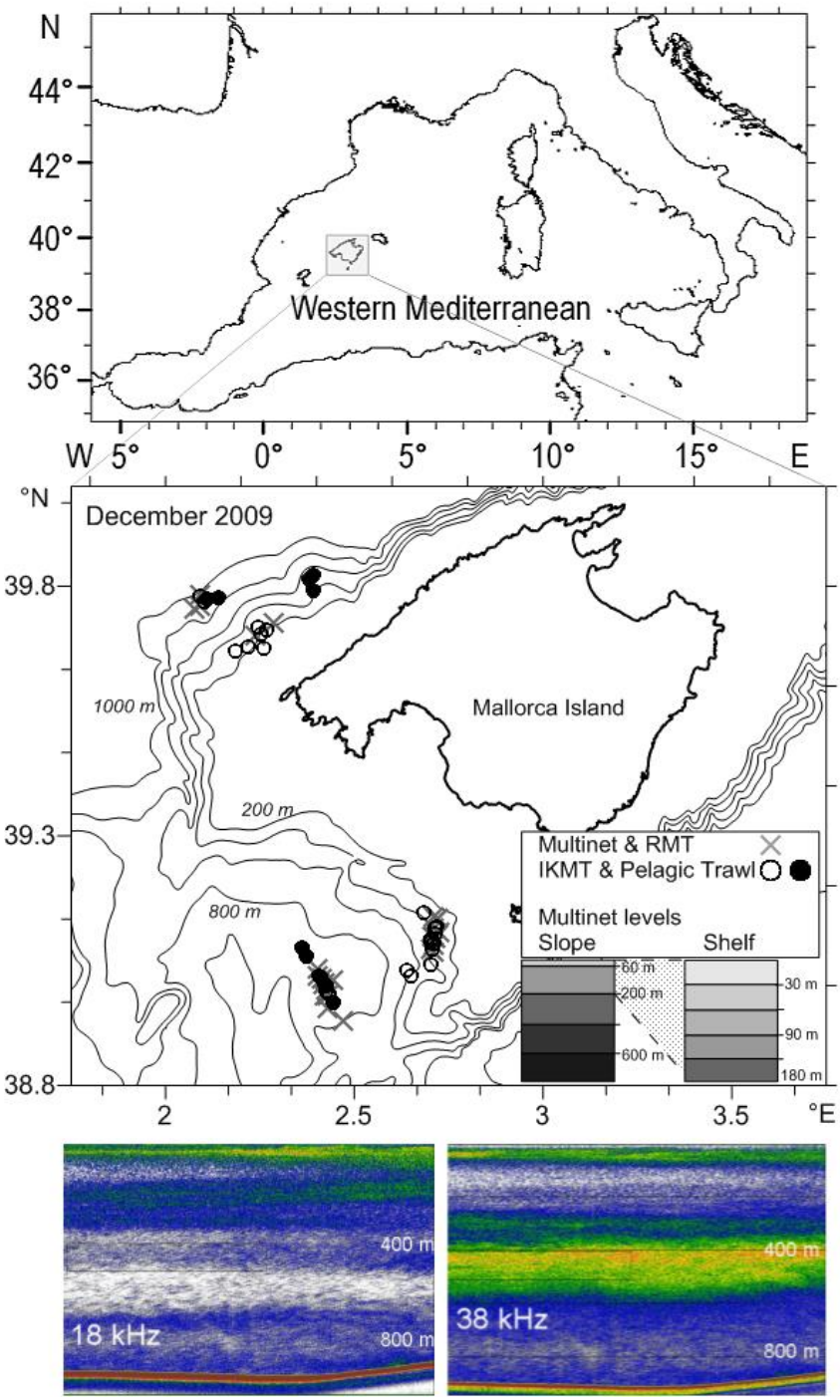


Fig. 3.1 Sampling grid covering the western Mediterranean off the coast of Mallorca during the cruise in December 2009 using different types of nets. Crosses: locations of larval sampling. Circles: locations of adult sampling. Filled circles indicate the presence of adults of *L. pusillus* and white circles the absence of the species in these hauls. Bottom images: acoustic echograms obtained at 18 and 38 kHz, showing the surface and 400 m Deep Scattering Layers.

rendered the main acoustic layers observed using 18 and 38 kHz echo sounding, which were the near-surface layer (ca. 60 m), 400 m deep scattering layer (400 m DSL) (Fig. 3.1) and benthic boundary layer (BBL, 50 m above the bottom). The ship speeds were 4 and 3 knots, respectively, during these collections. Because the pelagic trawl and the IKMT did not have opening and closing devices, these nets were lowered and retrieved as quickly as possible at a boat speed of ca. 1 knot to prevent contamination with specimens from the upper layers. The depth was controlled using a Scanmar sensor attached to the nets. The pelagic trawl was employed for 13 trawls during the night and 2 trawls at sunset, and the IKMT for 6-day and 11-night hauls.

The plankton samples were preserved in 5% buffered formalin. Adults were stored in 5-10% formalin or frozen on board at -20 ºC and then transferred to 5% formalin-buffered seawater prior to dissection.

3.2.2 Laboratory and statistical analyses

Microzooplankton samples (53-200 µm) were brought to a volume of 100 ml, and the nauplii from two 5-ml subsamples were counted and identified according to Fernández de Puelles *et al.* (1996). The mesozooplankton samples were analysed after subdivision with a Folsom Plankton splitter to determine the abundance of the main groups, especially copepod species, as the major contributors to the total zooplankton biomass and abundance (Fernández de Puelles *et al.*, 2007).

The gut contents of 156 larvae and 129 adults were examined to determine the changes in the diet during ontogenetic development (Table 3.1). Prior to dissection, the following measurements were recorded to the nearest 0.01 mm: (a) the distance along the midline of the body from the tip of the snout to the tip of the notochord in preflexion and flexion larvae (Notochord Length, NL) and to the posterior margin of the hypural elements in postflexion larvae and adults

(Standard Length, SL); (b) lower jaw length (LJ), measured from the tip of the snout to the junction with the maxilla; (c) upper jaw length (UJ), measured from the tip of the snout to the posterior end of the maxilla; and (d) mouth width (MW), measured ventrally as the widest distance between the posterior edges of the maxillae. MW, LJ and UJ were used to establish allometric relationships with NL/SL. Additionally, size frequency distributions were presented for the overall number of larvae and adults caught during the survey.

Table 3.1
Summary of *L. pusillus* analysed for stomach contents. All the catches came from the December 2009 survey. Exemplars from the 2010 survey were not included in this table. N.L.: number of larvae. N.A.: number of adults. SUR: Surface; DSL: 400 m Deep Scattering Layer; BBL1: Benthic Boundary Layer at the shelf stratum; BBL2: Benthic Boundary Layer at the slope stratum.

	N.L.				N.A.			
	prefexion & flexion		postflexion		immature		mature	
	Night	Day	Night	Day	Night	Day	Night	Day
Slope								
SUR2	20	28	12	40	44	0	28	0
400DSL	0	0	0	0	18	0	8	3
BBL2	0	0	0	0	0	0	11	0
Shelf								
SUR1	13	22	6	4	4	0	0	0
BBL1	5	4	23	1	0	1	0	0

In larvae, the entire gut tract was extracted and opened using a scalpel, whereas in adults the stomach was removed by cutting at the beginning of the oesophagus. To avoid including organisms that could have been consumed in the nets during catching, the prey items detected in the oesophagus were counted only if digested. Hence, four categories of digestion were considered: the digestion grade was 0 for digested unshaped prey; 1 for digested prey with a recognisable exoskeleton or shape (if gelatinous); 2 for moderately digested items; and 3 for whole, undigested items.

The gut contents were placed on a glass slide with a 50% mixture of water and glycerine and examined under a microscope (40-100-1000x) to allow the identification of each prey and the measurement of its maximum length and width (1000x). The food items were identified to the lowest possible taxonomic level. Two major categories of copepods were considered to easily represent the general trends in prey preferences: Calanoida and "Cyclopoida", emphasising those genera or species that were more representative in the diet and constituted minor individual categories associated with one of the major groups. We placed the genera *Oithona* and *Oncaea* inside the phylogenetic branch "Cyclopoida", as it is currently believed that they form a monophyletic group (Boxshall and Halsey, 2004). The oncaeids were identified to the genus level for the statistical analyses, as Böttger-Schnack and Schnack (2009) warned that species of *Oncaea* are often mistaken for similar organisms of the same genus in trophic studies.

In many cases, the food items could not be fully sized due to the advanced grade of digestion. If possible, apart from the maximum body width, the prosome length was given for both calanoids and "Cyclopoida", the total length for harpacticoids, the prosome length for euphausiids and the body length without the tail for larvaceans. The measurements for Urochordata and other gelatinous organisms found in the gut might be biased toward being smaller than live prey because they are affected by rapid digestion (Uchikawa *et al.*, 2001), resulting in subestimated sizes while counting. Faecal pellets were recognised based on their size, shape and colour. According to Wilson *et al*. (2008), ellipsoid, light brown pellets of c.a. 150-200 µm in length were identified as coming from larvaceans. Furthermore, in some cases, larvacean pellets appeared attached to their discarded houses.

The relationships between prey and predator sizes were analysed by classifying specimens into size intervals to produce the maximum number of size classes

containing 3 or more prey. To fit this criterion, intervals of 0.12 and 0.5 mm were used to regroup larvae and adults, respectively. Relationships were estimated via linear regression analysis (weighted by the number of prey items per size class). The changes in prey size were studied using Pearre's trophic niche breadth (1986). This model takes the SD of the $\log_{10}$-transformed prey size as a measure of the trophic niche breadth. The diet was analysed by combining specimens according to developmental stages. Larval stages were differentiated based on the bending of the notochord [i.e. preflexion (2.8-3.9 mm), flexion (4-4.9) or postflexion (5-8.5)]. Adults were separated by size between the "mature" and "immature" individuals according to Hulley (1984), who indicated that sexual maturity occurs beginning at approximately 36 mm (i.e., immature adults range from 14 to 35.9 mm and mature adults from 36 to 43 mm). The frequency of occurrence (%F) and the percentage of abundance (%N) of the diet items examined were calculated. The number of prey per larva as a function of time was compared among four time groups from sunrise (at 0610 hours GMT) to sunset (at 1623 hours GMT). The number of prey followed a Poisson distribution. Therefore, we used a Generalised Linear Model with the appropriate link function (in this case, the log link) to analyse the number of prey per larva as a function of the developmental stage and time. The feeding incidence (FI) was taken as the percentage of specimens examined containing at least one prey organism and was calculated separately for day and night.

The Shannon-Wiener Index was calculated to estimate the diversity of the prey items in the gut. The preflexion and flexion stages were considered together to avoid errors as a result of a small sample size. This index is defined as:

$$H' = - \sum p_i \ln (p_i)$$

Where p_i is the proportion of species i in the gut of each size group of *L. pusillus*. This index ranges from 1 to 4.5.

Selectivity was determined after zooplankton abundance was recorded by applying Chesson's electivity index (Chesson, 1978), calculated as follows:

$$\alpha_i = \frac{r_i / p_i}{\sum_{i=1}^{m} r_i / p_i}$$

Where r_i and p_i are the frequencies of a prey item in the diet and plankton, respectively, and m is the number of prey categories considered. The index ranges from 0 to 1. A value of 0 indicates that no individuals of a category were found in the gut, despite the presence of the item in the environment and the fact that that neutral selection would result in a constant 1 m^{-1}. Student's *t*-test was used for testing the differences in the mean selectivity values calculated for each prey type from the neutral value.

The selectivity values were calculated for individual specimens and for each food organism and then averaged for the developmental stages (preflexion, flexion and postflexion larvae, immature and mature adults). Thirteen prey categories were considered when analysing selectivity for the larval stages and 12 for the adults. Larval selectivity was estimated using zooplankton abundances from 6 daylight micro- and mesozooplankton samples (Calvet and 3WP2 nets). The information on zooplankton abundance required for adults referred to the temporally and spatially closest night-Multinet hauls, as meso- and macrozooplankton samples were not collected at the same time as most of the adult hauls.

3.3 Results

3.3.1 Vertical distribution of larvae and adults

Lampanyctus pusillus larvae appeared at both shelf and offshore stations, showing a similar vertical distribution in both zones, as they were found only

from the surface to 200 m of the water column. Larvae appeared mostly in surface layers (0-60 m) during daytime and displayed a wider range at night (0-200 m). Size frequency distributions revealed that the few larvae appearing below 60 m during daytime were of the preflexion and flexion stages, while in surface layers, postflexion larvae were also present (Fig. 3.2). No significant differences were found in diet composition of the different developmental stages of larvae between shelf and slope regions (MANOVA).

Adults appeared only at the slope stations. They were caught in all three sampled levels of the water column (near surface, 400 m DSL and BBL), with all the size ranges being found at the surface and DSL, whereas in the BBL, only the largest size classes (>35 mm SL) occurred (Fig. 3.3). The distribution of the adults during daylight hours remained unresolved because only 4 and 10 specimens were caught during the winter and summer surveys, respectively.

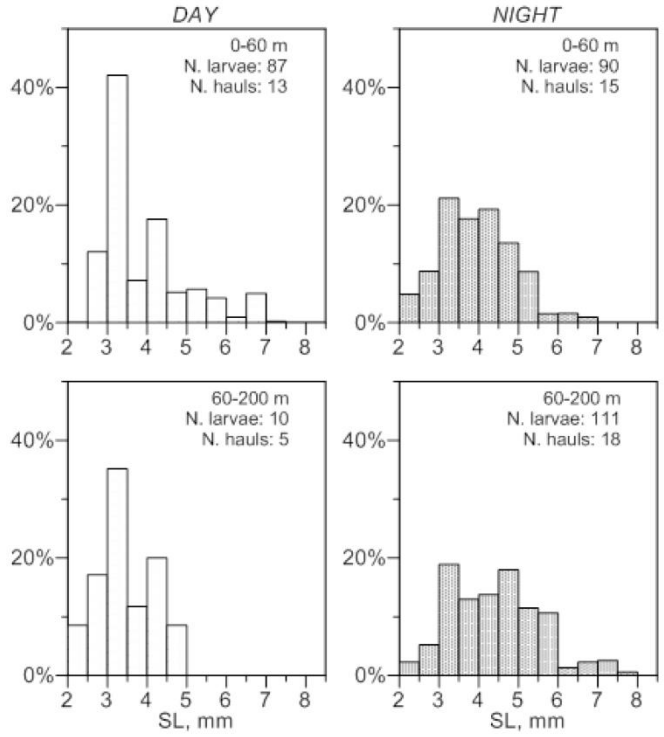


Fig. 3.2 Size frequency distribution of *L. pusillus* larvae caught by means of Multinet hauls, grouped into day and night captures and by depth layers (0-60 m and 60-200 m).

3.3.2 Feeding patterns

All of the larvae examined fed during daytime, except one of the largest specimens (7.4 mm SL, caught at 1843 hours GTM), which contained five moderately digested prey items (grade 1 or 2) in its gut. The smallest larva that contained prey in the gut measured 2.8 mm SL. There were significant

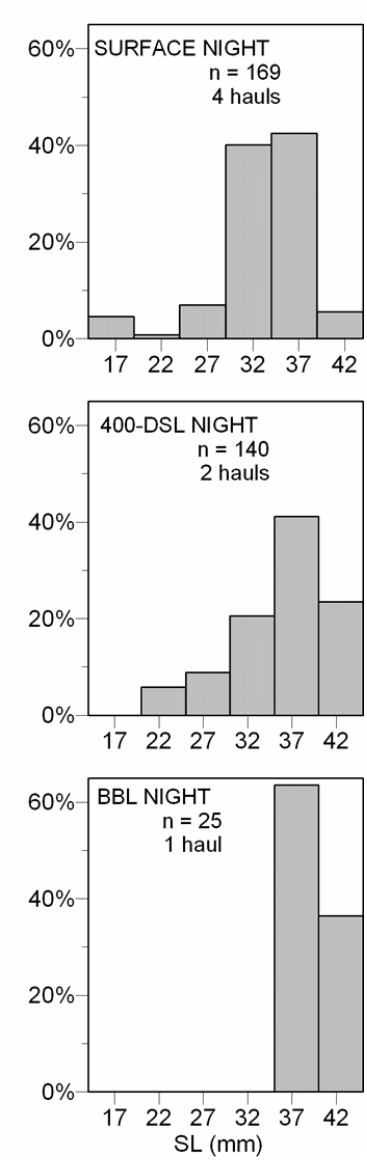


Fig. 3.3 Size frequency distribution by depth of adults of *L. pusillus* caught by means of horizontal pelagic hauls trawls during the night-time.

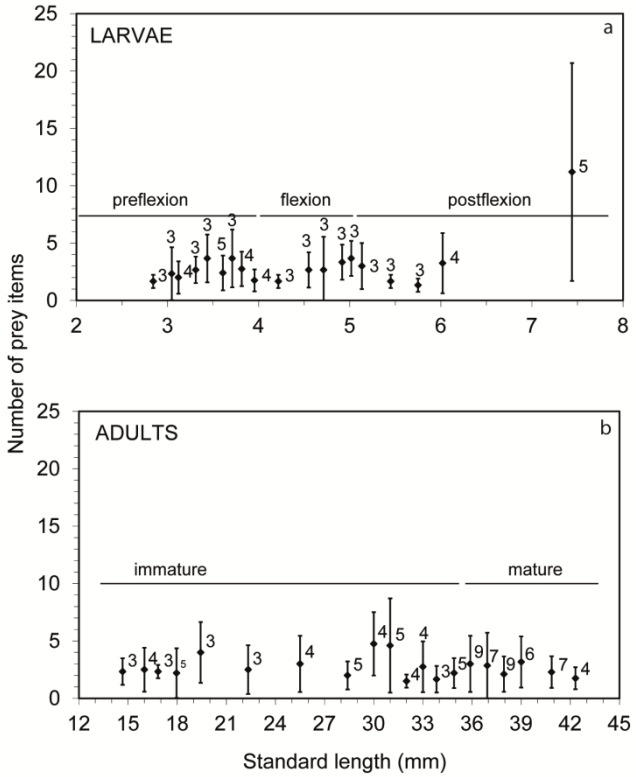


Fig. 3.4 Number of prey in the gut of *L. pusillus* across developmental stages (top figure) and an expanded graph of the larval stages (bottom image).

differences detected when considering the number of prey as a function of time ($\chi_3^2 = 74.047$, P<0.001), with a greater number of prey items per larva observed for the period between daybreak and midday, falling off from the last hours of light to sunset and during the night. The FI for larvae captured during the day was 71.3%.

The FI for night-collected adults was 83.5%. As adults were collected almost exclusively by means of night trawls during the study period (December 2009), the ten adults caught in the 31 day-hauls performed in a July 2010 survey were also examined. Their stomachs contained disaggregated remains and sclerotized body parts resistant to digestion (e.g. the knob of *Pleuromamma* spp., hard spines of *Centropages* spp. and a few hard exoskeletal regions from euphausiids). These results suggest that adults do not eat during the day.

3.3.3 Predator-prey interactions and trophic niche breadth

No significant differences were found in the number of ingested prey among the different larval stages ($\chi_3^2 = 1.599$, P = 0.45). The prey number did not change from early-feeding larvae to those of 7 mm SL (mean values from 1.7 to 3), although a noticeable increase in the number of prey was observed in the two largest larvae examined (8.5 and 8.7 mm SL), which contained more items (23 and 10 items) (Fig. 3.4). The number of food items ingested by the adults was fairly constant, and the mean ranged from 2 to 5 (Fig. 3.4).

The mouth width, upper jaw length and lower jaw length showed a significantly positive allometric relationship with SL (MW: b= 2.214, 95% CI, b= 0.185, r^2= 0.854; UJ: b= 2.027, 95% CI, b= 0.175, r^2= 0.849; LJ: b= 1.951, 95% CI, b= 0.174, r^2= 0.842), demonstrating that the size of the mouth increased at a relatively higher growth rate than the body length. The weighted regressions for the mean log size of prey items on larval MW and SL exhibited positive slopes

and significant correlations (Fig. 3.5). Figure 3.6 depicts the relationship between prey width and the standard length of all of the individuals analysed from the first larval stage to the largest adults (r^2 = 0.77, P<0.0001). The smallest larvae ingested prey of ca. 25 µm, but as the size of the larval mouth increased, the larvae fed on larger prey. Organisms of >400 µm in width were consumed by only larvae ≥6 mm, whereas larvae >8 mm SL consumed prey up to ca. 500 µm in width. The adults consumed larger items than the larvae, although they also fed on small organisms. The width of the prey ingested by the

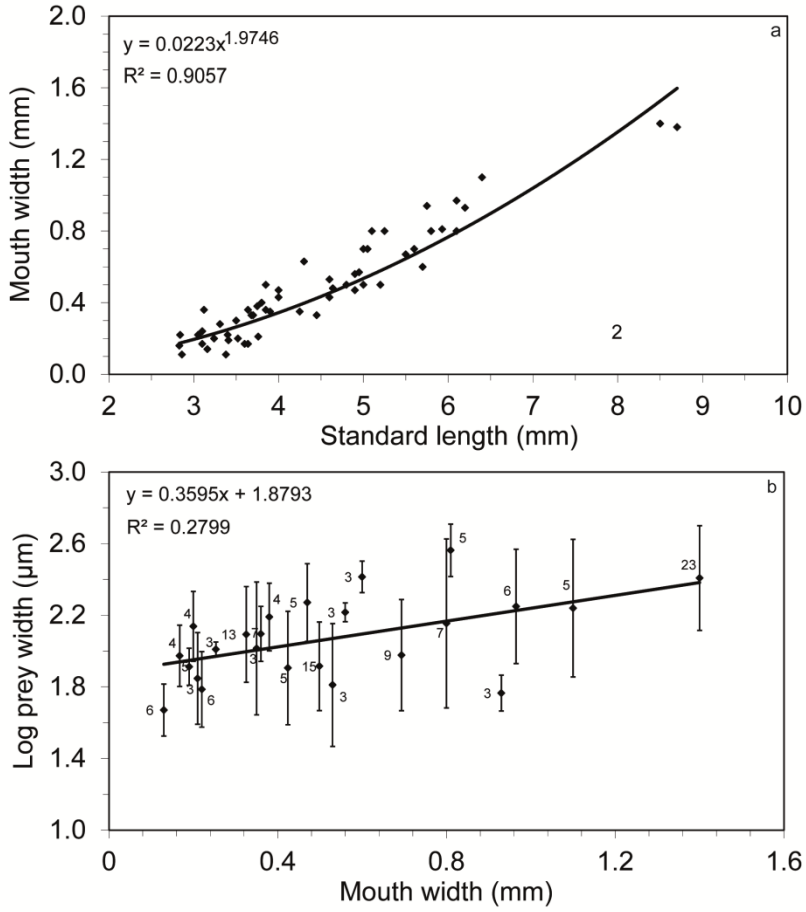


Fig. 3.5 a) Relationship between the standard length (SL) and mouth width (MW) in larvae; b) logarithmic mean prey item width (±SD) plotted against MW. The numbers over the data points indicate the number of prey items per larval size class.

adults ranged from 85 µm to 2.8 mm (Fig. 3.6). The weighted regressions for the mean log width of the prey items on adult SL had a positive slope, but it was lower than those for the larval stages (b = 0.0121; r^2 = 0.41 for the adults and b = 0.093; r^2 = 0.38 for the larvae).

The relationship between the niche breadth (standard deviation of the log of prey width) and SL did not change during development, although it did increase during the first stages of larval development up to 5 mm SL (Fig. 3.6). An

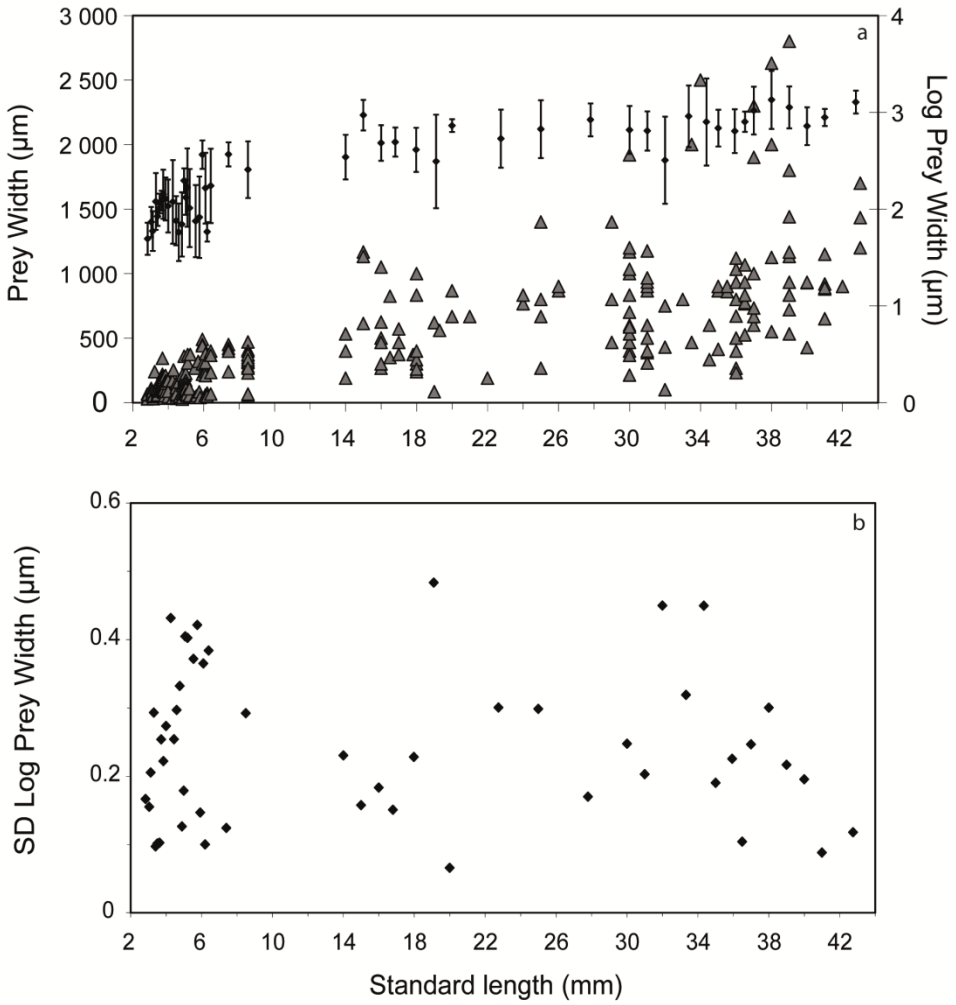


Fig. 3.6 a) Logarithmic mean prey width (µm) (± SD) plotted against the SL (mm) (right axis) and prey width (left axis) versus the SL for both larvae and adults. The triangles represent individual observations. b) Niche breadth, expressed as the SD log of prey in relation to the SL.

ANOVA confirmed that the SD log PW is independent of the SL for both larvae and adults ($r^2 = 0.14$; P = 0.066, and $r^2 = 0.002$; P = 0.827, respectively).

Table 3.2
Ingested prey items are summarised by numerical percentage (%N), frequency of occurrence in feeding individuals (%F) and index of relative importance (%IRI)

Developmental stage	**Preflexion**		**Flexion**		**Postflexion**		**Immature**		**Mature**	
Size class (mm SL)	2.8-3.9		4.0-4.9		5.0-8.5		14.0-35.9		36.0-43.0	
Nº guts with prey	29		10		22		51		35	
Diversity Index *H'*	2.17				2.51		2.50		1.77	
FOOD ITEMS	%N	%F	%N	%F	%N	%F	%N	%F	%N	%F
Tintinnida; Tintinnoinea										
Tintinnopsis spp.	5.7	10.3								
Polychaete; Annelida							1.4	2.0		
Ostracoda										
Conchoecia obtusata					4.5	9.1	4.8	11.8	2.2	5.7
Nauplii (copepods & euphasiids)	28.6	44.8	4.3	10.0	1.1	4.5				
Copepoda										
Egg	5.7	10.3	4.3	10.0	2.2	9.1				
Calanoid copepods										
Aetideus cf					1.1	4.5				
Acartia sp.									1.1	2.9
Calanus sp.							0.7	2.0		
Candacia sp.							0.7	2.0		
Mesocalanus tenuicornis							1.4	3.9		
Pleuromamma abdominalis							25.0	43.1	30.1	42.9
Pleuromamma gracilis							2.8	5.9	4.3	8.6
Pleuromamma spp							23.6	41.2	16.1	28.6
Paracandacia simplex							2.8	7.8	1.1	2.9
Nannocalanus minor							1.4	3.9	2.2	5.7
Temora stylifera							0.7	2.0		
Eucalanoidea							0.7	2.0		
Euchaetidae										
Euchaeta acuta							2.1	5.9	3.3	8.5
Euchaeta marina							0.7	2.0		
Euchaeta spp.							2.1	5.9	2.1	2.9
Paracalanidae										
Calocalanus pavo					1.1	4.5				
Calocalanus spp.	1.4	3.4			4.5	18.2				
Paracalanus spp.			4.3	10.0	2.3	9.1				
Clausocalanoidea										
Clausocalanus acuicornis					15.7	18.2	2.2	3.6		
Clausocalanus spp					10.1	13.6	1.3	6.2	1.1	2.9
Euchirella messinensis									1.1	2.9
Gaetanus sp							0.7	2.0		

Table 3.2 Continued.

Microcalanus cf							0.7	2.0		
Scottocalanus persecans							0.7	2.0	1.1	2.9
Unidentified calanoids	4.3	10.3	17.4	30.0	7.9	27.3			1.1	2.9
Non-Calanoida										
Poecilostomatoida										
Corycaeidae	1.4	3.4			1.1	4.5				
Oncaea spp	25.7	48.3	17.4	20.0	5.6	18.2	4.9	11.8	2.2	5.7
Cyclopoida										
Oithona spp.	8.6	17.2	4.3	10.0	3.4	9.1				
Harpacticoid copepods										
Microsetella rosea							0.7	2.0		
Unidentified Non-Calanoida	2.9	6.9			1.1	4.5				
Unidentified copepods	2.9	6.9			2.2	9.1	6.9	13.7	2.2	5.7
Mysidacea							0.7	2.0		
Euphausiidae										
Calyptopis/furcilia	1.4	3.4			2.2	9.1				
Meganyctiphanes norvegica							0.7	2.0	3.2	5.7
Meganyctiphanes spp.					2.2	4.5	1.4	3.9	4.4	11.4
Unidentified Euphausiacea							2.8	5.9	20.7	45.8
Unidentified crustaceans	1.4	3.4	4.3	10.0	3.4	9.1	2.1	5.9		
Pteropoda							0.7	2.0		
Appendicularians										
Discarded houses or body parts			8.7	20.0	10.1	31.8	0.7	2.0		
Faecal pellets	1.4	3.4	34.8	40.0	15.7	36.4				
Undetermined gelatinous	1.4	3.4			1.1	4.5				
Salpida										
Salpa spp							1.4	3.9		
Teleostei										
Myctophidae									1.1	2.9
Unidentified remains	7.1	17.2			1.1	4.5	0.7	2.0		

3.3.4 Dietary shifts and selectivity

A total of 36 prey taxa, which were almost exclusively pelagic organisms, were identified within the larval guts and the stomachs of adults (Table 3.2). The gut diversity increased with larval development. The highest value for gut diversity was observed in postflexion larvae and immature adults, while the lowest was found for mature adults (Table 3.2).

The preflexion larvae fed selectively on nauplii of different crustaceans (mainly copepods) and oncaeids, which represented more than 70% of the gut contents

(Figs. 3.7 and 3.8). The flexion specimens exhibited a wide range of prey categories, and small calanoids (<0.7 mm), oncaeids and faecal pellets from larvaceans made the greatest contributions to the diet in terms of abundance and the frequency of occurrence; however, no significant positive selectivity was observed. The diet of postflexion larvae comprised a variety of items (Fig. 3.7); several genera of calanoid copepods composed more than a third of the total prey in the gut, with some genera being notably more representative (e.g. *Clausocalanus* or *Pleuromamma*). Other minor contributors included larvaceans, cyclopoids, Paracalanidae and calanoids other than *Paracalanus* spp. The calanoid genus *Clausocalanus* was highly represented in postflexion larvae, followed by larvacean faecal pellets. Both categories were positively selected (Fig. 3.8). A shift in food intake was observed in immature adults. The contribution of small prey items such as *Oncaea* spp. diminished, whereas the euphausiid postfurcilia appeared for the first time in the diet. The order Calanoida, comprising diverse species of calanoids, was the most important; within this category, the genus *Pleuromamma* was the favoured selected item (Fig. 3.8). Other calanoids constituted minor prey categories eaten by immature adults, usually with lower frequencies. Calanoids were also the dominant group in mature adults, dominated by *Pleuromamma* spp., decreasing slightly from immature to mature individuals as euphausiids became more abundant and representing the only positively selected items for mature adults. The abundance of calanoids (including *Pleuromamma* spp.) was thirteen times the abundance of euphausiids in the stomachs of immature adults and twice the abundance of euphausiids in the stomachs of mature adults.

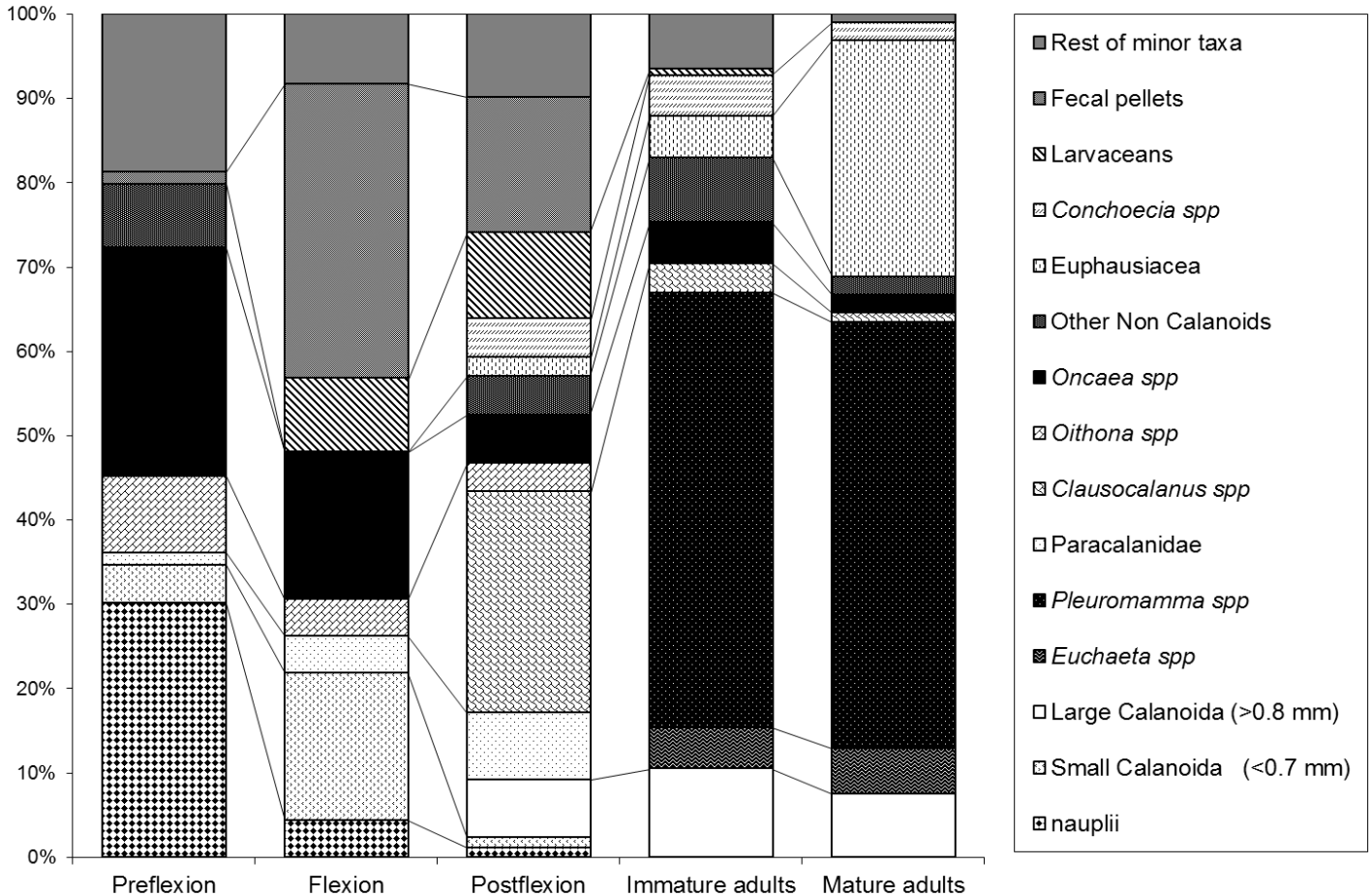


Fig. 3.7 Food composition grouped into major categories. The food category "Euphausiacea" also includes the furcilia phase, which appeared exclusively in *L. pusillus* postflexion larvae. The vertical axis indicates the percentage of prey consumed.

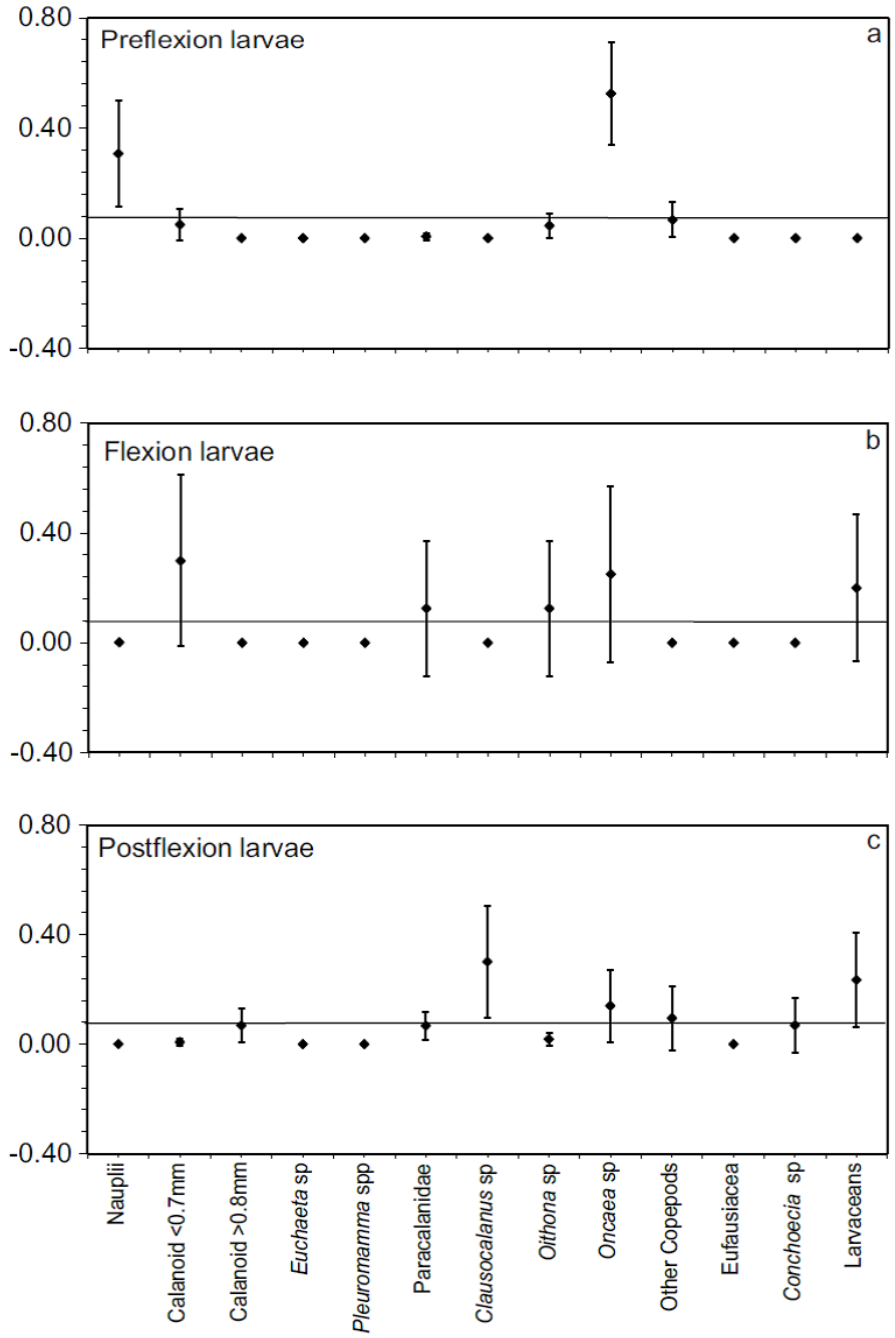
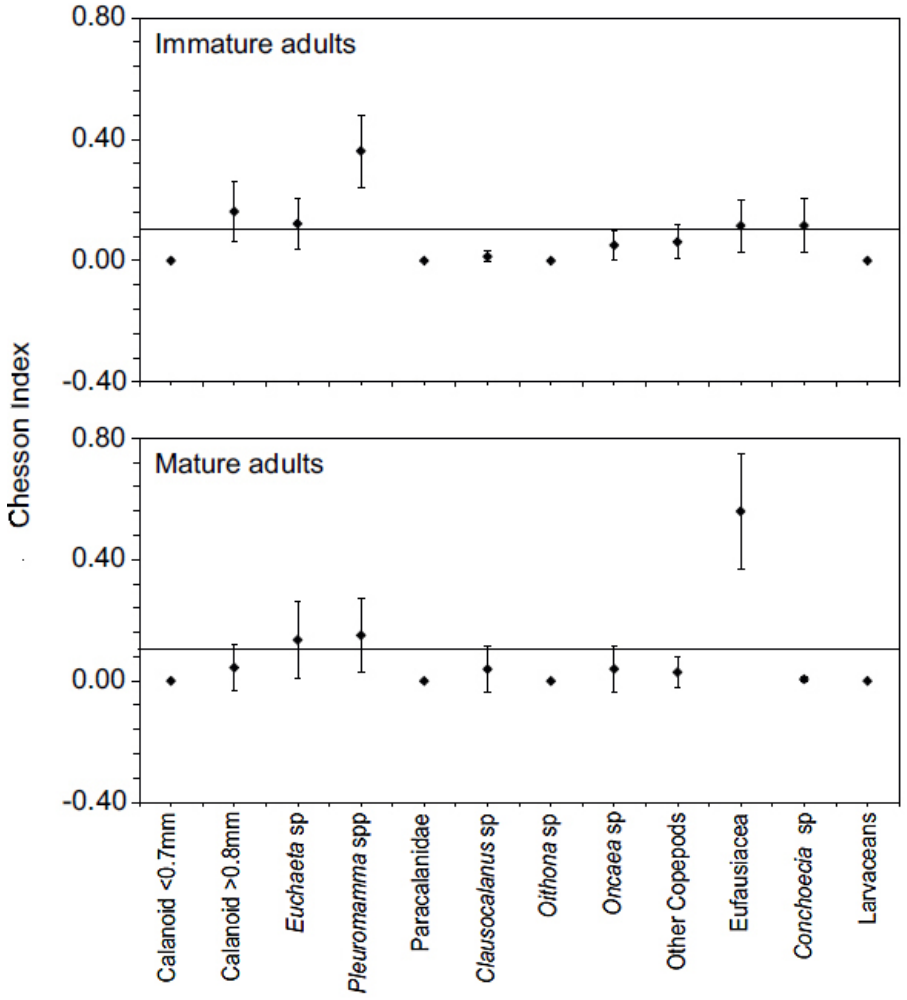


Fig. 3.8 Mean Chesson's α values (±95% confidence interval) for the major zooplankton categories found in the stomachs of the different size classes. The horizontal line denotes neutral selectivity.

Fig. 3.8 Continued



3.4 Discussion

Extensive literature on lanternfishes documents that nycthemeral migrations to the upper layers have generally been interpreted as feeding displacements following the night-time ascension of their potential zooplankton prey (Merrett and Roe, 1974; Gartner *et al.*, 1987; Moku *et al.*, 2000; Watanabe *et al.*, 2002), notwithstanding the possibility that this behaviour might also be related to other factors, such as the species population density or avoidance of predators

(Mehner and Kasprzak, 2011). Similar to many other myctophids, it has been reported that *L. pusillus* in the adult stages undertake such nycthemeral migrations (Badcock, 1970). Very little was previously known about the trophic ecology of *L. pusillus*, particularly in relation to vertical displacements. Thus, a comprehensive study of the feeding ecology of this species over its entire life cycle was carried out in the western Mediterranean to ascertain whether these migrations are related to feeding and whether this behaviour differs among developmental stages.

3.4.1 Vertical distribution

The present study showed that adults of *L. pusillus* were distributed throughout the water column, while the larvae displayed a more restricted distribution, being confined to the upper 200 m, in agreement with the vertical patterns of other myctophids (Moser and Ahlstrom, 1996). Despite the narrow vertical range observed for larvae, differences in abundance between day and night were apparent, with a shallower larval distribution (in the upper 60 m) observed during the day. Examination of the gut contents of larvae found at the near surface during the day and the finding of empty guts at night demonstrate the relationship between the distribution of the larvae and the search for food.

Previous data on the vertical migration of adult specimens of *L. pusillus* have shown that they are distributed between 500 and 1000 m during the day and that the most of the population migrate to the upper 300 m at night (Hulley, 1984). Nevertheless, Goodyear *et al*. (1972a) found that some large individuals do not migrate upward as far as the main body of the population, which is in accord with our observation of large individuals at the benthic boundary layer at night. As will be discussed below, adults appear to feed at night in the upper 400 m, further supporting the association between the vertical displacements and trophic behaviour.

3.4.2 Feeding patterns

The larvae and adults of *L. pusillus* exhibited a relatively high feeding incidence, as has previously been reported for other myctophids (Suntsov and Brodeur, 2008; Sassa, 2010).

Similar to most fish larvae, the feeding activity of *L. pusillus* took place in the epipelagic zone during daylight hours (Sabatés and Saiz, 2000; Sabatés, 2004; Rodríguez-Graña *et al.*, 2005; Morote *et al.*, 2008a; Sassa, 2010). This type of daylight feeding pattern for larvae is generally explained by the lack of an adapted retinacular system with rods and the migration of the retinomotor pigment required for low light intensities (Conley and Hopkins, 2004; Morote *et al.*, 2011). Although larvae from preflexion stages up to 7.5 mm SL fed during the day, the two largest examined larvae fed nocturnally, suggesting that *L. pusillus* must shift its diurnal strategy by the late postflexion stage. In other myctophids, nocturnal feeding begins after transformation (e.g. Sassa, 2010), which is associated with changes in the visual system (Evan and Browman 2004 in Sassa 2010). In adults, the observation of full stomachs and slightly digested prey in specimens from night hauls, both in the near-surface layer and DSL (Dec. 2009), indicates recent feeding at these levels. However, the advanced stage of digestion of the prey found in adults collected in the BBL does not shed light on their preferred location for obtaining food: they could have eaten at the bottom, after migration to the epipelagic zone, or in any other strata. Further comparison with the disintegrated remains observed in the stomachs of individuals caught during the day at the DSL level (July 2010) points to a night-time feeding pattern. Nevertheless, due to the limited data set for individuals captured in day hauls and the scarcity of information on food vacuity in myctophids, we cannot completely dismiss the possibility of diurnal feeding. Previous investigations into other myctophids found that adults preferably fed at night (e.g. Holton, 1969; Pakhomov *et al.*, 1996; Williams *et al.*, 2001), but

other authors have shown the opposite trend for several species of lanternfish (Paxton, 1967; Kosenok *et al.*, 2006). Similar studies on *Symbolophorus californiensis* (Connan *et al*., 2007) and *Lampanyctus australis* (Williams *et al*., 2001) found that both species behaved as nocturnal predators but showed some feeding activity at dusk and dawn, respectively.

The major prey categories found in the stomachs of the ten fish collected during summer were similar to those from the winter. Therefore, we think that there must not be any seasonal influence on the time of day in which these fish feed (at night), even though seasonality could be associated with different concentrations of zooplankton. The consumed prey taxa could change with the time of year and predator size, without affecting the diel pattern. For instance, Gjøsaeter (1981) found that the diet of the mesopelagic fish *Maurolicus muelleri* differed seasonally and with size but did not find evidence of any change in the diel feeding pattern.

3.4.3 Predator-prey interaction and trophic niche breadth

The obtained mean values of 2-3 ingested prey per gut were comparable to those reported for other myctophid larvae (Conley and Hopkins, 2004; Rodríguez-Graña *et al.*, 2005; Sassa and Kawaguchi, 2005; Sassa, 2010) and did not change during development, although the adults depended on larger prey items to supply their higher energetic requirements. The mean prey number was similar to that of pelagic species such as *Sardinella aurita* (Morote *et al.*, 2008b), *Sardinella pilchardus* (Morote *et al.*, 2010), *Thunnus maccoyi* and *T. alalunga* (Young and Davis, 1990) but lower than that observed in *Merluccius merluccius*. The last species is also a night feeder with sensitivity to low light levels (Morote *et al.*, 2011). The only difference found in the mean prey number in *L. pusillus* was observed for the oldest larvae, which contained 10-20 prey items, potentially meeting a higher energy demand to promote the growth and metamorphosis of these larger larvae.

The maximum prey size increases with the growth of *L. pusillus*; however, the adults of this species continue ingesting small items. Consequently, the niche breadth from the larval stages to adulthood was independent of the body size, showing no trend towards the exclusive consumption of larger items, similar to what has been observed in a broad range of pelagic fishes (Pearre, 1986).

3.4.4 Dietary shift and selectivity

Different stages of copepods were prevalent in the *L. pusillus* gut contents throughout larval life, with a shift from cyclopoids to small calanoids observed in the postflexion stage. Other studies have also shown that nauplii and small copepods are the main prey items for myctophid larvae (Sabatés *et al.*, 2003; Sassa *et al.*, 2004). Moreover, remains of larvaceans, either their houses or faecal pellets, were also noted in flexion larvae. The presence of larvaceans (mostly *Oikopleura* spp.) may be higher than reported due to rapid digestion (Sassa and Kawaguchi, 2005), and their houses could constitute a receptacle for the faecal pellets that were found individually as separate prey items. Thus, it was difficult to distinguish the two categories as independent items. It is of particular note that positive selection has been observed for nauplii, oncaeids and larvaceans in these early stages. Selection in larvae is a compromise between their energy requirements and the morphometric limitations of their mouths (Hunter, 1981). However, other factors, such as nutritional quality, organ development and an improving swimming ability, also influence prey selection.

Large copepods (mainly *Pleuromamma* spp.) and euphausiids constituted the dominant prey items for adults (60-80%), although they also continued to feed on oncaeids and small calanoids. Immature adults fed on various epipelagic species, such as *Clausocalanus acuicornis* (Massutí, 1942); mature adults preyed on other surface species of copepods, such as *Paracandacia simplex* (rare below 200 m, according to Vives 1978; Scotto di Carlo *et al*. 1984),

Nannocalanus minor (Vives, 1978; Yahia *et al.*, 2004), *Euchaeta marina* and the more dispersed species *E. acuta* (50-500 m, according to Vives 1978), which also migrate to the upper 100 m at night. There was a superposition of some prey taxa for adults and larvae (e.g. *Clausocalanus* spp., *Oncaea* spp.); however, diet overlap was common when examining the prey at the genera level (Burghart *et al.*, 2010). Based on the zooplankton data and the identifiable euphausiids found in the examined stomachs, we believe that *Euphasia krohnii* and *Meganyctiphanes norvegica* were the two preferred euphausiids consumed. Piscivory also cannot be disregarded, as a fish belonging to the Myctophidae family was found in the gut of an adult. The advanced digestion stage of this prey showed that it was eaten before the fish was captured and suggests piscivory as an occasional and opportunistic behaviour, as has been observed sporadically in other myctophids (Hopkins *et al.*, 1996; Gaskett *et al.*, 2001).

Other studies on myctophids support the existence of a dietary shift from small organisms in early stages towards a diet based on large calanoids and euphausiids in adults (e.g. Tyler and Pearcy, 1975; Scotto di Carlo *et al.*, 1982; Young and Blaber, 1986; Kozlov, 1995). This ontogenetic shift is related to an improved swimming capacity and enlargement of the mouth width (Scharf *et al.*, 2000; Masuda, 2009).

The large calanoid *Pleuromamma* spp. was found more frequently than other calanoids in adults and was positively selected by immature adults. The selection for this genus might be partly determined by detection of the pigmented spot on one side of the body or by the luminosity of these species (Goswami *et al.*, 1992). Clarke (1978) found that the intensively pigmented oncaeids were the only zooplankton category eaten regularly by some mesopelagic fishes. A larger body size is another factor that could favour the capture of *Pleuromamma* spp. The proportion of euphausiids in the diet of *L. pusillus* gradually increased with the size of the fish, as euphausiids are

positively selected by mature adults. An increasing presence of euphausiids in the diet must indicate a decreased energetic effort with respect to the previous developmental stages, filling the stomach with one prey instead of catching and ingesting various items of smaller size.

Our findings do not correspond to the description proposed by Suntsov and Brodeur (2008) for lanternfishes; myctophids categorised as less active swimmers required a high lipid content diet, which can be obtained from *Pleuromamma* spp. (Kotani, 2006), while active myctophids consumed significantly higher amounts of protein-rich prey, for example, euphausiids (Kulka and Corey, 1982). The authors of the study cited above confirmed that a number of species of *Lampanyctus* are inactive, which agrees with the results obtained by Stefanescu and Cartes (1992) for adults of *L. crocodilus* in the NW Mediterranean, but not the results provided in the present study, where all size ranges of *L. pusillus* adults reached the upper layers of the water column to feed.

In summary, the present study demonstrated that the vertical distribution of larvae and adults of *L. pusillus* was partly induced by their feeding habits. The fact that the larvae appeared to be concentrated in the upper 60 m during the day indicates that they are visual feeders. In adults, the better swimming skills, larger mouth size and presumably more developed sensory organs should explain the observed night-time feeding pattern within the upper 400 m of the water column. Our results demonstrate that larvae feed selectively on small zooplankton, whereas adults select larger taxa that undergo nycthemeral migrations, in addition to small prey. A decrease in the frequency of small prey was found in favour of larger and more nutritive prey. Further research is needed to ascertain the influence of morphological and sensorial characteristics on the diet and feeding patterns of this species in comparison to other myctophids.

Chapter 4

TROPHIC

ECOLOGY (II)

4. Diet and feeding strategy of mesopelagic fishes in the western Mediterranean

Abstract

Myctophids, gonostomatids and sternoptychids are the most abundant mesopelagic fishes worldwide and constitute an important assemblage of mesopelagic ecosystems, functioning as vehicles of energy and matter fluxes through trophic webs. This study concentrates on the trophic ecology of the most abundant mesopelagic fishes of the western Mediterranean (WM) based on stomach content analysis. The myctophids (*Ceratoscopelus maderensis, Benthosema glaciale, Hygophum hygomii, H. benoiti, Lampanyctus crocodilus, L. pusillus, Lobianchia dofleini, Myctophum punctatum and Notoscopelus elongatus*) perform extensive nycthemeral migrations across the water column, between the surface and 1000 m depth, interacting with plankton and micronekton at multiple depths, and generally feeding in the epipelagic layers at night. On the contrary, the gonostomatids *Cyclothone braueri, C. pygmaea*, and the sternoptychid *Argyropelecus hemigymnus* remain below epipelagic layers, feeding intermittently throughout the day and night. The diet composition, trophic niche breadth and prey selectivity of 11 of these fish species were determined for juvenile and adult individuals from two surveys performed in December 2009 and July 2010 in the western Mediterranean Sea. We found strong heterogeneity in diet composition within each species, with high dietary overlap among species, relatively low selectivity and incorporation of larger prey throughout ontogeny. The number of prey items varied among species, e.g. *Myctophum punctatum* was the species with the highest feeding intensity, reaching ca. 700 prey items, while the mean number of prey in *Cyclothone braueri* was 1.4 and a maximum of 8 prey items. A dietary shift towards larger prey was evident from the juveniles to the largest adults, but trophic niche breadth did not increase with Standard Length (SL) for any of these mesopelagic species. The diet of the small gonostomatids and the sternoptychid, and early juvenile myctophids were dominated by non-calanoids, ostracods, and small mesozooplankton, while medium-size myctophids, i.e. *L. dofleini* or *H. benoiti*, preyed mainly on calanoid copepods. The oldest stages of *L. crocodilus* and *N. elongatus* fed mostly on macrozooplankton and micronekton. There was high diet overlap among myctophids, although some species showed certain degree of segregation by size and positive selection towards particular prey items. Chesson's selectivity index showed that *L. dofleini, N. elongatus, L. crocodilus* and *L. pusillus* preyed selectively on euphausiids; *B. glaciale* on the dominant calanoid *Pleuromamma* spp. *C. maderensis* preferred larvaceans in autumn. The two *Hygophum* spp. consumed a high number of food items, but *H. hygomii* showed positive selection for more nutritive prey, such as euphausiids, while *H. benoiti* preferred the small corycaeid copepods. Overall, the main trophic difference among

mesopelagic fishes in the WM was observed between the small non-migratory species that feed continuously on small zooplankton and the largest myctophids, which fed on the largest mesozooplankton and, occasionally, on fishes. Mediterranean midwater fishes can be characterized by the adoption of mixed feeding strategies, with varying degrees of specialisation on different prey types, allowing flexibility in a changeable environment.

4.1 Introduction

The mesopelagic fish community includes those species that inhabit the portion of the water column from the surface to ca. 1000 m in the high-seas pelagic environment (Gartner *et al.*, 1997). Myctophids, gonostomatids and sternoptychids form most of the substantial biomass of such assemblage, and have a ubiquitous occurrence worldwide (Gjøsæter and Kawaguchi, 1980). The vertical migratory behaviour up to the surface of some species make them an important component of the food webs, constituting a common prey for demersal and large pelagic fishes (e.g. Gibbs, 1984; Anastasopoulou *et al.*, 2013; Fanelli *et al.*, 2014), cephalopods, marine birds (Cherel *et al.*, 1993) and marine mammals (Pereira *et al.*, 2011). Mesopelagic fishes exert notorious feeding pressure in the oceanic ecosystem due to their high abundance, e.g. myctophids have been reported to remove more than 10% of the surface zooplankton biomass per night (Watanabe *et al.*, 2002). Moreover, these fishes can be selective upon certain types of prey (e.g. Hopkins and Gartner, 1992; Van Noord *et al.*, 2013), and consequently, constitute an important top-down control on the zooplankton community structure in open oceans. Recent studies claim that the biomass of these midwater fishes might be widely biased and underestimated due to the generalised effect of fish net avoidance (Watanabe *et al.*, 1999; Kaartvedt *et al.*, 2008). Thus, the efficiency of the energy transferred from primary production to top predators in the high sea through these mesopelagic migrators might be higher than previously reported (Davison *et al.*, 2013).

Most groups of zooplankton and micronekton, the latter dominated by mesopelagic fishes in terms of biomass, exhibit strong diel variations in their vertical distribution, increasing the possibilities of interaction amongst trophic levels and, therefore, enhancing the complexity of the food web structure.

Information on the vertical food usage of these midwater fishes is scarce, although it is generally accepted that most species perform nycthemeral migrations towards the epipelagic layers to forage, following the nocturnal ascension of potential planktonic prey (Watanabe *et al.*, 1999; Benoit-Bird and McManus, 2014). However, there are different opinions on the reasons why they return to deeper waters. Two alternative hypotheses were suggested to explain their return to the depths; firstly, that fishes shelter from larger predators in deeper layers (Childress and Nygaard, 1973; Childress, 1995) and, secondly, that their energy consumption decreases in colder waters (Enright, 1977; Marshall, 1979). The extent of the diel vertical migrations (DVMs) varies depending on the species, the ontogenetic developmental stage, and other environmental factors, such as the thermocline and oxycline depths, and the gradient strength (Parker-Stetter and Horne, 2009).

The western Mediterranean (WM) is an oligotrophic region characterized by high species diversity (Estrada, 1996); however, for mesopelagic fishes the number of species is much lower than in large oceans (Hulley, 1984). Mediterranean myctophids migrate to the epipelagic zone at night, with the exception of the oldest and largest individuals of some species (*Lampanyctus crocodilus*, *Benthosema glaciale* and *Notoscopelus elongatus*) (Olivar *et al.*, 2012). The gonostomatids *Cyclothone braueri*, *C. pygmaea* and the sternoptychid *Argyropelecus hemigymnus* do not migrate to the epipelagic waters, being partly responsible for the permanent acoustic response at the 400-600 m, i.e., the Deep Scattering Layer (DSL) reported in the Mediterranean continental slope (Olivar *et al.*, 2012). As in other regions, the distributions of these mesopelagic fishes extend from the continental slope, where they co-inhabit with many other pelagic and demersal species, to the open ocean, where they constitute the dominant fish biomass of this typically oligotrophic system. Low primary production in the open ocean may be expected to induce the

mesopelagic fish community to exhibit partitioning of food resources by adopting alternative behaviours, feeding on different food items or showing a particular spatial distribution that minimizes overlap (Hopkins and Gartner, 1992).

Studies on the feeding habits of juveniles and adults of midwater fishes from the Pacific and Atlantic oceans (Hopkins and Baird, 1973; Clarke, 1974; Gorelova, 1974; Merrett and Roe, 1974; Tyler and Pearcy, 1975; Clarke, 1978) showed that these fishes constitute the intermediate trophic levels of the food web and due to their high abundance are key components of open ocean food webs. In contrast, the information about their feeding habits in the WM is very limited. The oldest work in the WM described the diet of stranded individuals of *Hygophum benoiti* and *Myctophum punctatum* (Scotto di Carlo *et al.*, 1982), thus, the possible factors involved in their feeding dynamics could not be evaluated. Stomach content analyses of the near-bottom adult stages of *Lampanyctus crocodilus* (Stefanescu and Cartes, 1992), and from individuals occurring at depths below the DSL to the bottom have been reported (Fanelli *et al.*, 2014). Finally, the diet of *Lampanyctus pusillus* was studied throughout its complete life history from larvae to late adults in a recent study (Bernal *et al.*, 2013). Data on the feeding ecology of the stomiiforms (which includes the gonostomatids and sternoptychids) in the WM are even more scarce, restricted to the gonostomatid *Cyclothone braueri* (Palma, 1990). All these studies supply valuable information on the feeding of particular species; however, they do not integrate feeding interactions for the whole mesopelagic fish assemblage. A recent study (Valls *et al.*, 2014) based on stable isotope analysis of Mediterranean mesopelagic fishes, as alternative to stomach contents analysis, provided data on the trophic positions (TrL from 3 to 4) of mesopelagic Mediterranean fishes and elucidated their diet sources through isotopic mixing models, providing a complementary approach to the diet of these fishes.

Determining the diet of mesopelagic fishes is important to establish the topology of pelagic food webs, the flows of biomass across compartments and, eventually, assess the impact of environmental changes on pelagic systems, by means of ecosystem models, such as Ecopath. Due to the key role of mesopelagic fishes and the paucity of data on their tropic ecology, the study of the stomach contents of midwater fishes is a potential source of the information required for modelling the active fluxes of matter from zooplankton and microbial loops to top predators and back in the pelagic environment.

This study was focused on the diet of the most abundant and frequent mesopelagic fishes of the WM, based on the identification and quantification of the different prey items consumed and changes in the prey size spectra throughout fish ontogeny. The aims of this study were: firstly, to define their diet composition, prey selectiveness and feeding strategy; secondly, to determine the factors responsible for significant differences in diet composition at both intra- and interspecific levels; thirdly, to examine the hypothesis of high levels of food partitioning, expected for oligotrophic regions such as the WM; and finally, to compare our data with those estimated from isotope analyses of previous studies, which provided the trophic position of the species in a particular ecosystem.

4.2 Materials and methods

4.2.1 Area of study and sampling

The study was carried out in the western Mediterranean (WM) over the shelf-break and slope off Mallorca Island (39º N, 2º E) during two surveys conducted in December 2009 and July 2010 (Fig. 4.1). The WM is a semi-enclosed basin characterized by moderate oligotrophic condition and seasonal alternation of the hydrodynamic conditions; in winter, the mixing of the water column occurs and

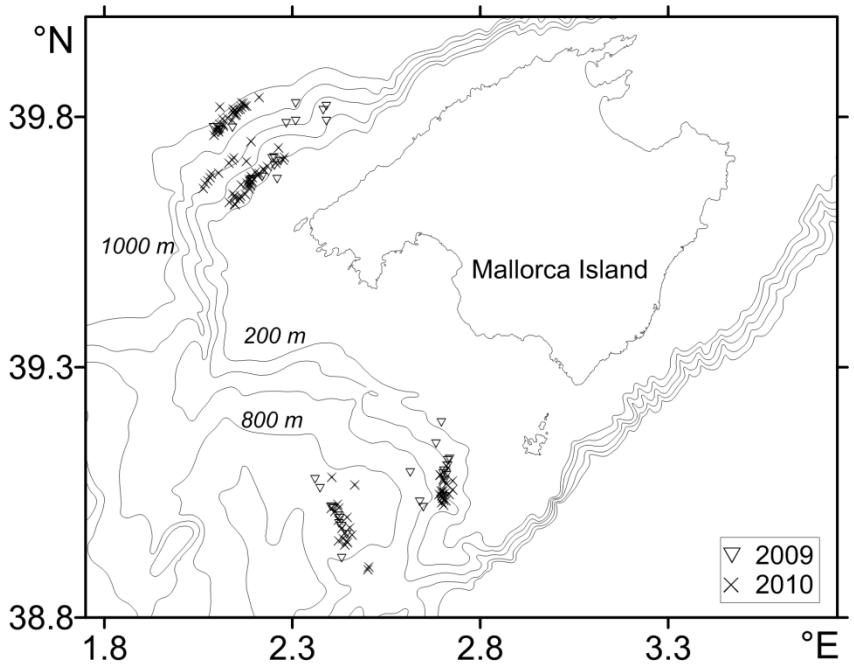


Fig. 4.1 Map of the study area showing the pelagic trawls, and hauls performed with different devises during the surveys of December 2009 and July 2010.

the highest productivity levels are reached in the first 50 m depth. The mixing period is followed by a marked stratification of the water column in summer, with the highest levels of primary production and zooplankton biomass in subsurface layers (Deep Chlorophyll Maximum) (Sabatés *et al.*, 2007). The study was focused on the 9 most abundant and frequent fish species of the pelagic hauls (more than 90% of the total number of fish captures, and at least 50% of occurrence in the hauls) within the family Myctophidae (Table 4.1). This study also included three stomiiforms, the dominant species of the family Gonostomatidae, *Cyclothone braueri*, as well as its congener *Cyclothone pygmaea*, and the most common species of the family Sternoptychidae, the hatchetfish *Argyropelecus hemigymnus* (Table 4.1). Fish samples were collected using large Pelagic Trawls (100 and 200 m^2) with 10 mm cod-end, a 3 m^2 Isaacs-Kidd Midwater Trawl (IKMT) with 3 mm mesh size of the cod-end, and a 1 m^2 MOCNESS net with 1.5 mm mesh size (Fig. 4.1). The fish specimens

came from hauls performed during both day and night near the surface (from 40 to 80 m), in the 400 m DSL, and in the Benthic Boundary Layer (BBL) at 50 m above the bottom. Further details on sampling are described elsewhere (Olivar *et al.*, 2012). A rectangular net of 1.25 and 0.3 m was attached to the upper part of a beam trawl to collect samples of supra-benthos over the sea bed (500 µm mesh size) during the daylight hours.

4.2.2 Laboratory and data analysis

Fish samples were sorted on board and identified to species level. Specimens destined for stomach content analyses were measured (Standard Length, SL±1 mm) and stored in 5% buffered formalin. The size range for each developmental fish stage (juvenile, immature and mature adults) was extracted from literature (Badcock, 1984; Hulley, 1984).

Stomach content analyses were carried out on a selection of 1202 individuals (Table 4.1). Each stomach was removed by cutting at the beginning of the oesophagus, using a fine scalpel and placing the contents on a Petri dish with a mixture of glycerin 50% and distilled water. A high number of eversions of the stomach contents was detected was detected in *A. hemigymnus* and only the specimens without signs of regurgitation were considered for the analyses. Empty stomachs (Table 4.1) were counted just to determine feeding incidence during the daylight (Dec.: 0610 to 1623 hours GMT; July: 0427 to 1929 hours GMT) and night-time (Dec.: 1723 to 0610 hours GMT; July: 2030 to 0330 hours GMT), but were not considered for the subsequent statistical analysis. Feeding incidence (FI) was determined as the percentage of specimens containing at least one prey in the stomach. Data from both cruises was pooled for some fish species in order to obtain a representative sample size for the FI calculations. Stomach fullness was qualitatively determined by a range from 0 to 4 (0: empty stomach; 1: one prey or less than 25% full; 2: 50% full; 3: 75% full, stomach

with distinguishable *rugae*, and 4: 100% full, the stomach is completely expanded and *rugae* are not evident).

Table 4.1
Number of individuals used for the examination of the diet composition.

Species	N. stomachs				SL (mm) range
	December		July		
O. Stomiiformes	Total	Empty	Total	Empty	
Argyropelecus hemigymnus	37	3	26	2	7.3-33.5
Cyclothone braueri	80	37	165	115	12-28
C. pygmaea			26	17	17-24
O. Myctophiformes					
Benthosema glaciale	98	20	88	33	12-46
Ceratoscopelus maderensis	58	3	36	1	16-65
Lobianchia dofleini	40	1	29	1	23-46
Myctophum punctatum	28	2	18		18-57
Hygophum benoiti	41	5	17		13-64
H. hygomii			20		39-56
Notoscopelus elongatus	55	4	63		30-172
Lampanyctus pusillus	114	23	15	6	14-86
L. crocodilus	81	7	17	7	20-60
L. crocodilus (bottom)	119	71			67.5-283

Prey items were identified to the lowest possible taxonomic level under the power resolution of the binocular microscope up to 1600x. Prey richness for each fish species was calculated and plotted against the number of examined individuals in order to assess whether the number of sampled stomachs was adequate for diet description. The number of predators was considered sufficient when cumulative prey richness reached the asymptote of the rarefaction curve. The Shannon diversity index (*H'*) was calculated using PRIMER 6 (Clarke and Gorley, 2006) in order to estimate the mean prey diversity per fish species and survey. Only those individuals with at least 50% gut repletion and recognizable prey items were selected for prey diversity calculations.

Body length and width of prey items were measured to the nearest 0.01 mm, when possible, using Leica Application Suite (LAS) imaging software. Body Prey Width (PW) was used in all cases, instead of prey length, for regression relationships against fish size (SL), as PW would approximate better the prey aspect from which predators ingest animals, at the time it is less vulnerable to degradation and deformation than prey length, and subsequently, less likely to introduce bias in further calculations. In order to analyse relationships between PW and SL, fish species were grouped in size intervals to produce the maximum number of size classes containing 3 or more prey items and PW was log10 transformed. Trophic niche breadth (TN) was then calculated, according to Pearre (1986), as the standard deviation (±SD) values of the log10 transformed PW plotted against size classes, with the aim of determining the relationship of prey size with the predator feeding strategy throughout development. The SD of PW per size class was used to ensure that this measure was independent from sample size. Specimens of each predator species, and from both cruises, were pooled for the calculation of niche breadths to avoid seasonal effects on the size spectra of prey organisms (Pearre, 1986), since it provides a wider range of sizes for predators.

Abundance of each prey taxa in the fish diet (%N) was calculated as the percentage of total prey number for a specific fish species, and its frequency of occurrence (%F) as the number of times a prey item occurred in the stomachs divided by the total number of stomachs for that species. The particulate organic matter (POM) and digested remains were recorded once per stomach, in order to report their presence/absence, since they cannot be properly quantified. Selectivity was calculated by applying Chesson's electivity index (Chesson, 1978) as follows:

$$\alpha_i = \frac{r_i / p_i}{\sum_{i=1}^{m} r_i / p_i}$$

Where r_i and p_i are the respective frequencies of a prey item in the diet and plankton, and m is the number of prey categories considered. Positive or negative electivity were determined when the α-values ±95% CI fell above or below the line defining the neutral α-value for selectivity, respectively.

4.2.3 Statistical analyses

Multivariate analyses were performed to determine the similarity of diets among 11 mesopelagic fish species on a matrix of prey items as variables (columns) and individual predator as samples (rows). *C. pygmaea* was not included for statistical analysis because of the small sample size for this species. Prey taxa were grouped by genus within copepods, by developmental stages in euphausiids, and into broader taxonomic groups for the remaining prey categories: cladocerans, ostracods, amphipods, mysids, decapods, crustacean eggs, chaetognaths, polychaetes, larvaceans, tunicates, gastropods, cephalopods and fishes. Unidentified material and prey categories accounting for less than 5% of the total abundance and therefore were deleted from the matrix. Furthermore, samples with only unidentified digested food and empty stomachs were deleted from the original matrix. Different tests were run using PERMANOVA+ for PRIMER 6 (Anderson *et al.*, 2008) to determine the influence of diverse factors such as species, size class, developmental stage, season and depth on the diet composition of mesopelagic fishes. Non-metric multidimensional scaling (MDS; Clarke and Warwick, 2005) performed on the whole dataset, and based on Bray-Curtis distances, showed broad diet overlap among the individuals of different species. In addition, PERMANOVA detected significant differences ($p<0.0001$) between cruises, but also dispersion of data

($p<0.0001$). The homogeneity of multivariate dispersion was tested using a PERMDISP routine. Both procedures were run with 9999 unrestricted permutations. Homogeneity of dispersions could not be assumed; in this way, because within-species variability was higher than the variability due to the studied factors. To avoid dispersion effects, mean abundance matrix based on the number of prey (fourth root transformed) was calculated, averaging data depending on the predator species, stage of development and season, in order to run a cluster analysis that allows discerning dietary associations, at species level, within the fish community. The cut off for low contributions was established at 60%. Hierarchical clustering was computed on the basis of Bray-Curtis similarity coefficient along with SIMPROF ($p<0.05$) permutation routine. Individual PERMANOVA analyses by predator species were also performed (9999 unrestricted permutations) to discern possible tendencies with development, vertical distribution, zone or seasonality. Analysis of covariance (ANCOVA) was used to test for differences in slopes and intercepts of the regression relationships of prey number, prey width and TN breadth with predator SL.

Additionally, the diets of the 11 species of mesopelagic fishes were analysed by means of canonical correspondence analysis (CCA: Ter Braak, 1986). CCA is an ordination technique to analyse the relationships between biological assemblages and their environment. We used the multivariate diet response of each predator as a biological assemblage matrix, and several explanatory variables as an environmental matrix. Prey number for each prey category was converted to relative values (% diet composition) for stomachs containing at least one prey item and for prey categories being present in 5% or more of the stomachs analysed. Relative number of prey was subsequently arcsine-square root transformed, which is appropriate for percentages (Zar, 1996) to normalise the data and reduce heteroscedasticity. The explanatory variables contained two

continuous variables (SL: fish Standard Length in mm, PN: Number of Prey items per stomach) and four categorical variables (SP: Species code; CAMP: season/year, with two levels: 2009 and 2010; day period= day/night; depth stratum with 3 levels (surface, DSL and 50 m from the bottom of the shelf-break). The categorical variables were recorded as a set of dummy variables (Legendre and Legendre, 1998). Only k-1 dummy variables created from the categorical variables with k categories were used because of the model dependence among the k dummy variables (Zar, 1996). The significant explanatory variables determining the multivariate diet response of these mesopelagic fishes were found using a forward stepwise section procedure (Ter Braak and Verdonschot, 1995). First, CCA was computed with one explanatory variable at a time. The significance of each variable (at the *p*-level=0.05) was assessed with a randomisation test. A partial CCA was conducted using the variable with the highest amount of variation explained as a conditioning factor and the other variables one at a time to determine the second explanatory variable in order of importance (Ter Braak and Verdonschot, 1995). The procedure was repeated until the explanatory variables were not statistically significant anymore. Finally, a CCA with all the selected variables was carried out. CCA, partial CCA and permutation tests were carried out with Vegan package (Oksanen, 2005) of the R statistical and computing language. The results of the CCA are presented in the form of an ordination diagram with the continuous explanatory variables shown as arrows that demote the direction of maximum change, and the categorical variables as centroids of the samples belonging to each category.

4.2 Results

4.3.1 Feeding Incidence

The stomiiforms *A. hemigymnus* and *C. braueri* fed indistinctly during day- and night-time, while most myctophid species were nocturnal feeders. Likewise, feeding intensity was very low in *C. braueri* during the whole 24-hour period, and higher in myctophids at night, when they occur aggregated in epipelagic depths. During the day, myctophids were found to be more dispersed in the water column, and the FI was 0 or very low for most species, except for the two *Hygophum* spp. that fed, also, during daylight hours away from the photic zone (Table 4.2). The limited number of specimens collected at daytime in the epipelagic and DSL revealed presence of food remains in *B. glaciale*, *L. dofleini*, and *M. punctatum*. Furthermore, the largest and non-migratory specimens of *L. crocodilus* collected in bottom hauls showed daytime feeding. Interestingly, no significant differences were found in the ingested prey types between day and night for those species feeding through the whole day.

Table 4.2
Feeding incidence (FI) of mesopelagic fishes during daylight and night-time. The asterisk denotes feeding activity at daytime, although small sample size to provide unbiased calculations. Numbers in brackets indicate the number of examined individuals.

Species	FI night (%)	FI day (%)
A. hemigymnus	85.3 (34)	86.2 (30)
C. braueri	29.4 (132)	34.4 (147)
B. glaciale	67.8 (171)	*
C. maderensis	97.8 (90)	0
L. dofleini	96.4 (68)	*
M. punctatum	97.1 (47)	*
H. benoiti	75 (36)	83 (21)
H. hygomii (July)	No data	95.2
N. elongatus	96.5 (113)	0
L. pusillus	83.5 (131)	0
L. crocodilus	82.4 (103)	0
L. crocodilus Bottom (Dec.)	No data	62.18 (128)

4.3.2 Prey number and prey size trends through predator development

The number of ingested prey fluctuated widely among predator species and developmental stages, from >5 prey items per stomach in *Cyclothone braueri* to a maximum of 696 items in the myctophid *M. punctatum.* The prey number (PN) and diversity of zooplankton in the stomachs was generally higher in the intermediate size classes of predators (Appendix 4.1), except for *H. benoiti.* The slopes of mean PN with SL were never significant (ANCOVA), suggesting that prey number does not increase with size for any species.

In the sternoptychid *A. hemigymnus*, PN varied broadly from juveniles to adults. Maximum PN (67 items) was found in a medium-sized specimen (20 mm SL). After this peak, the mean PN decreased and remained constant with narrow standard deviations. The gonostomatid *C. braueri*, characterised by a small body size (maximum SL=30 mm), showed low PN throughout the entire body size (SL) range, with a maximum of 8 food items and a mean PN=1.4.

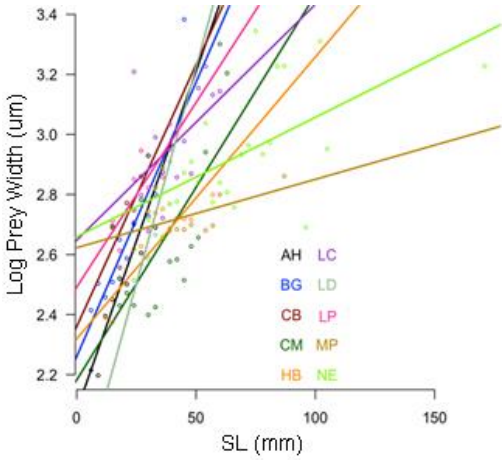
In the myctophid *B. glaciale*, the maximum PN (21 items) was found in a medium-sized individual (32 mm SL), and decreased in the larger size classes. PN was quite variable among the *C. maderensis* size classes. The mean PN increased up to 47 in a medium-sized specimen (23 mm SL), and decreased afterwards between 50 mm and 70 mm SL, while SD reduced. In *L. dofleini*, the maximum PN (55 items) occurred in a medium-sized individual of 23 mm SL. In *M. punctatum*, PN varied widely across size classes, and was considerably higher than in the other mesopelagic species. The mean PN increased in the medium-sized individuals, and reached a peak in a specimen of 50 mm SL and decreased to a low number in the largest analysed specimen (88 mm SL). PN was also broadly variable across size classes in both *Hygophum benoiti* and *H. hygomii*. *H. benoiti* was found to have a low PN in medium-sized individuals,

but consumed loads of prey in larger-sized individuals. The maximum PN, 363 items, was found in an individual of 45 mm SL, while in *H. hygomii*, the maximum PN was 179 food items for a 42 mm SL individual.

In *N. elongatus*, PN was greatly variable among individuals. Maximum PN (65 items) was reached in a medium-sized predator (65 mm SL), and clearly decreased in the larger-sized individuals of mature adults. The pelagic specimens of *Lampanyctus crocodilus* showed low PNs, with a maximum of 5 food items (SL=51 mm SL).

Table 4.3
Regression models for PW *versus* SL. ANCOVA tests for differences in intercepts (**a**) and slopes (**b**) of the regression relationships. Residual standard error: 0.1385 on 97 degrees of freedom. Multiple R-squared: 0.716; adjusted R-squared: 0.6604. F-statistic: 12.87 on 19 and 97 DF, p-value <2.2e-16.

Species	a	b	Significance (slope)
A. hemigymnus	2.080	0.025	0.001
C. braueri	2.360	0.017	0.001
B. glaciale	2.260	0.018	0.001
C. maderensis	2.182	0.018	0.001
L. dofleini	1.782	0.029	0.001
M. punctatum	2.627	0.002	0.001
H. benoiti	2.319	0.009	0.001
N. elongatus	2.659	0.004	0.001
L. pusillus	2.492	0.013	0.001
L. crocodilus	2.648	0.008	0.001



All the species analysed exhibited asymmetric predator body size *versus* prey size distributions, i.e., the upper limit of prey width increased with predator SL, while prey of the minimum sizes were also ingested with increasing predator body lengths. The slope of TN breadths throughout development was not significant for any of the predator species (ANCOVA), despite some of them showing increasing or decreasing non-significant slopes (see Appendix 4.1). Nevertheless, the ANCOVA model detected significant slightly positive slopes

and intercepts for the relationships between log PW and predator SL for all the fish species (Table 4.3). The smallest-sized mesopelagic fishes with the lowest intercepts showed the highest positive slopes (*A. hemigymnus*, *C. braueri*, *B. glaciale*, *C. maderensis*), reaching the uppermost value in *L. dofleini*. On the contrary, the largest-sized myctophids (*M. punctatum*, *L. crocodilus*, and *N. elongatus*) showed the highest intercepts and the lowest slopes.

Not enough data were recorded for PW in *H. hygomii*, due to the advanced stage of digestion of most items. Additionally, the largest benthic specimens of *L. crocodilus* showed a considerable degree of digestion of the stomach contents, which prevented measuring prey items. Although prey sizes were not quantitatively assessed, the identified prey categories found in the oldest *L. crocodilus* associated to the sea bottom had substantially larger dimensions than those of the pelagic individuals.

4.3.3 Prey diversity, diet composition and selectivity

Prey richness was plotted against the number of analysed fish individuals of a species to assess whether the number of samples per species was adequate for diet description. The cumulative prey richness curves approached an asymptote in most cases. Only for the species *L. crocodilus* and *C. braueri*, which have a high number of digested contents and undetermined/particulate organic matter (UOM/POM), the prey richness curves, while close to an asymptote, still showed a slight increasing gradient.

The diversity of prey items in the stomachs was outstanding, when considering the numerous copepod species/genera (Table 4.4) together with other unrelated taxa. The index of diet diversity *H'* reached the highest values in *A. hemigymnus*, *Hygophum* spp., *C. maderensis*, *M. punctatum*, and *L. dofleini*, all of them with comparable values. Relatively high values for prey diversity were indicative of generalist behaviours, when compared with species showing low

prey diversity, which might indicate specialisation on specific prey taxa. The lowest *H'* value was for *L. crocodilus*, in particular, the largest individuals from bottom hauls.

Table 4.4
Mean diversity index of Shannon-Weaver per species and season. Stomachs with repletion below 50% were not included for calculations. Abbreviated names corresponded to the two first letters of the species scientific name. Autumn 2009: 09; summer 2010: 10.

Species Abbrev.	H' (log e)	$\overline{PN}$	Species Abbrev.	H' (log e)	$\overline{PN}$
Ah 09	1.373	8.3±7.2	Hb 09	1.615	25.0±41.7
Ah 10	1.367	10.3±14.1	Hb 09 (early juveniles)	1.510	69.4±87.5
Cb 9 10	0.828	5.6±1.7	Hb 10	1.247	94.3±128.5
Bg 09	0.866	3.8±3.3	Hh 10	1.171	50.5±50.5
Bg 10	0.771	3.8±4.1	Ne 09	1.329	10.9±12.6
Cm 09	1.652	12.0±9.9	Ne 10	0.868	8.4±9.2
Cm 10	1.262	8.6±9.2	Lp 09	0.654	3.1±2.2
Ld 09	1.746	16±15.1	Lp 10	0.791	6.8±10.0
Ld 10	1.613	16.7±9.6	Lc 09	0.309	1.7±0.9
Mp 09	1.006	86.3±141.0	Lc 10	0.395	2±1.2
Mp 10	1.779	40.1±76.9	Lc 09 (bottom)	0.123	1.2±0.4

The results of the stomach content analyses are summarized in the %N and %F graphs (Fig. 4.2). Sixty-two prey categories were identified in total (Appendix 4.2). The mesopelagic fishes were mainly zooplanktivorous, preying on diverse taxa of meso- and macrozooplankton. Copepods, particularly the order Calanoida, were the most frequent and abundant component in the diet of mesopelagic fishes, with the exception of *L. crocodilus*. The dominant copepod species ingested were the medium-sized calanoids *Pleuromamma gracilis*, *P. abdominalis*, *Temora stylifera,* and *Clausocalanus* spp., the small calanoids *Paracalanus*-*Calocalanus* spp. and the cyclopoid genus *Oncaea*. Larvaceans (mainly *Oikopleura dioica*), euphausiids (mainly *Meganyctiphanes norvegica*), and ostracods (*Conchoecia* spp.) were the next most important taxa. Prey sizes seemed to be correlated with predator body lengths; the diets of the small-sized

hatchetfish *A. hemigymnus* and juvenile myctophids were dominated by small calanoid and non-calanoid copepods along with diverse unrelated mesozooplankton. Large-sized classes of adult myctophids contained medium-sized calanoids such as *Pleuromamma* spp. and adult stages of euphausiids as major food contributors, or alternatively, showed evidence of occasional feeding upon more nutritive prey, such as small fishes. Chesson's selectivity index for each mesopelagic fishes is depicted in Fig. 3 and referred to below for each mesopelagic fish species.

The stomiiforms, *A. hemigymnus* and *C. braueri*, fed almost exclusively on calanoids (55% N, 52% F (Dec. and July) and 28% N (Dec.)- 47% N (July), 50% F (Dec.)-72% F (July), respectively), ostracods (8% N, 9% F and 15-8.8% N, 38-32% F, respectively) and non-calanoids (5% N, 5% F and 37-27% N, 50-60% F, respectively). The oncaeids were of particular importance in both seasons in *A. hemigymnus*, which show positive but low selectivity for this group, while different corycaeid species (mainly *Farranula rostrata*) were eaten by most summer specimens, and the rest of its diet was composed by other diverse but rare taxa and small calanoids (*Clausocalanus*, *Calocalanus*). *C. braueri* showed high positive selectivity for the ostracod *Conchoecia obtusata*. Some stomachs of *Cyclothone* contained particulate matter, which was difficult to ascribe to marine snow, digested remains, detritus, or a mixture of all the above.

The diet of *B. glaciale* was represented by copepods in more than 50% N and F. Other prey categories such as larvaceans, gastropods or chaetognaths, were rare. The diet was quite similar between the two seasons, although during the summer the ingestion of euphausiids (8.4% N, 13% F) and fishes (5.16% N, 14.3% F) increased, which were almost absent in the stomachs from the autumn, while the ingestion of copepods decreased (calanoids: from 69% to 33% F; non-calanoids:

from 30% to 6.5% F) and ostracods (from 13% to 5.8% N, and from 28.9% to 10% F). The abundance and frequency of consumption of *Conchoecia* was higher in autumn, coinciding with the ostracod abundance peak in the environment. Dietary shift with body size was clear. Juveniles up to 25 mm SL relied mainly on small cyclopoids (*Oncaea* spp.) and ostracods (*Conchoecia* spp.), while medium-sized classes from 26 to 40 mm SL preyed selectively on the dominant calanoid *Pleuromamma,* and the larger-sized classes from 41 to 46 mm SL mainly fed upon euphausiids.

C. maderensis contained an assortment of different types of prey, with positive and considerable selectivity for larvaceans (*Oikopleura* spp.). Different species of calanoids, mainly from the genera *Pleuromamma, Paracalanus* and *Clausocalanus*, represented about the 35% of the diet, followed by larvaceans, non-calanoids, gastropods and ostracods with similar contribution each in both periods. A notable change was detected in the presence of euphausiids that increased in number and frequency of occurrence from 1% N and 4% F in autumn to 8% N and 15% F in summer. Likewise, the diet of *L. dofleini* was composed of miscellaneous calanoids and, regularly, non-calanoids, larvaceans and ostracods. Seasonal differences were plausible in its diet composition, where the abundance and frequency of ostracods increased from 16.9% N and 2.36% F in autumn to 53.85% N and 35.7% F in summer. Calanoids and non-calanoids had similar values of abundance and frequency of occurrence in the summer individuals (32% N, 19%F and 9% N, 10% F, respectively); euphausiids increased the occurrence in the stomachs and were positively selected in summer. The genus *Pleuromamma* was the dominant food item, being significantly high in summer.

Prey composition was quite similar between *Hygophum* congeners. Calanoids constituted more than 85% prey abundance in a third of the stomachs of *H.*

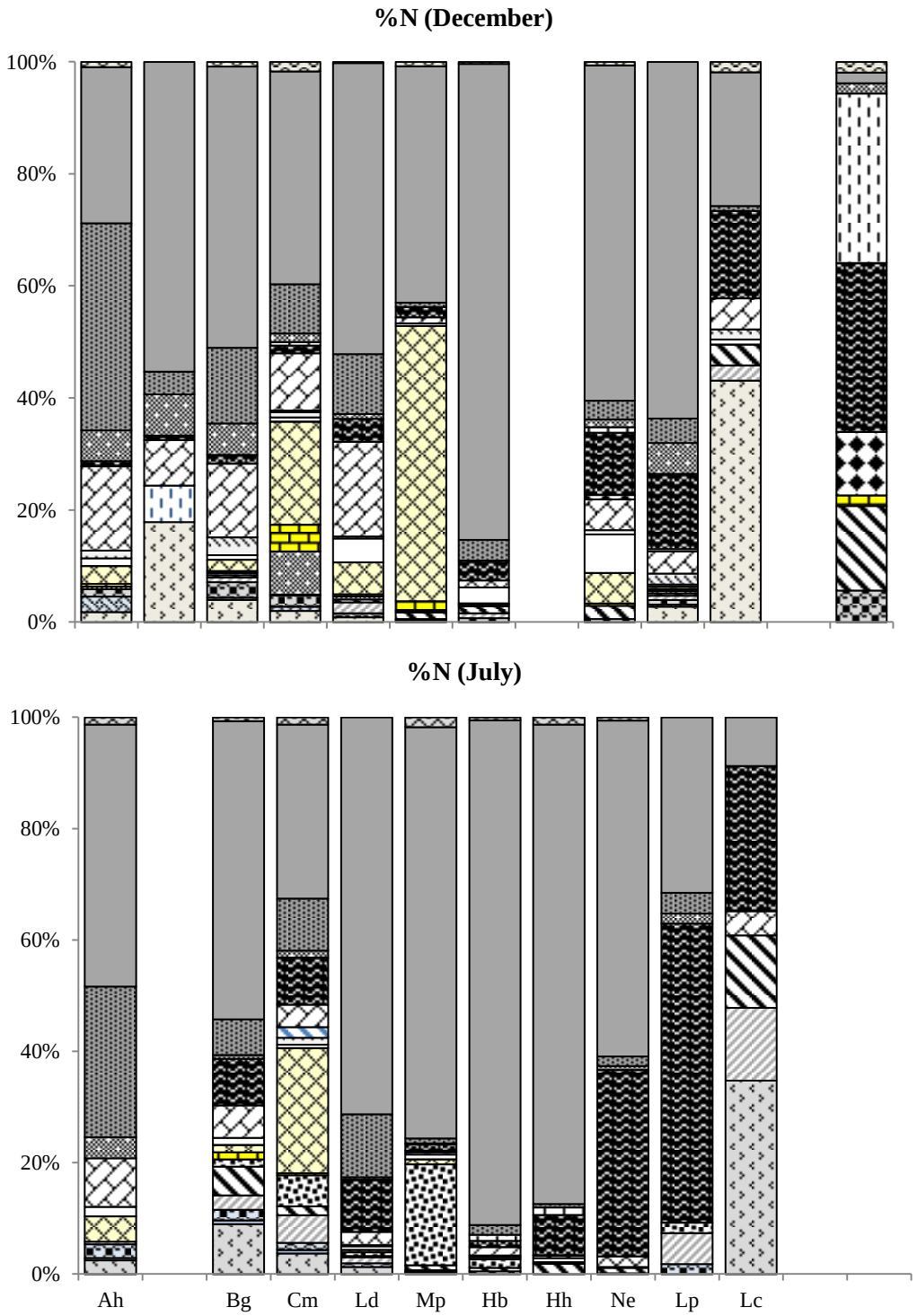


Fig. 4.2 Percentage abundance and frequency of occurrence of the prey categories found in the analyzed stomachs of the 11 species of mesopelagic fishes captured during the December and July surveys. Abbreviated names corresponded to the two first letters of the species scientific name.

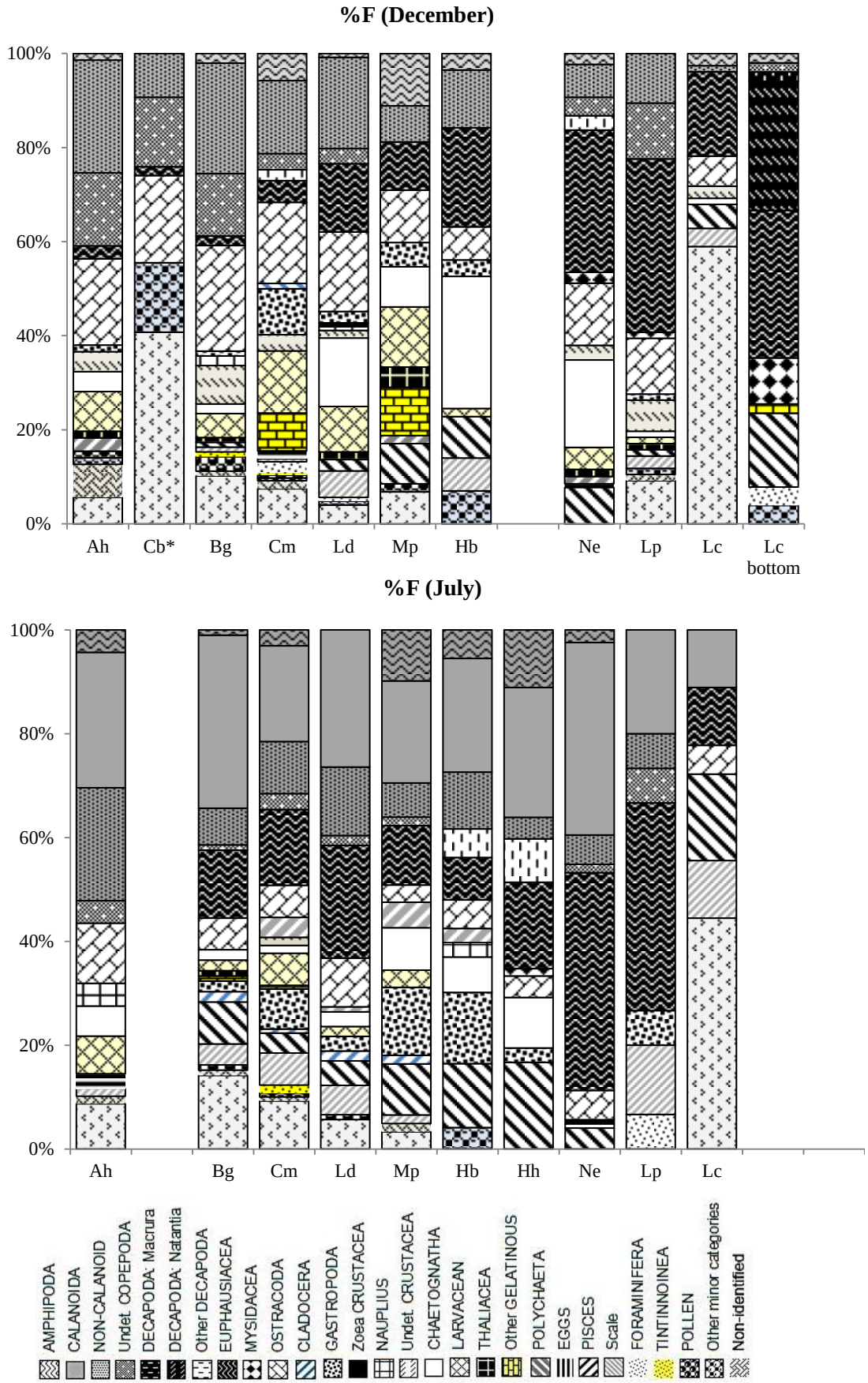

%F (December)
100%
80%
60%
40%
20%
0%
Ah
Cb*
Bg
Cm
Ld
Mp
Hb
Ne
Lp
Lc
Lc bottom
%F (July)
100%
80%
60%
40%
20%
0%
Ah
Bg
Cm
Ld
Mp
Hb
Hh
Ne
Lp
Lc
AMPHIPODA
CALANOIDA
NON-CALANOID
Undet. COPEPODA
DECAPODA: Macrura
DECAPODA: Natantia
Other DECAPODA
EUPHAUSIACEA
MYSIDACEA
OSTRACODA
CLADOCERA
GASTROPODA
Zoea CRUSTACEA
NAUPLIUS
Undet. CRUSTACEA
CHAETOGNATHA
LARVACEAN
THALIACEA
Other GELATINOUS
POLYCHAETA
EGGS
PISCES
Scale
FORAMINIFERA
TINTINNOINEA
POLLEN
Other minor categories
Non-identified

Fig. 4.2 Cont.

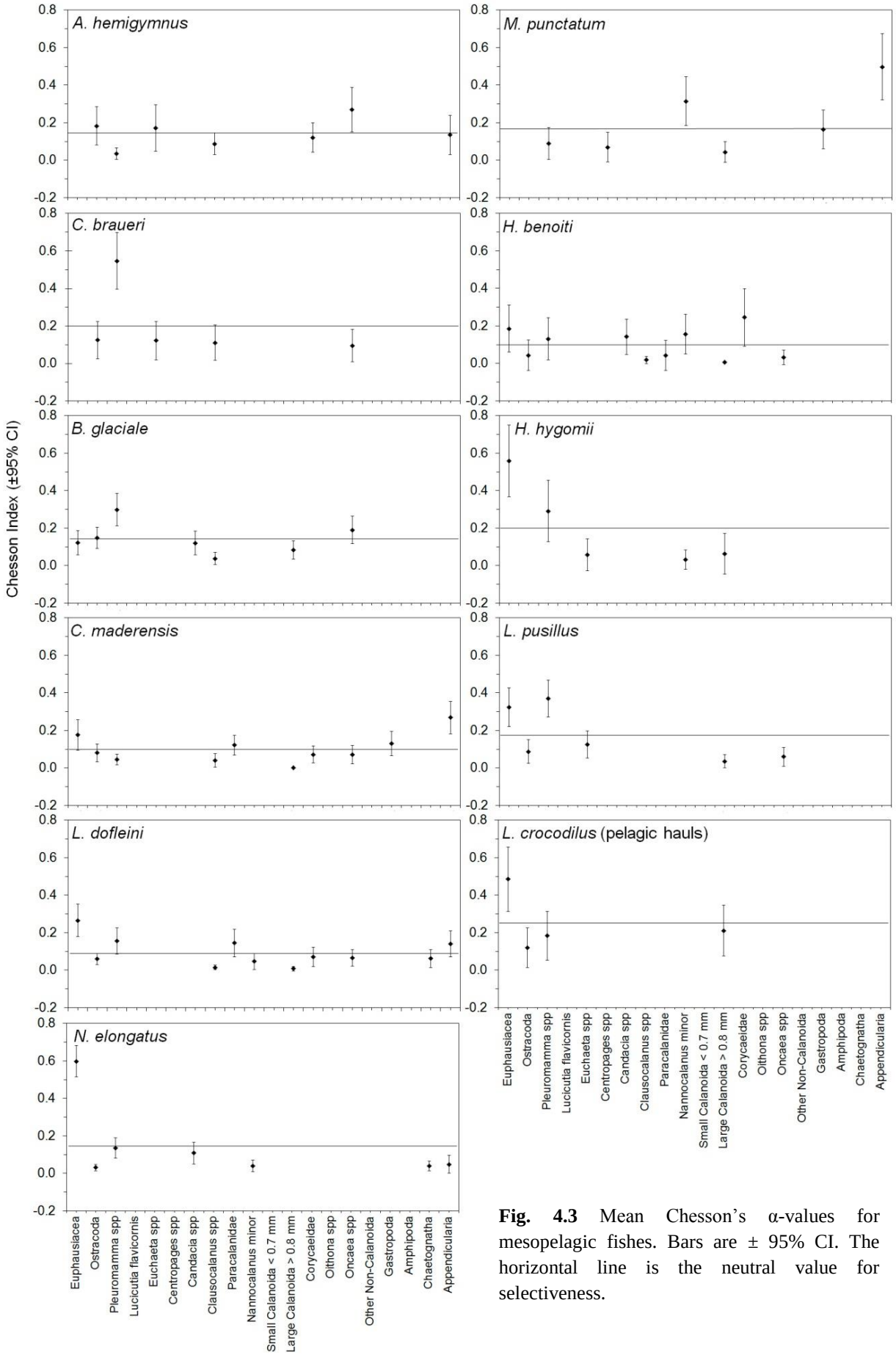


Fig. 4.3 Mean Chesson's α-values for mesopelagic fishes. Bars are ± 95% CI. The horizontal line is the neutral value for selectiveness.

was the most important prey by far, followed by *Nannocalanus minor* and *Euchaeta marina*. The euphausiids and hooks of chaetognaths were frequent in *H. benoiti* in autumn, but consisting of less than 3% N, while non-calanoids, gastropods and fishes were frequent in summer and constituted less than 2% N. The euphausiids and fishes were common in *H. hygomii* in summer (23% F, 15% F, respectively), but with a low abundance (less than 8% N). Adult specimens of *H. hygomii* presented selective feeding on euphausiids.

M. punctatum had a variable mixture of calanoids, euphausiids, amphipods, ostracods, gastropods, larvaceans, chaetognaths, and fishes (Fig. 2). This myctophid species showed great fluctuations in its diet composition between seasons and in the individual prey abundance, with some specimens depicting an individual preference for *N. minor* and *Oikopleura dioica* and negative selectivity for *Centropages* spp. Larvaceans were only important in autumn (49% N, 44% F), while gastropods (*Atlanta* or *Limacina* spp.) only in summer (49% N, 57% F). Two specimens from the autumn ingested an exceptional high number of larvaceans (185 and 676 each), and likewise two specimens from the summer ingested high numbers of *Temora stylifera* (148 and 178). Even disregarding these specimens with many prey items in their stomachs, calanoids were dominant (Dec.: 42% N, 92% F; July: 73% N, 67% F), with *N. minor, T. stylifera, Pleuromamma abdominalis, P. gracilis* and *Centropages typicus* as the most important items.

The diet of *L. crocodilus* was significantly changeable throughout development. Immature individuals of *L. crocodilus* had a mixture of UOM and digested remains that formed almost half of the stomach contents (between 30% and 45% N). It is difficult to accurately determine the relative abundance of food items. If considering only identifiable food items, *Pleuromamma* spp. and euphausiids were dominant. Bottom-dwelling individuals showed an evident shift towards larger prey, feeding on a different assortment of prey types from the migratory

stages of *L. crocodilus* and all other myctophid species from the water column. Decapods with pelagic and benthic distributions (42% N; 31% F) (e.g. *Gennadas elegans*, *Sergestes* spp. and Natantia), euphausiids (44% N; 35.6% F), mysids (11.1% N; 14.8% F), and small fishes (8% N; 17.8% F), i.e. myctophids, were, in this order, the preferred food items. In contrast with the other myctophid species, pelagic and bottom-dwelling *L. crocodilus* consumed significant proportions of small fishes, at least representing between 4% (pelagic) and 15% (bottom) of the stomach bulk. The pelagic individuals of *L.crocodilus* fed selectively on euphausiids coinciding with the adults of congener *L. pusillus*, which also preyed selectively on *Pleuromamma* spp. The Chesson α-value could not be calculated for those *L. crocodilus* associated with the sea bottom because we lacked samples from the suprabenthos to compare with the stomach contents.

Table 4.5
Results of the general *PERMANOVA* analysis on the average matrix by species, stage of development and season. DF: degrees of freedom; SS: Sum of Squares; MS: Mean Square.

Source	DF	SS	MS	Pseudo-F	P (perm)	Unique perms	P (MC)
Species	11	3.8991^{E5}	35446	11.099	0.0001	9825	0.0001
Development	15	1.1643^{E5}	7762	2.4304	0.0001	9722	0.0001
Season	1	16309	16309	5.1303	0.0001	9930	0.0001
Sp.*Season	10	83748	8374.8	2.6344	0.0001	9789	0.0001

The diet of *N. elongatus* was based on calanoids and euphausiids across all different size classes. Approximately, 60% N and >60% F (both December and July) of its diet was composed by calanoids, with dominance of *Pleuromamma* spp. Euphausiids were common in the diets from both seasons (>75% F Dec. and July) and the preferred prey, with a greater ingestion in summer (from 11.2% N in Dec. to 33.5% N in July). Before maturity, food composition was more varied in the smaller size classes, which preyed more intensely on

Pleuromamma spp. and furcilia and juvenile euphausiid. In size classes from 80 mm SL to 103 mm SL, mature fishes reduced the number and the variety of ingested prey to positively selecting adult euphausiids. The abundance of *Pleuromamma* decreased successively across larger size classes. Other taxa, including gelatinous, calanoids such as *N. minor, Euchaeta* spp., and small proportions of *Corycaeus* spp. and oncaeids, were frequent prey in winter. Moreover, *N. elongatus* from both seasons caught at night in the DSL fed, basically, on euphausiids (80-90% diet contribution).

Table 4.6 Results of various PERMANOVA tests performed separately for each species. The asterisk symbol denotes a "P value" P(perm) <0.0005, and a double asterisk denotes P(perm) <0.05. Groups with significant differences between them are indicated in brackets. Abbreviations: J: juvenile; I: Immature; M: Mature; SUP: Surface; DSL: Deep Scattering Layer; BBL1: layer at 50 m over the shelf (200 m depth).

Species	Season	Season (develop. stage)	Develop. stage	Size class (restricted to species)	Depth
A. hemigymnus				** [<10 mm SL, 20-34 mm SL]	
				** [10-19 mm SL, 20-34 mm SL]	
B. glaciale	*		** [J, M]	** [12-17 mm SL, 31-46 mm SL]	
				** [21-30 mm SL, 41-46 mm SL]	
C. maderensis	*	** [level 'M']			
L. dofleini	*			** [23-36 mm SL, 39-46 mm SL]	
M. punctatum	*				
H. benoiti			* [J, M]	* [13-19 mm SL, 31-50 mm SL]	
			* [J, I]	** [13-19 mm SL, 20-30 mm SL]	
				** [13-19 mm SL, 51-64 mm SL]	
N. elongatus				** [30-80 mm SL, 91-172 mm SL]	* [SUP, DSL]
				** [30-50 mm SL, 31-172 mm SL]	* [SUP, BBL1]
					* [DSL, BBL1]
L. crocodilus				**	** [SUP, DS]
					* [SUP, Bottom]
					** [DSL, Bottom]

There was no evidence of piscivory in the stomachs of *A. hemigymnus* and *C. braueri*. On the contrary, the nine lanternfish species revealed, at least once, the occurrence of fish remains. The highest degree of piscivory was found in *H. hygomii, H. benoiti, L. dofleini,* and *M. punctatum* as well as in *L. crocodilus* from bottom trawls. Due to the advanced stage of digestion of most of the fishes found in the stomachs, cod-end feeding has been disregarded.

4.3.4 Seasonal, spatial, and ontogenetic variations in diet

A general PERMANOVA was run on the whole dataset, showing significant differences ($p<0.001$) between fish species, the developmental stages within the species, the two seasonal periods and the interaction species*seasonality (Table 5). However, as mentioned in the material and methods, PERMDISP routine was also found to be significant ($p<0.001$), manifesting a dispersion effect over the analysis on how these factors influence fish diets. Non-metric MDS showed clear overlap of diet compositions for the different mesopelagic species. Alternative PERMANOVA tests for each species were performed, and a summary of the significant outputs is shown in Table 6. There were significant differences between the interspecific diet compositions of some size classes in most mesopelagic fishes. Our results exhibited a clear shift in the diet composition with size within the species *A. hemigymnus, B. glaciale, H. benoiti, L. dofleini, L. pusillus,* and *N. elongatus*. Nevertheless, no dissimilarities were found between the diet of mature and immature stages of *L. dofleini, H. benoiti H. hygomii, M. punctatum, N. elongatus,* and *C. braueri.*

The effect of seasonality was evident for *L. dofleini, B. glaciale, M. punctatum* and between mature individuals of *C. maderensis*. A lower number of immature individuals were analysed in summer as a consequence of the population size structure, which could be affecting these small dissimilarities between mature *C. maderensis* from different seasons. Diet composition was also indirectly

affected by depth. The dissimilarities in diet were significant between the largest specimens of both *L. crocodilus* and *N. elongatus*, which remain close to the sea bottom, and the pelagic adults of the respective species. Therefore, the dietary shift experienced in mature individuals of both species entailed depth stratification of food resources.

Table 4.7 Prey items and percentage of occurrence (F) in predator stomachs. Only items present in more than 5% of the stomachs were used in the CCA analysis.

Prey item	**Code**	**F (%)**
Nannocalanus minor	nan	18.94
Temora stylifera	tem	6.46
Candacia spp.	can	13.92
Euchaeta spp.	euc	13.77
Clausocalanus spp.	cla	17.36
Centropages spp.	cen	8.75
Lucicutia spp.	luc	5.16
Pleuromamma spp.	ple	53.23
Paracalanidae (*Calocalanus, Paracalanus* spp.)	par	10.33
Undetermined Calanoida	cau	20.52
Corycaeidae	cor	12.63
Oncaea spp.	onc	21.38
Undetermined Copepoda	cou	10.19
Ostracoda (*Conchoecia* spp.)	ost	26.54
Amphipoda	amp	8.61
Euphausiid furcilia/juvenile	eu1	14.2
Euphausiid adult	eu2	27.4
Chaetognatha	cha	15.06
Appendicularia (*Oikopleura dioica*)	app	12.34
Gastropoda (*Atlanta, Limacina, Cresseis* spp.)	gas	7.03
Fish scale	sca	7.89
Pisces (small mesopelagic fishes)	pis	10.76

A final CCA ordination diagram is shown in Fig. 4 and the CCA diagnostics in Table 7, complementary to the mentioned statistical results. Total inertia (variance) was low, i.e. 11.8%, reinforcing the hypothesis of a high degree of

diet overlap among mesopelagic fishes. The explanatory power of the variables was low; only the first two variables selected had an r^2>0.3 (Table 4.8). The 1-axis in the CCA is mainly correlated with SL, and the 2-axis with the number of prey items (PN) in the stomachs.

Table 4.8
Results of the forward selection procedure to determine significant explanatory variables in the CCA. The total inertia explained by the constrained model was 11.8%.

Explanatory variable	**Codes**	**r^2 (envfit)**
+Species	AH, BG …(in Fig. 4)	0.359
+Standard Length	SL	0.3127
+Cruise/season	2009 2010	0.0188
+Stratum DSL	DSL	0.0118
+Prey Number	PN	0.0947
+Stratum Surface	SUP	0.0075
CCA1% variance		31.7%
CCA2% variance		19.4%

4.4 Discussion

Midwater fishes are important forage and predatory species, whose role in pelagic ecosystems is not fully understood due to the lack of direct data available in relation to the commonly used ecological models. Our study investigates the diet preferences and feeding dynamics of Mediterranean mesopelagic fishes through stomach content analysis.

4.4.1 Resource partitioning *versus* diet overlap

Food web interrelationships can be affected by the oligotrophic conditions of the WM, characterised by low primary and secondary productions, with an accentuated impoverishment in summer and high species diversity (Estrada, 1996). In demersal fish communities from oligotrophic areas, sporadic

concentration or scarcity of food resources determines specialised foraging strategies on particular food items, which reduces niche overlap (Macpherson, 1981). The degree of diet specialisation found in the Mediterranean fish species from the present work was low, except for the oldest stages of some myctophids (*B. glaciale*, *L. crocodilus*, *L. pusillus*, *N. elongatus*, *H. hygomii*, all with a mean prey diversity <1; Table 4); hence, the whole group of mesopelagic fishes was rather typified by high prey diversity, and generally a low level of selectivity on one or two preys, according to Chesson's index. Broad diet overlap, which is

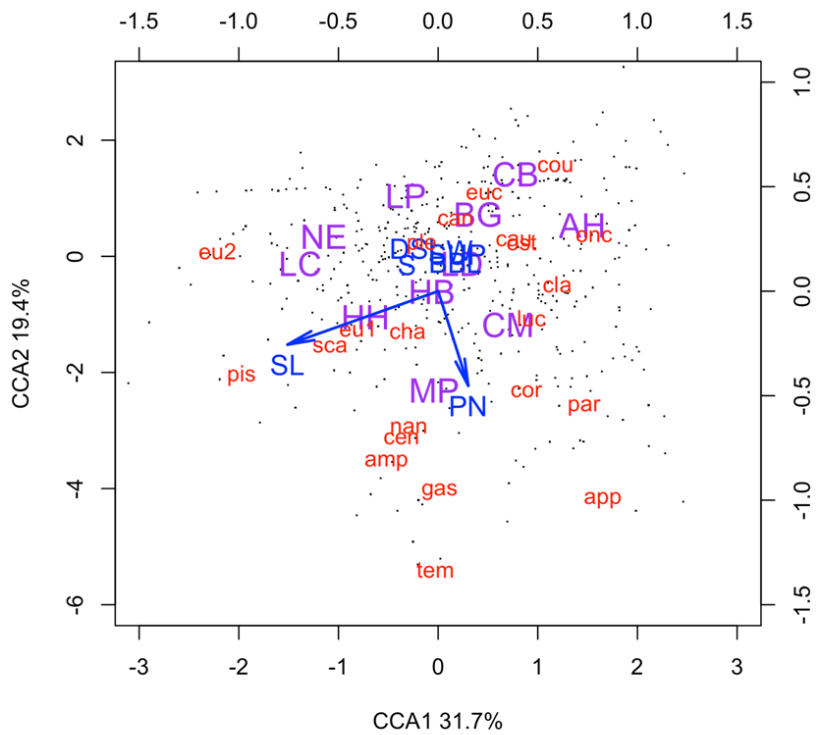


Fig. 4.4 CCA ordination diagram for mesopelagic species. The arrows (blue) indicate the significant explanatory variables, with arrowheads indicating the increase in gradient. Categorical explanatory variables (blue letters) are located at their respective centroid positions. Red labels are the prey items (Table 6). Codes: see table 6. AH, *Argyropelecus hemigymnus*; CB, *Cyclothone braueri*; BG, *Benthosema glaciale*; CM, *Ceratoscopelus maderensis*; HB, *Hygophum benoiti*; HH, *H. hygomii*; LC, *Lampanyctus crocodilus*; LD, *Lobianchia dofleini;* LP, *L. pusillus*; MP, *Myctophum punctatum*; NE, *Notoscopelus elongatus.*

distinctive of regions with abundance of food resources (e.g. Tyler and Pearcy, 1975; Pakhomov *et al.*, 1996), was evident among myctophid species from the oligotrophic WM. The overlap was large between all species, revealing that prey taxa were not very limiting (Holbrook and Schmitt, 1986). A particular predator might obtain its energetic requirements from the available prey, and within that spectrum of organisms, selectively capture those with a higher energetic value. None of the analysed environmental factors were discriminatory between the diet compositions of the different mesopelagic species. This suggests that the interspecific similarities in the diet prey proportions would, to a large extent, be determined by zooplankton availability in the environment, and that the small dissimilarities between these diets are difficult to assign to a unique factor.

Resource partitioning at both, intraspecific and interspecific levels, has been shown in previous studies for the mesopelagic fish assemblage in low-latitude oligotrophic ecosystems (Hopkins and Gartner, 1992; Hopkins and Sutton, 1998; Burghart *et al.*, 2010). The WM is also characterised by oligotrophic waters, thus some partitioning of the exploitation of food resources by predators of the lowest trophic levels (TrLs) might be expected. In this regard, the most abundant mesopelagic fishes, i.e., the non-migratory gonostomatid *C. braueri* and the surface-migrant myctophid, *C. maderensis*, have overlapping diets, but showed clear segregation of their feeding habitat. This suggests that resource partitioning between the two most abundant mesopelagic fishes in the WM was mainly addressed by their differential vertical distributions. Hopkins and Gartner (1992) considered that partitioning in the myctophid community of the Gulf of Mexico was regulated by two parameters, space and food availability. Assumptions on a relative oligotrophic environment inducing partitioning in the diet composition seem not to be applicable for the WM midwater fishes, and competition among them could be mitigated by ways such as feeding at different depths or times, or depicting a certain degree of selectiveness. In fact, fishes

feeding on the same prey species, but segregated in the water column, are partitioning a common food resource (Hopkins and Sutton, 1998). Defining a spatial niche for these migratory species is complex and would require a detailed chronological effort, short-time scale sampling, and higher spatial resolution in the vertical sampling strata.

An alternative, but nonetheless conceivable, explanation for the high diet overlap among midwater fishes in the WM could be that the Mediterranean fish assemblage is not saturated with diverse range of species and, therefore, it is less inclined to compete within their own community than other mesopelagic communities. In particular, the oligotrophic waters in the Gulf of Mexico (Hopkins and Gartner, 1992; Hopkins and Sutton, 1998; Burghart *et al.*, 2010) have a high species diversity of stomiiforms (69 reported species) and myctophids (over 50 spp.), thus, resource partitioning could be decisive to avoid strong interespecific competition.

Diet estimated through isotopic mixing models (SIAR) (Valls *et al.*, 2014) suggested differences between sympatric DVM species, i.e. *L. crocodilus* and *L. pusillus*, and between *H. hygomii* and *H. benoiti*. Such dissimilarities in the diet were unclear from the direct analysis of stomach contents. *H. hygomii* and *H. benoiti* from the same size range showed comparable diets, with a major contribution of fish remains and euphausiids in *H. hygomii*, in agreement with the higher mean δ15N value (Valls *et al.* 2014) for this latter species. Diet overlap was found between *L. pusillus* and *L. crocodilus* from the water column, which shared similar proportions of prey taxa. However, when considering the large contribution of scattered organic matter (UOM, 43% N) in the diet of the pelagic individuals of *L. crocodilus*, food partitioning was strong between these congeners, and coincided somehow with the prediction of SIAR isotope mixing models and the relatively distinct trophic values estimated for each species (Valls *et al.*, 2014). The results of the isotope analysis for these two

Lampanyctus spp. fitted this last consideration on the exploitation of different trophic niches, defining a lower TrL for the juvenile specimens of *L. crocodilus*, whose diet seemed partly dependent on UOM, than for *L. pusillus.* The spatial segregation of larger individuals of *L. crocodilus*, also determines differential feeding habits. Margalef (1974) considered that the evolution of sympatric and close species leads to feeding segregation, which results in differential sizes: for example, if applying this to the genus *Lampanyctus* from the WM, we can describe *L. crocodilus* as a relatively large myctophid that feeds on larger prey than the smaller *L. pusillus*.

4.4.2 Ontogenetic, spatial, and seasonal variability

Shifts of the diverse food usage with development partly modulated the feeding dynamics in the mesopelagic fish community. Ontogenetic dietary shifts are usual in most mesopelagic fishes (e.g. Gartner *et al.*, 1997; Uchikawa *et al.*, 2001; Tanimata *et al.*, 2008; Borme *et al.*, 2009) as well as in other organisms experiencing large increases in size along its development (Jackson *et al.*, 2004). Most marine fishes consume larger prey items with increasing predator body sizes (Popova, 1967; Juanes, 1994; Scharf *et al.*, 2000), expanding the range of prey sizes eaten by incorporating large organisms and, occasionally, feeding on the available smaller plankton sizes, rather than becoming size-dependent. In this study the sizes of the different prey items varied notably among the predator species, and all fish species showed significant differences from small to large size classes. However, a lack of relation between the TN breadths and growth was found in all the fish species, which is characteristic of predators that are not size-selective, usually described as "engulfing types" (Pearre, 1986). Growth manifested in larger body and mouth sizes is an essential feature in the election of a broader range of prey sizes. In agreement with this, we found that adults with larger mouth widths consumed a wider range of prey. Those myctophids

with the smallest body sizes in early juvenile stages (e.g. *C. maderensis*, *L. dofleini*) consumed the smallest prey items, but reached the same size proportions than the rest of the species in larger sizes. It is probably due to a limitation in the capacity of capture determined by mouth width in the earlier stages. By widening prey size spectra, predators acquired a flexible condition in feeding, moving out from intraspecific competition within earlier developmental stages, and rapidly satisfying their energetic requirements (e.g. Gartner *et al.*, 1997; Uchikawa *et al.*, 2001; Tanimata *et al.*, 2008; Borme *et al.*, 2009). Although small prey were consumed throughout the entire development, they were less common than larger preys. Euphausiids appeared as the more common prey in the diet of the oldest myctophids, partly displacing copepods, which tend to dominate in immature stages. Similar observations were found in myctophids from other regions (Gjøsæter, 1973; Pearcy *et al.*, 1979; Pusch *et al.*, 2004).

Main differences among myctophid species, and with the stomiiforms, were related to the differential number of ingested organisms. The variability in the number of prey had an ontogenetic component at an intraspecific level with the oldest individuals of myctophids usually eating a low number of larger prey items.

The migratory behaviour of some mesopelagic fishes along with their occurrence in a particular depth layer is intimately related to ontogenetic changes in the feeding habits of the increasing-size classes. Trophic functional groups can be defined by the vertical dynamics of active-migrants, weak-migrants, and permanent species in conformity to the spatial dimension of the open ocean. For instance, the stomachs of adults of some species with an active migrant behaviour (*B. glaciale, Hygophum* spp., *L. pusillus*) contained large micronekton organisms with high swimming abilities, such as euphausiids and small mesopelagic fishes. In this study, non-significant differences were found in the diet composition of the different myctophids with depth, except for the

oldest individuals of *L. crocodilus* and *N. elongatus* with low or non-migratory activity. The largest size classes of some myctophids have been reported to be relatively more frequent in deeper waters (Badcock and Merret, 1976; Willis and Pearcy, 1980; Gartner *et al.*, 1987; Auster *et al.*, 1992; Stefanescu and Cartes, 1992). However, our study shows that Mediterranean active migratory fishes displaced upwards to feed on zooplankton near the surface and had a more diverse diet in terms of prey richness than those living close to the bottom. *A. hemigymnus* and *C. braueri* dwell in the more stable environment at the 400-600 m DSL, with minor energetic requirements, which is manifested by small prey in their stomachs and the lower trophic position determined by isotope analyses (Fanelli *et al.*, 2014; Valls *et al.*, 2014).

Seasonal changes in the zooplankton community are likely to affect the structure of the fish population. Variations in relative species occurrence by seasons (Fernández de Puelles *et al*., 2014) might be reflected in the mass bulk and frequency of prey in the stomachs of the myctophids *B. glaciale, C. maderensis, L. dofleini,* and *M. punctatum.* For instance, the zooplankton groups *Pleuromamma* spp., *Clausocalanus,* Paracalanidae and pteropods were more abundant both in the stomachs of *C. maderensis* and in the environment during the autumn. A higher feeding on a particular prey taxa restricted to one season can be attributed to the fact that those food resources have an abundance peak for that period (Macpherson, 1981; Cartes, 1998). This is reflected in fish species that tend to exhibit opportunistic behaviour. On the contrary, *N. elongatus, L. crocodilus* and *L. pusillus* had higher abundance of euphausiids in July, when the biomass of these crustacean populations, for which these myctophids were selective, was less concentrated in the surface and spread in deeper waters (Sardou *et al.*, 1996).

4.4.3 Diet and feeding strategy

In the open ocean, meso- and bathypelagic fishes have been categorised within three major trophic guilds; a) 'zooplanktivores'; b) 'micronektivores', which feed upon small fishes and cephalopods; and c) 'generalists', which use a broad assortment of unrelated taxa of crustaceans, gastropods, gelatinous and fishes (Gartner *et al.*, 1997). The generalist description encompasses, to a large extent, most of the midwater fishes in the present study. Our results confirm the relevant status of midwater fishes in the western Mediterranean basin as mainly zooplankton and micronekton consumers. Isotopic studies place these species in the third-fourth TrLs of the food web (Fanelli *et al.*, 2014; Valls *et al.*, 2014). In other oceanic regions, myctophids and stomiiforms also occupy an intermediate position in the food web (Kozlov, 1995; Cherel *et al.*, 2010; Flynn and Kloser, 2012) by feeding on meso- and macrozooplankton, and micronekton.

Most mesopelagic fishes are nocturnal carnivores (Merrett, 1974; Kinzer and Schulz, 1985; Pakhomov *et al.*, 1996; Williams *et al.*, 2001) that forage in the upper layers coinciding with the main zooplankton concentrations. The ocean surface has been found to be the most productive layer of the water column, essential for trophic interactions with the surface-migratory midwater fishes (Gray and Kingsford, 2003). Night feeding in the epipelagic layers was observed in the present study in myctophid species, in agreement with reports from low-latitudes (e.g. Gorelova, 1974; Tyler and Pearcy, 1975; Moku *et al.*, 2000; Watanabe *et al.*, 2002). Daytime feeding was rare and has elsewhere been documented only in a few studies from productive areas (e.g. Kinzer and Schulz, 1985; Young and Blaber, 1986; Moku *et al.*, 2000). The genus *Hygophum* (juveniles to mature adults) and the near-bottom-dwelling specimens of *L. crocodilus* were the only myctophids that showed active daytime feeding away from the photic zone. Nevertheless, the stomach contents of *H. benoiti* and *H.*

hygomii from day- and nighttime hauls were in an advanced state of digestion. Our results showed that, as other lanternfishes, most individuals of both *Hygophum* species feed during the night in the epipelagic layer, but may also sporadically feed in deeper waters at other times, where the digestion processes occur slowly. This behaviour may also happen in individuals of *L. dofleini* and *M. punctatum*, which were captured below the photic zone during the day with a mixture of fresh and digested food in their stomachs, suggesting intermittent feeding throughout the day. In other oceanic regions, daytime feeding has also been reported for non-migratory myctophids that remain close to the sea bottom and for a few species inhabiting subarctic regions and relatively high productive zones (Kinzer and Schulz,1985; Young and Blaber 1986; Moku *et al.*, 2000), however, this is not the usual pattern for oligotrophic regions, such as the Gulf of Mexico (Hopkins and Gartner, 1992; Hopkins and Sutton, 1998; Burghart *et al.*, 2010), the central-western Indian Ocean (de Alwis and Gjøsæter, 1988), and the western North Pacific (Watanabe *et al.*, 2002).

In contrast to myctophids, *C. braueri* and *A. hemigymnus* fed during day- and nighttime. These two species do not migrate to the surface as they can adapt to a dim environment in relatively stable conditions; both species are linked to DSL (Olivar *et al.*, 2012) at 400-600 m depth, with non-significant variations in sunlight and temperature. Their strategy consisted of foraging on the available zooplankton in these deeper layers, saving considerable energy not migratiing to the surface. A lack of feeding periodicity has also been reported for some other stomiids in the Atlantic (Sutton and Hopkins, 1996). However, this is not a general pattern, because more active foraging dynamics during the daytime have been reported for a Pacific non-migratory phosichthyid (Williams *et al*. 2001), as well as for various sternoptychids from the equatorial Atlantic (Kinzer and Schulz, 1985). Apart from a distinctive and continuous foraging pattern, *C. braueri* usually presented low stomach fullness, with a coefficient of vacuity of

63%, similarly to that reported by Palma (1990). Low prey number and high evacuation rate can be partly explained by gut morphology, with a straight-shape and medium length, in which food items remain for a short time. Moreover, as stated in previous studies, the low consumption of prey is affected by the lethargic nature of the species (Barham, 1970), with a considerable quantity of lipid reserves.

Midwater fishes fed upon organisms widely distributed throughout the water column. In terms of prey importance copepods were the dominant category in the stomachs, specifically, the genus *Pleuromamma* spp. Species of this genus have been important in the diet composition of many myctophids from other latitudes (e.g. Williams *et al.*, 2001; Pusch, 2004). Prey taxa other than the copepods *N. minor, T. stylifera, Clausocalanus* spp., Paracalanidae and *Oncaea* spp., the ostracod *Conchoecia obtusata*, the euphausiids *Meganyctiphanes norvegica* and *Euphausia krohnii* and the larvacean *Oikopleura dioica,* were rare in these predators (Appendix 2). In some instances, the diversity of prey taxa reflected part of the feeding strategy of the migratory fish species, as they feed in the time period in which the energetic effort is maximized. In a spatial and temporal scale, it means that predators move upwards to the productive epipelagic zone at night (Clarke, 1974; Kinzer and Schulz, 1985; Watanabe *et al.*, 2002).

Myctophids and stomiiforms have been reported showing diverse feeding patterns, and particular species adapt their behaviour depending on a region or latitude range. For instance, Paxton (1967) attributed a high specific feeding behaviour to some lanternfish species off southern California, and other authors concluded thats forage upon a broad array of prey taxa and sizes, suggesting that lanternfishes were opportunistic (Collard, 1970; Cailliet, 1972; Hopkins and Baird, 1973). However, our study shows that most Mediterranean mesopelagic

species in the Sea exhibit diverse feeding conducts from generalists to more specialised feeding patterns, particularly in the older developmental stages.

The stomiiforms *A. hemigymnus* and *C. braueri* were found to be quite opportunists, with a dominance in their stomachs of conspicuous and available prey, such as *Pleuromamma* and *Conchoecia*, which are bioluminescent crustaceans that can easily be detected. The diet composition of *C. braueri* was consistent with the observations previously documented by Palma (1990), with *Pleuromamma*, *Conchoecia* and detritus frequently recorded. Other prey taxa reported by Palma (1990) such as *Euchirella messinensis, E. marina*, *E. acuta*, *Candacia* sp., *Calanus* spp. and euphausiid furcilia stages were found with low frequency. The diet composition of *A. hemigymnus*, based primarily on copepods and ostracods, coincided with that reported for the same species from other oceanic regions (Hopkins and Baird, 1985). The foraging strategy of *Cyclothone* spp. has been suggested in other studies as opportunistic based on the capture of organisms within a short distance due to a limited hunting capability (Palma, 1990; Uchikawa *et al.*, 2001), unlike *A. hemigymnus*, which has an adapted stereoscopic vision for prey detection from beneath (Hopkins and Baird, 1973; Badcock, 1984; Hopkins and Baird, 1985). Furthermore, *Cyclothone* usually preyed on one or two large copepods, filling its stomach at once, while *A. hemigymnus* relied on the consumption of a broad taxonomic spectrum and larger number of small prey. The absence of large-sized items (e.g. adult euphausiids, amphipods, fishes) in both stomiiforms is in line with the lowest nitrogen isotope signatures (Fanelli *et al.*, 2014; Valls *et al.*, 2014) reported within the whole mesopelagic fish populations.

The myctophid *B. glaciale* fed upon a diverse array of copepods, ostracods and euphausiids, with a diversity index lower than other generalist myctophids from this study. Similar prey taxa were found to important in *B. glaciale* from other remote areas (Gjøsæter, 1973; Kinzer, 1977; Clarke, 1978; Kawaguchi and

Mauchline, 1982; Roe and Badcock, 1984; Dalpadado and Gjøsaeter, 1988; Sameoto, 1988; Dypvik *et al.*, 2011; García-Seoane *et al.*, 2013), with preference for the common genus *Pleuromamma* in the WM, and for the abundant glacial amphipod *Themisto* spp. in high latitudes (García-Seoane *et al.*, 2013). The frequent occurrence of large euphausiids and, to a lesser extent, small fishes, in the stomachs might be the reason for a moderately high TrL value of *B. glaciale* within the mesopelagic community (Valls *et al.*, 2014).

The medium-sized myctophids *C. maderensis*, *M. punctatum* and the small *L. dofleini* were euryphagic and active predators that fed upon a wide assortment of unrelated taxa and seemed to have a mixed strategy affected by the seasonal differences of the available zooplankton. The high prey diversity in the stomach contents of these three species suggests a tendency towards generalist behaviour. To our knowledge, no detailed study on the feeding habits of *C. maderensis* from other regions has been carried out to allow comparative analysis, except for a brief study in the North Atlantic (Podrazhanskaya, 1993) that reported on a diet based on the dominant genus *Themisto*. The feeding strategy of *L. dofleini* was studied in the North Atlantic region by Pusch *et al.* (2004) and Merret and Roe (1974), who determined this myctophid as a random feeder with preference for the genera *Pleuromamma* and *Euchaeta*, while we found dominance of *Pleuromamma* spp. and positive selection for euphausiids. As for *C. maderensis*, our results support the conclusion that *L. dofleini* behaves with some flexibility between two foraging strategies, neither behaving strictly as a random feeder nor as a specialist. Isotopic analysis based on different sets of individuals from the same cruises as our study, assigned *L. dofleini* the highest TrL within the mesopelagic fish community (Fanelli *et al.* 2014; Valls *et al*. 2014), while our results from stomach content analysis are not consistent with this high trophic value, and do not show particularly high consumption of fishes or euphausiids than in the other WM myctophids. A possible explanation for these dissimilar

results may be related to the small fish sample size in the isotope analyses (3 individuals in Fanelli *et al.* 2014, and 3 per season in Valls *et al.* 2014) which may have resulted in poor estimation of their nitrogen isotopic signature (Van der Lingen and Miller, 2011). However, the isotope signatures from both studies (Fanelli *et al.* 2014; Valls *et al.* 2014) were consistent; thus, it is possible that *L. dofleini* is physiologically different than the rest of MW myctophids, with a smaller round body shape that results in a faster metabolism. In contrast with the former two myctophid species, *M. punctatum* seems to be even more generalist. The apparent high number of prey in most specimens of *M. punctatum* indicates that its foraging strategy consists of ingesting as many food items as its stomach can fit. We found similarities with the observations of Scotto di Carlo *et al.* (1982) in Messina (Mediterranean Sea) for the most common prey, such as the calanoids *N. minor, T. stylifera, P. abdominalis* and the larvacean *O. dioica*, except for the calanoid genus *Centropages*, which was not found in the Messina specimens. Scotto di Carlo *et al.* (1982) reported that *M. punctatum* became more opportunistic with age, preying on a broader range of taxa that included gastropods, larvaceans and chaetognaths. Our study does not support this statement, finding the same type of prey in the whole size spectrum from 23 mm to 60 mm SL. However, we think that *M. punctatum* eats a great number of prey during the early juvenile and adult stages, leading to quick growth, and the oldest specimens start feeding on a low number, but more nutritive and larger organisms.

The two species of *Hygophum* had a similar diet dominated by *Pleuromamma*, similar to the results for the specimens from Messina (Scotto di Carlo *et al.*, 1982) and Southern Ocean (Pakhomov *et al.*, 1996). The dietary preference of *H. hygomii* on euphausiids along with relatively frequent feeding on amphipods and small fishes can explain a higher TrL than in congener *H. benoiti* (Valls *et al.*, 2014). The two *Hygophum* species shared a similar spatial distribution, and

foraged upon the same diet categories, however the frequency of prey taxa found in each species was different, so the differential consumption of prey taxa may reduce competition within these congeners.

The oldest specimens of *N. elongatus* and *L. crocodilus* were specialised on the consumption of large euphausiids. The preference of *L. crocodilus* for larger and more nutritive organisms is consistent with the dietary and isotope results of previous studies (Stefanescu and Cartes, 1992; Fanelli *et al.*, 2014; Valls *et al.*, 2014). Piscivory in some myctophids was evident by the presence of whole fish or several fish scales and ocular lenses in the same stomach.

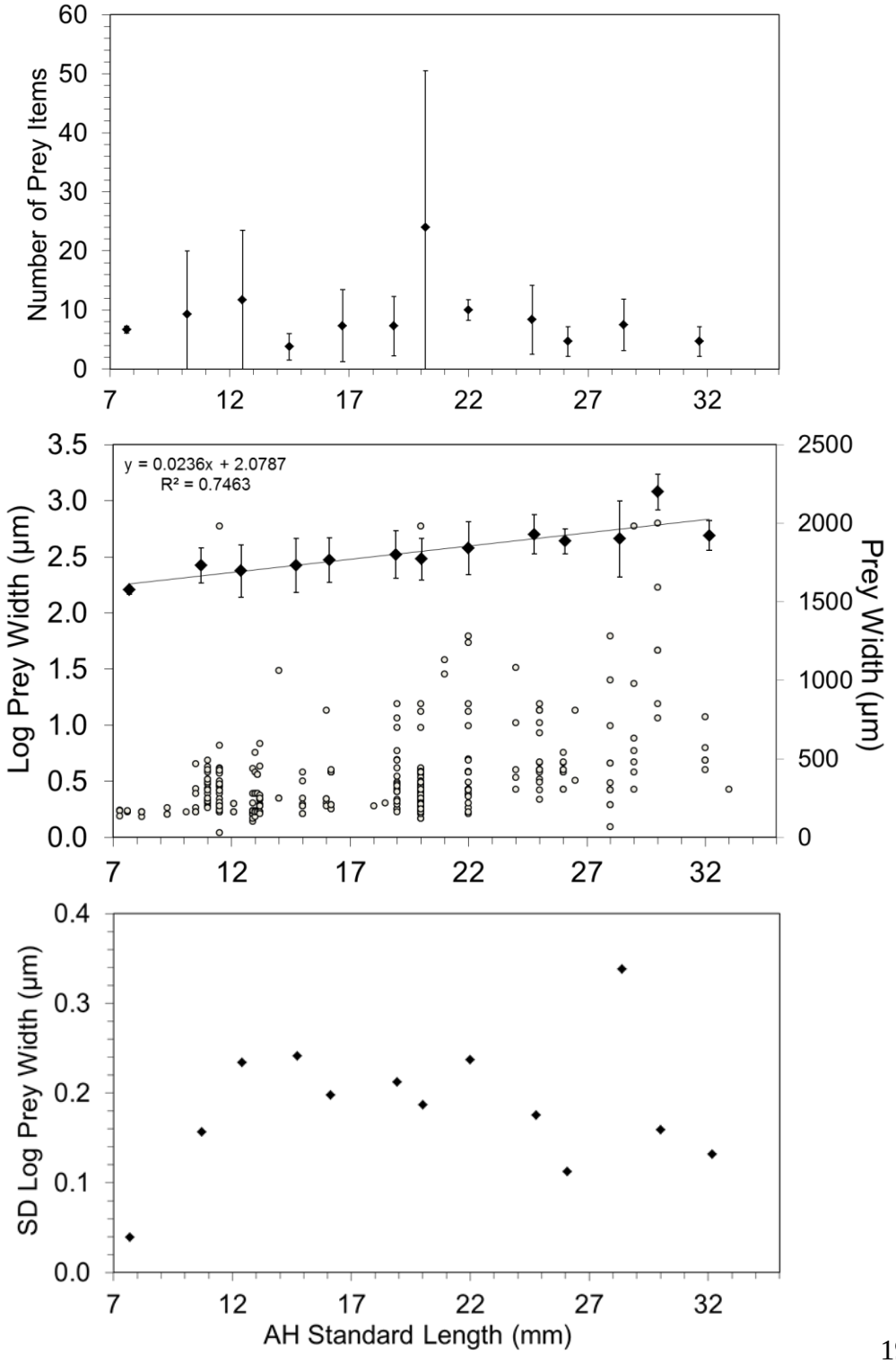
Finally, it is worth mentioning that some studies (Boerger *et al.*, 2010; Davison and Asch, 2011; Van Noord *et al.*, 2013; Rochman *et al.*, 2014) have suggested that mesopelagic fishes can be responsible for the ingestion of large quantities of plastics, particularly in the North Pacific Central Gyre. However, our data did not show consumption of plastic debris by mesopelagic species in the western Mediterranean. Plastic debris was not detected in noticeable concentrations in the zooplankton samples from the same area, as it was found in the cited studies under the gyre influence.

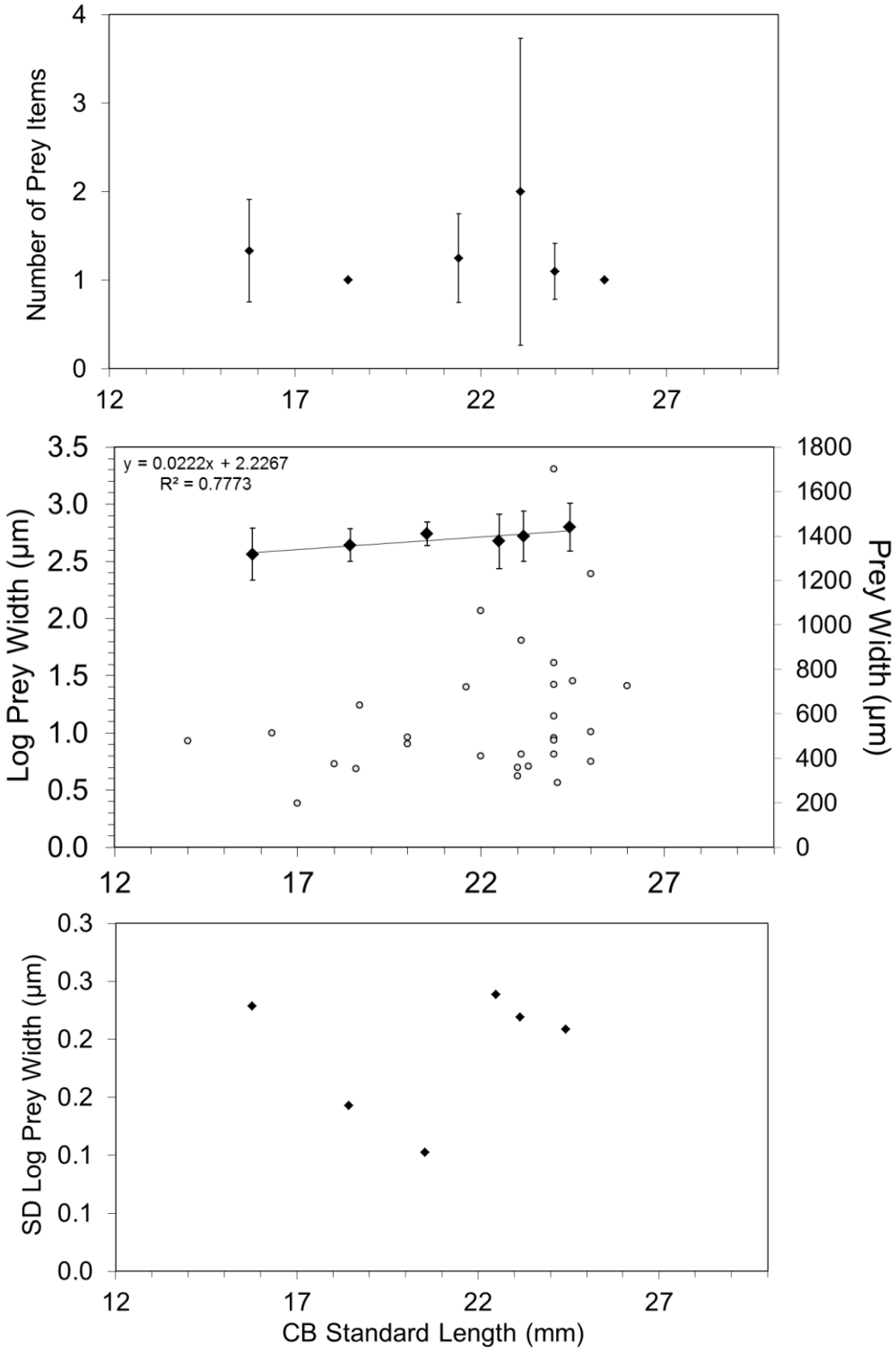
4.5 Conclusions

Our results suggest that, Mediterranean mesopelagic fishes exert an important feeding pressure on the zooplankton community, in accordance with classic studies of midwater fishes in other regions (e.g. Hopkins and Gartner, 1992), since they consume a high number of copepods and other taxa. In presence of moderate oligotrophic conditions these fishes were found to feed mainly on the most abundant prey categories (80% surface and/or migratory copepods), with notorious impact on particular copepod species, e.g. *P. gracilis* and *P. abdominalis*, which are frequent migrants to the epipelagic waters at night. The

main interspecific differences in the feeding patterns were due to differential voracity and vertical segregation among groups of species, with the gonostomatid *C. braueri* and the sternoptychid *A. hemigymnus* feeding intermittently throughout the day near the DSL, and myctophids feeding mostly during nighttime in the near-surface layers. As a result of its high abundance, *C. braueri* has an important role in the trophic dynamic of the mesopelagic community of WM. Mediterranean mesopelagic fishes constitute an assemblage with a broad feeding spectrum, shifting from the capture of varied prey taxa to the selection of particular prey organisms through ontogeny. These mixed strategies allow them to deal with greater environmental fluctuation and constitute an advantage for survival (Margalef, 1974). The observed high diet overlap between the studied fish species is contrary to the general assumption of the existence of strongly selective feeding strategies in oligotrophic regions, where competition pressure for the scarce food resources is expected (e.g. Van Noord *et al*. 2013). It might be due to the existence of "vacant niches" since the mesopelagic assemblage is not saturated (with a low number of species compared to other low or mid latitude assemblages). Still some degree of segregation was detected in the feeding patterns of the Mediterranean mesopelagic fishes, which could explain diet associations by a combination of several factors, including prey type, vertical use of the habitat (DSL *versus* surface), prey number, and ontogeny of predators. Therefore, we agree with Kozlov (1995) that the main differences observed in the diets of myctophids from various areas depend on regional and seasonal variability in zooplankton composition. Plasticity to adapt feeding strategy to external conditions contributes to the high numerical abundances of midwater fishes in the pelagic environment.

Appendix 4.1 Prey number (a), prey width (b) and trophic niche breadth (c) trends plotted against predator body length (mm SL) for mesopelagic fish species from this study. Species name abbreviations are given in Fig. 4.




Number of Prey Items
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2
3
4
12
17
22
27
Log Prey Width (µm)
Prey Width (µm)
y = 0.0222x + 2.2267
R² = 0.7773
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0
200
400
600
800
1000
1200
1400
1600
1800
SD Log Prey Width (µm)
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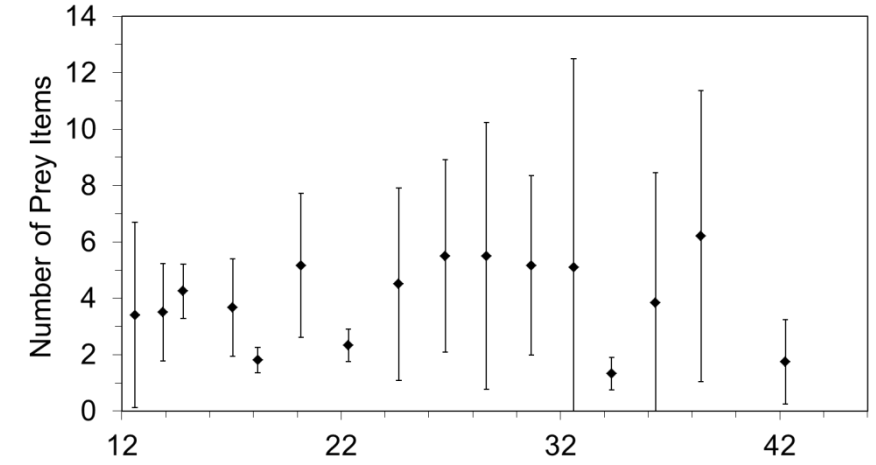
0.1
0.1
0.2
0.2
0.3
0.3
CB Standard Length (mm)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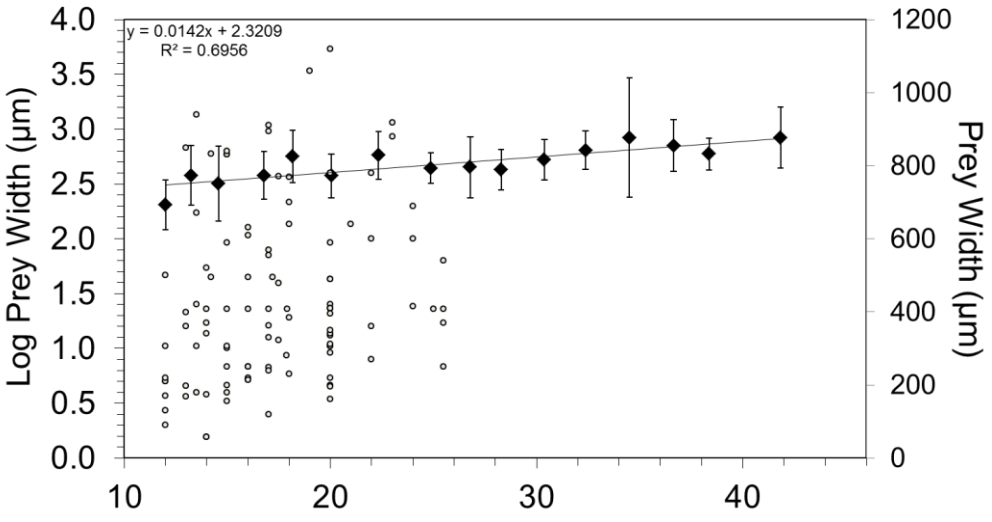
4
6
8
10
12
14
12
22
32
42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R² = 0.6956
Log Prey Width (µm)
Prey Width (µm)
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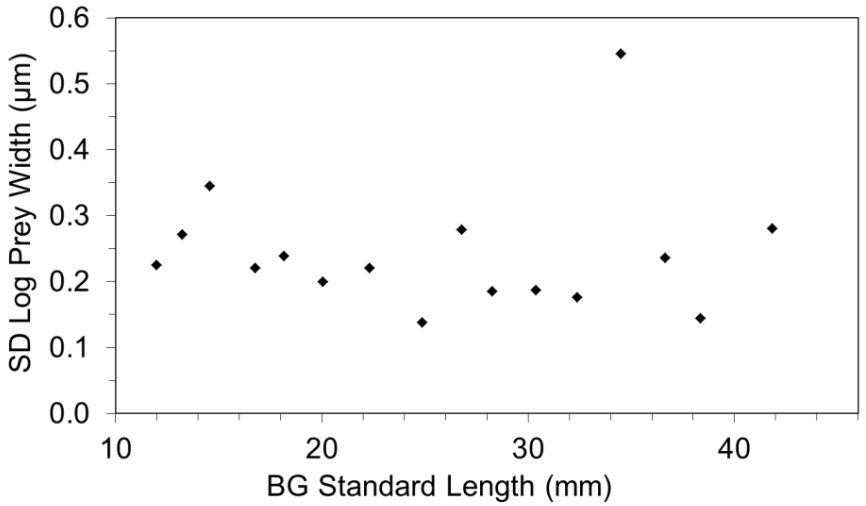
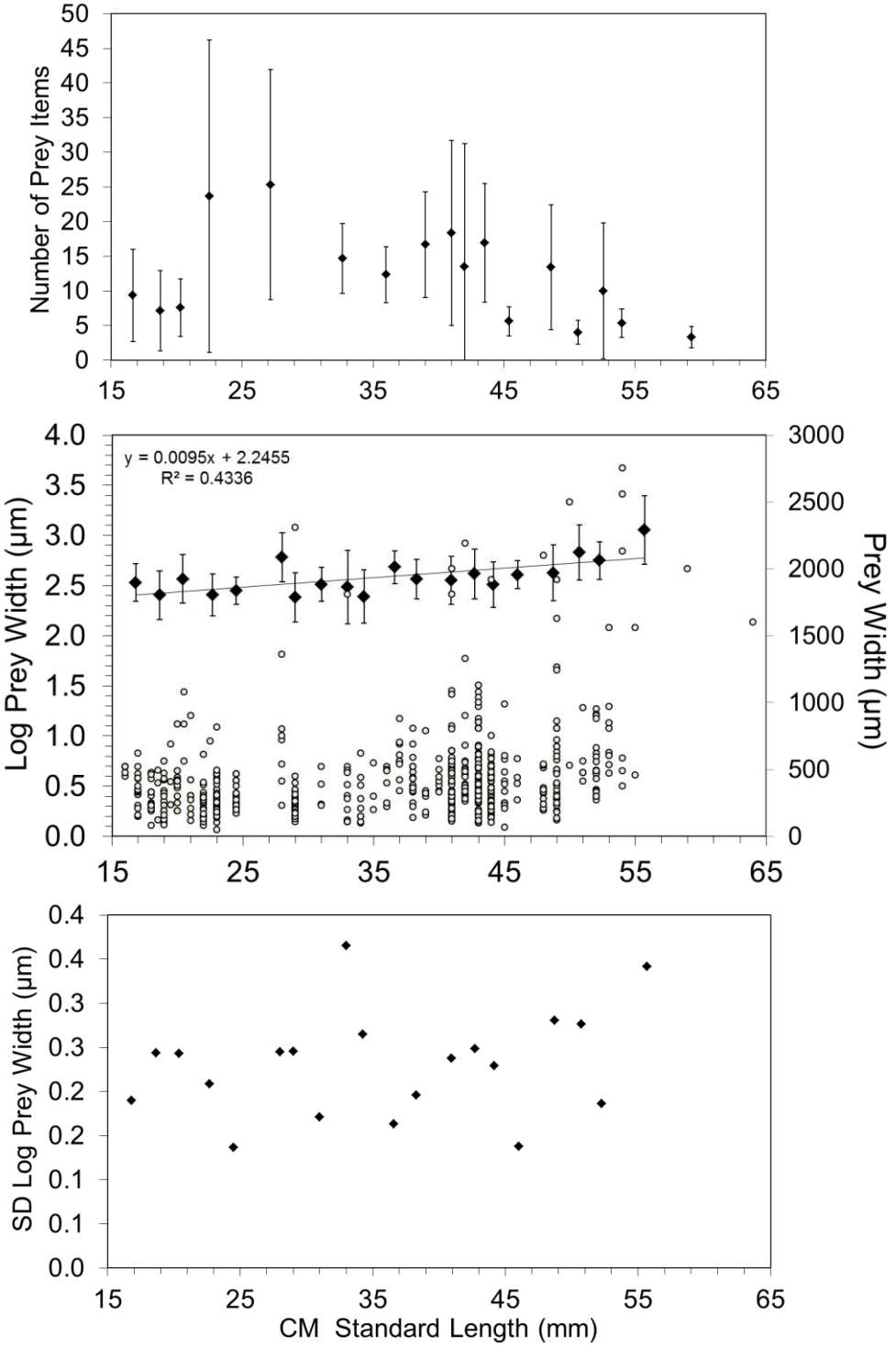
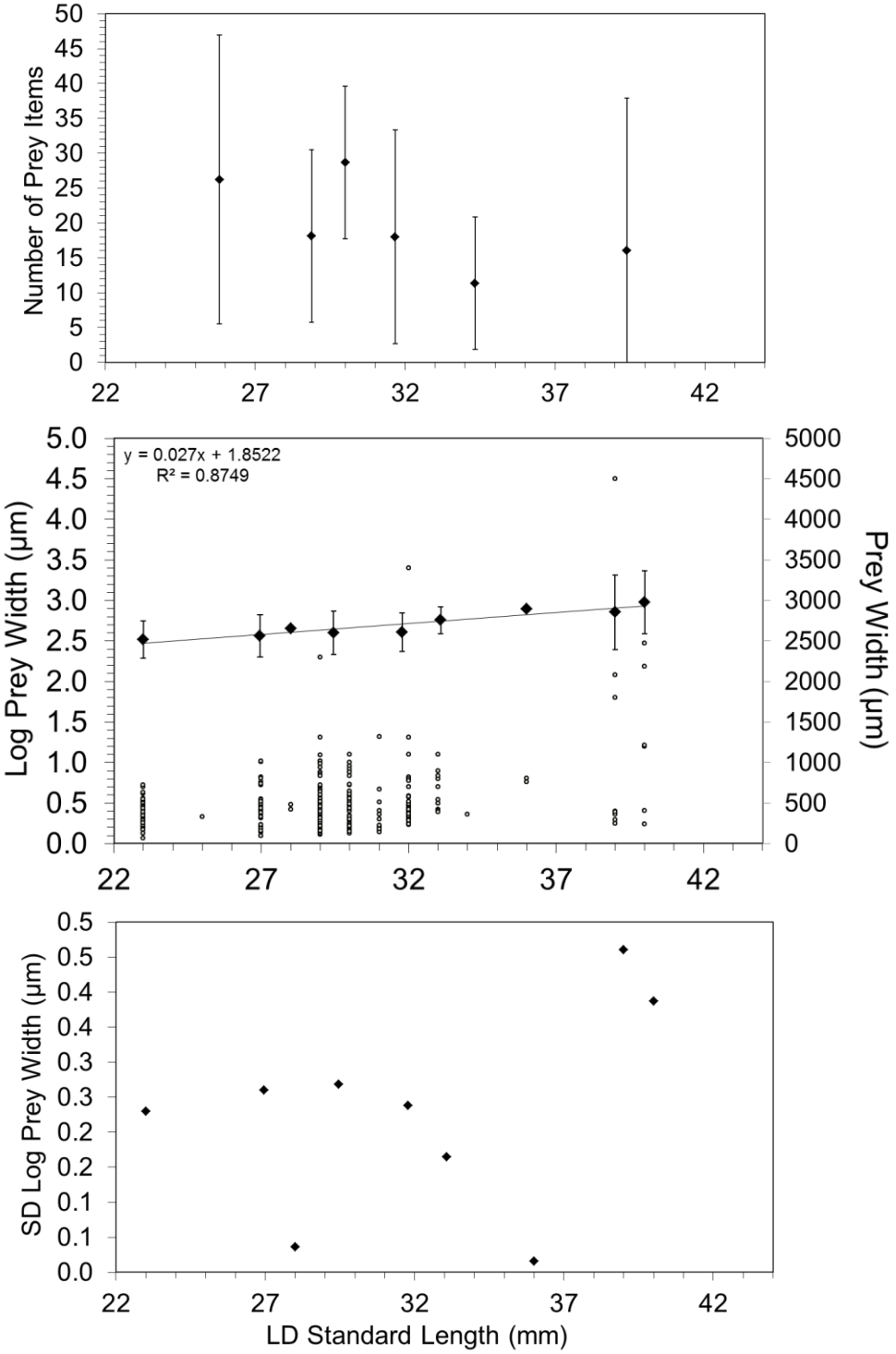
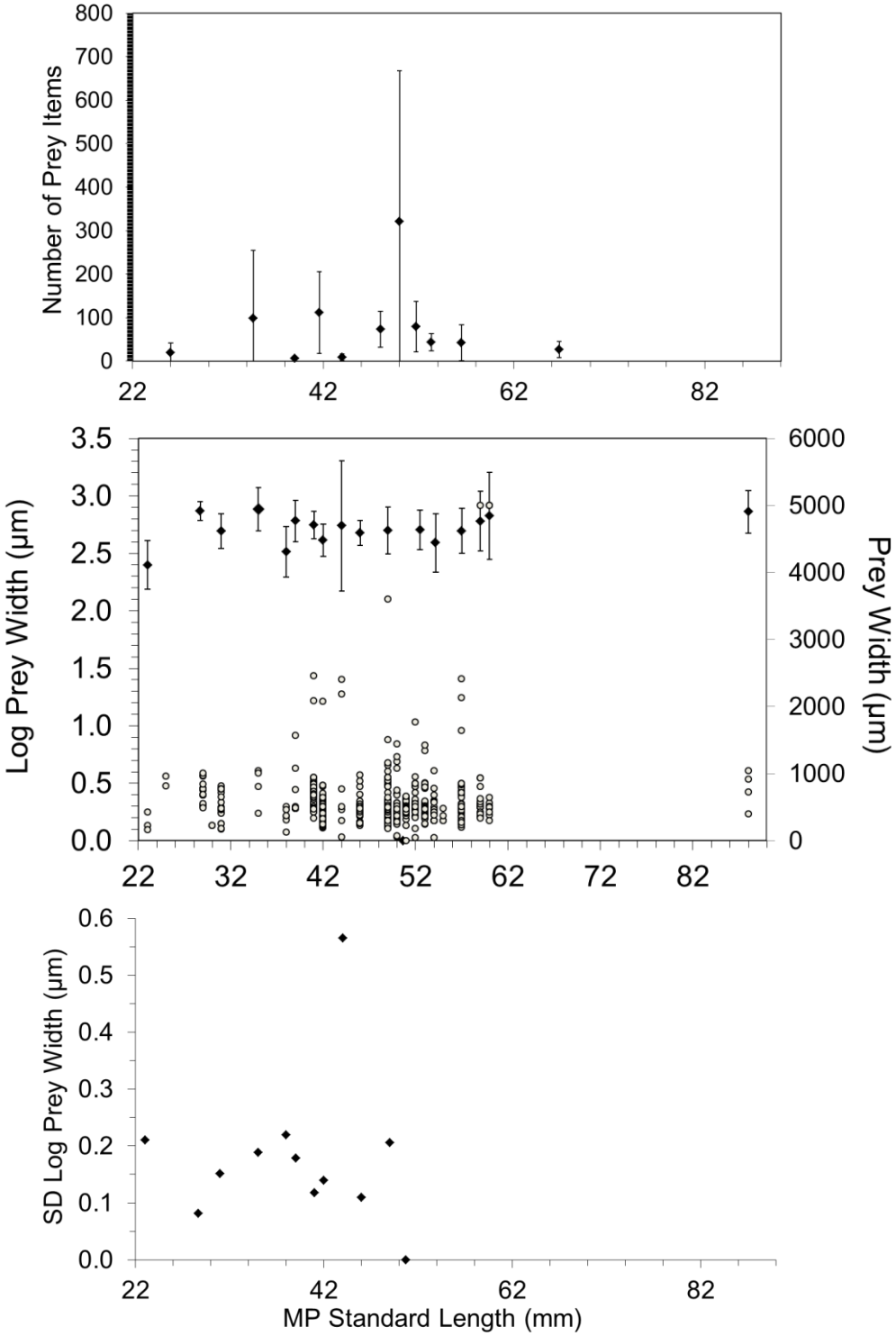
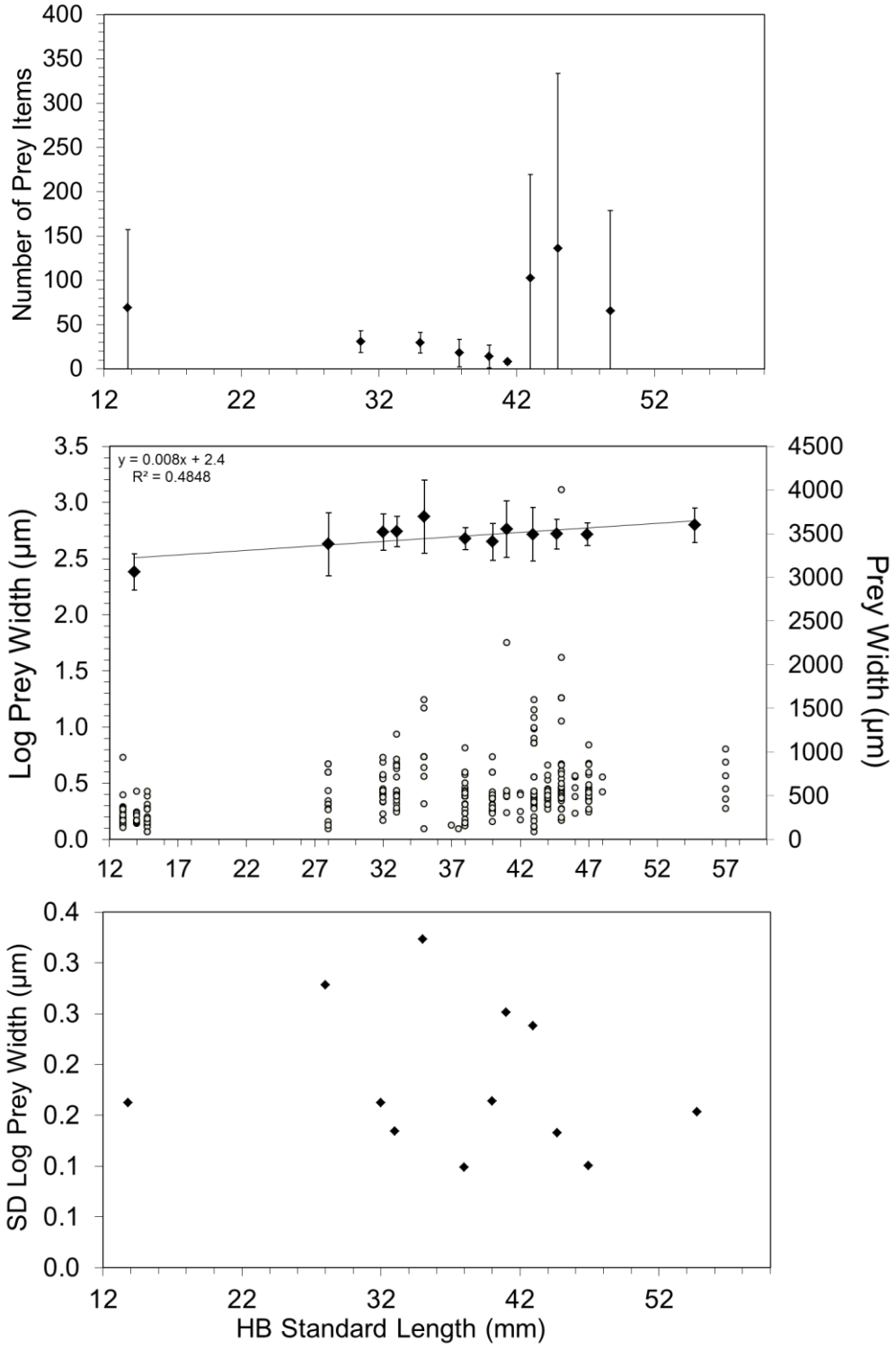
1.0
1.5
2.0
2.5
3.0
3.5
4.0
0
200
400
600
800
1000
1200
10
20
30
40

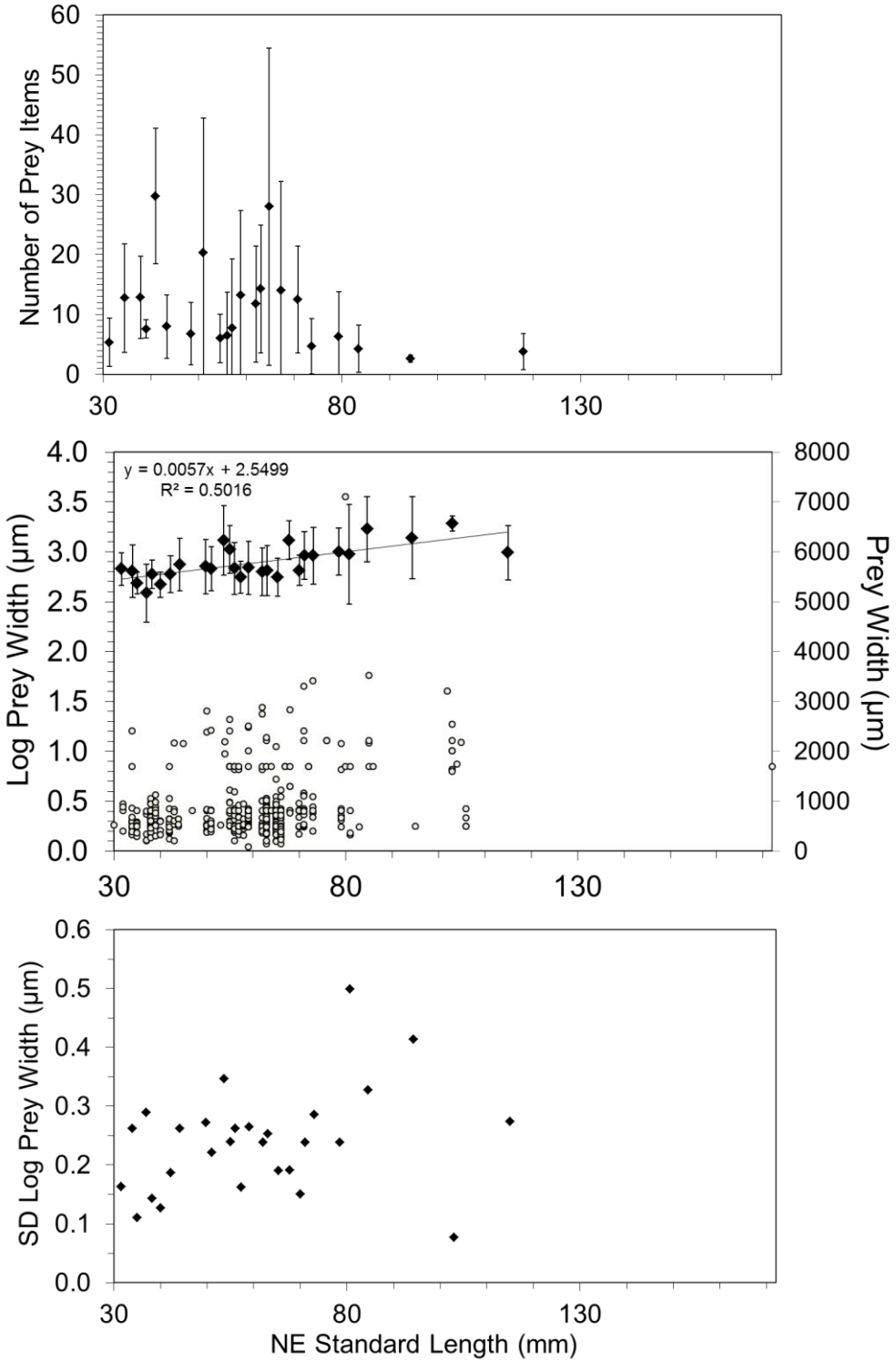

SD Log Prey Width (µm)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
10
20
30
40
BG Standard Length (mm)

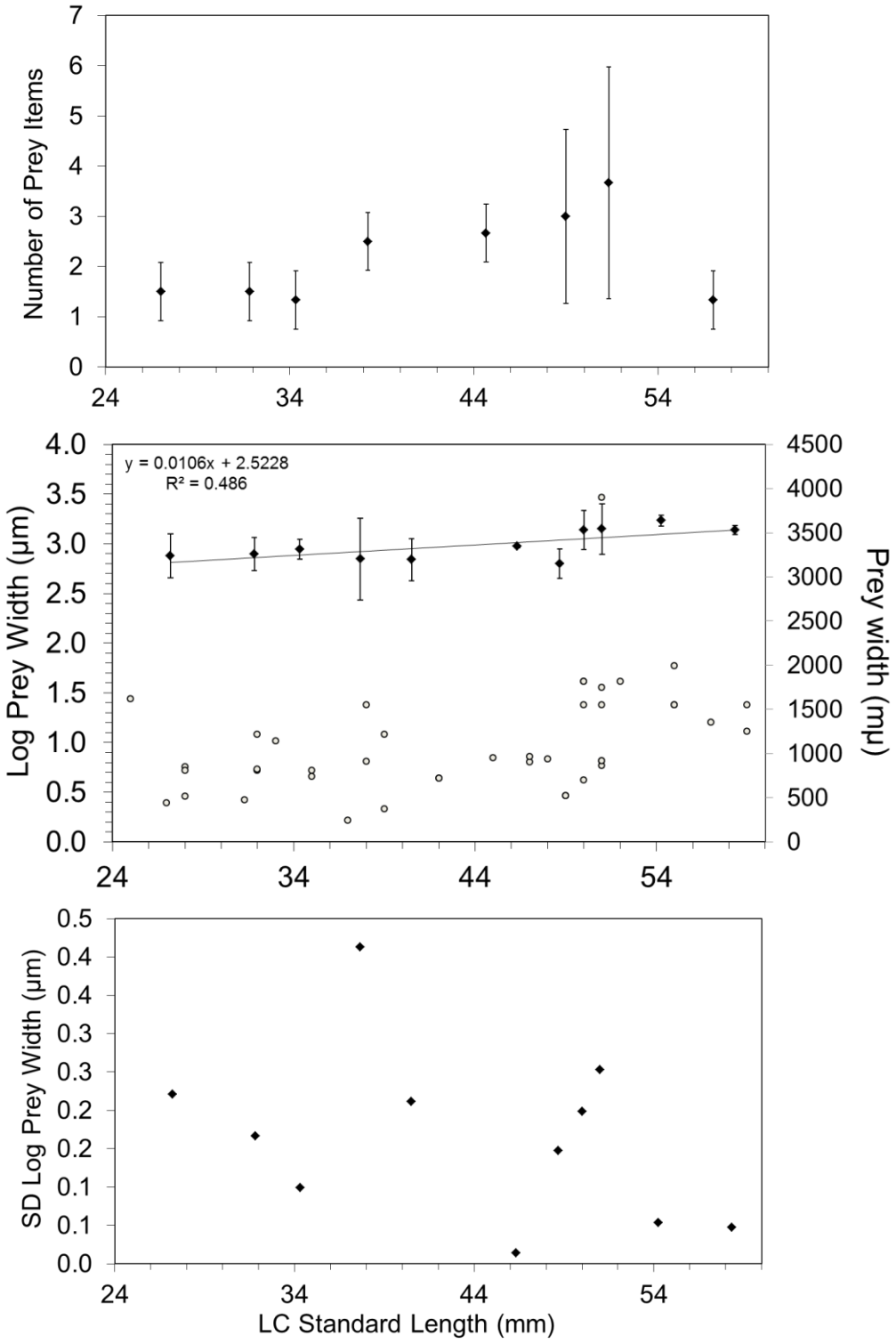

Number of Prey Items
y = 0.0095x + 2.2455
R² = 0.4336
Log Prey Width (µm)
Prey Width (µm)
SD Log Prey Width (µm)
CM Standard Length (mm)


Number of Prey Items
50
45
40
35
30
25
20
15
10
5
0
22
27
32
37
42
y = 0.027x + 1.8522
R² = 0.8749
Log Prey Width (µm)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Prey Width (µm)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
SD Log Prey Width (µm)
0.5
0.5
0.4
0.4
0.3
0.3
0.2
0.2
0.1
0.1
0.0
LD Standard Length (mm)


Number of Prey Items
800
700
600
500
400
300
200
100
0
22
42
62
82
Log Prey Width (µm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Prey Width (µm)
6000
5000
4000
3000
2000
1000
0
22
32
42
52
62
72
82
SD Log Prey Width (µm)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
22
42
62
82
MP Standard Length (mm)


Number of Prey Items
400
350
300
250
200
150
100
50
0
12
22
32
42
52
Log Prey Width (µm)
Prey Width (µm)
y = 0.008x + 2.4
R² = 0.4848
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
4500
4000
3500
3000
2500
2000
1500
1000
500
0
12
17
22
27
32
37
42
47
52
57
SD Log Prey Width (µm)
0.4
0.3
0.3
0.2
0.2
0.1
0.1
0.0
12
22
32
42
52
HB Standard Length (mm)


Number of Prey Items
60
50
40
30
20
10
0
30
80
130
Log Prey Width (µm)
Prey Width (µm)
y = 0.0057x + 2.5499
R² = 0.5016
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
8000
7000
6000
5000
4000
3000
2000
1000
0
SD Log Prey Width (µm)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
NE Standard Length (mm)


Number of Prey Items
0
1
2
3
4
5
6
7
24
34
44
54
y = 0.0106x + 2.5228
R² = 0.486
Log Prey Width (µm)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Prey width (mµ)
0
500
1000
1500
2000
2500
3000
3500
4000
4500
SD Log Prey Width (µm)
0.0
0.1
0.1
0.2
0.2
0.3
0.3
0.4
0.4
0.5
LC Standard Length (mm)

Appendix 4.2

Composition of diet as determined from gut analysis is summarized by numerical percentage (%N) and frequency of occurrence in feeding individuals (%F). All diet categories are mutually exclusive. Species name abbreviations are given in Fig. 4.2.

	AH		CB		BG		CM		LD		MP		HB		HH		NE		LC		LC_{bottom}	
Stomach contents	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**% N**	**% F**	**%N**	**%F**
Copepoda: Calanoida																						
Euaugaptilus sp.													0.04	1.96								
Haloptilus sp.	0.22	1.69																				
Heterorhabdus sp.									0.19	1.49	0.04	2.27	0.04	1.96								
Heterorhabdus abyssalis	0.22	1.69																				
Heterorhabdus papilliger	0.22	1.69																				
Lucicutia flavicornis	1.08	5.08	0.81	1.10	0.49	0.76	0.21	2.20	0.49	4.48	0.35	9.09	0.04	1.96			0.38	2.61				
Lucicutia longicornis									0.10	1.49												
Lucicutia (copepodite)											0.04	2.27										
Lucicutia spp.			0.81	1.10	0.74	2.27	0.52	5.49	0.29	4.48	0.14	6.82	0.17	3.92			0.28	2.61				
Metridia macrura			0.81	1.10							0.04	2.27										
Pleuromamma abdominalis	0.43	1.69	4.03	5.49	9.58	20.45	5.93	28.57	6.13	44.78	1.93	27.27	4.41	39.22	7.59	30.00	10.81	33.91	9.02	10.00		
Pleuromamma gracilis	1.94	10.17	8.06	9.89	8.11	16.67	2.19	16.48	18.09	49.25	6.21	52.27	55.19	43.14	53.01	65.00	17.86	42.61				
Pleuromamma spp.	2.80	10.17	12.90	16.48	6.39	14.39	2.81	23.08	8.17	55.22	2.25	47.73	2.98	33.33	0.94	15.00	13.25	40.00	0.75	1.11	1.85	2.22
Acartia danae							0.10	1.10														
Acartia sp.	0.22	1.69			0.49	1.52					0.04	2.27					0.09	0.87				
Candacia armata					1.97	2.27					0.11	4.55	0.09	3.92			0.85	6.09				
Candacia giesbretchi							0.10	1.10	0.19	2.99							0.28	2.61				
Candacia longimana					0.49	1.52							0.13	5.88								
Candacia tenuimana			0.81	1.10			0.10	1.10									0.09	0.87				
Candacia cf. *varicans*											0.04	2.27										
Candacia spp.	0.65	5.08	2.42	3.30	1.97	4.55	0.31	3.30	0.39	5.97	0.18	6.82	0.17	7.84			1.41	11.30	0.75	1.11		
Paracandacia simplex	0.22	1.69			0.74	2.27	0.31	2.20	0.88	10.45	0.28	13.64	1.04	21.57			1.13	7.83				
Centropages brachiatus																	0.09	0.87				
Centropages cf. *chierchiae*											0.04	2.27										
Centropages ponticus											0.11	6.82										
Centropages typicus					0.74	2.27	0.73	5.49	2.33	10.45	1.68	34.09	0.65	13.73	1.04	25.00	1.03	6.96				
Centropages cf. *typicus*											0.04	2.27										
Centropages *violaceus*	0.22	1.69									0.88	22.73	0.26	7.84								
Centropages spp.							0.10	1.10	0.39	4.48	0.53	15.91	0.09	1.96			0.28	2.61				
Isias claviceps									0.10	1.49	0.04	2.27	0.04	1.96								
Temora stylifera	0.22	1.69					1.25	10.99	0.39	5.97	17.62	36.36	0.61	14.53	0.62	15.00	0.09	0.87	0.75	1.11		
Temoropia mayumbaensis									0.10	1.49												
Aetideus armatus					0.49	1.52																
Aetidius giesbrechti					0.25	0.76											0.09	0.87				

	AH		CB		BG		CM		LD		MP		HB		HH		NE		LC		LC_{bottom}	
Stomach contents	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**% N**	**% F**	**%N**	**%F**
Aetidius sp.									0.19	2.99	0.04	2.27			0.10	5.00	0.09	0.87				
Aetididae (copepodite)									0.10	1.49												
Chiridius gracilis					0.25	0.76																
Chiridius poppei	0.22	1.69	0.81	1.10	1.97	4.55			0.10	1.49												
Chiridius sp.					1.72	2.27			0.10	1.49												
Euchirella messinensis			1.61	2.20			0.21	2.20	0.19	2.99					0.83	25.00			0.75	1.11		
Euchirella sp.									0.10	1.49												
Gaetanus minor			0.81	1.10																		
Gaetanus spp.					0.49	1.52											0.09	0.87	0.75	1.11		
Clausocalanus arcuicornis	1.51	5.08					0.94	9.89	2.34	17.91	0.60	15.91	0.91	21.57			0.28	2.61				
Clausocalanus furcatus	0.22	1.69					1.25	10.99	0.58	7.46	0.21	11.36	0.17	3.92								
Clausocalanus jobei					0.49	1.52	0.21	1.10	0.10	1.49	0.04	2.27										
Clausocalanus mastigophorus																	0.09	0.87				
Clausocalanus parapergens	2.16	8.47	0.81	1.10			0.10	1.10	0.39	5.97	0.25	9.09										
Clausocalanus paululus / parapergens	5.82	5.08					0.21	2.20	0.49	7.46	0.25	4.55	0.26	9.80			0.09	0.87				
Clausocalanus spp.	3.88	13.56	2.42	3.30	2.21	6.06	1.98	14.29	2.24	17.91	0.67	18.18	1.47	9.80			0.38	3.48				
Clausocalanus / Paracalanus s	0.22	1.69			0.49	1.52	0.42	4.40					0.13	5.88	0.10	5.00						
Pseudocalanus elongatus							0.21	2.20														
Clausocalanidae							0.10	1.10														
Euchaeta acuta	0.65	3.39	0.81	1.10	0.49	1.52	0.10	1.10	0.10	1.49	0.04	2.27	0.04	1.96	0.10	5.00	0.09	0.87				
Euchaeta marina	1.94	15.25	2.42	3.30	1.23	3.03	0.31	2.20	0.49	5.97	0.18	6.82	0.52	13.73	5.41	15.00	0.94	6.09				
Euchaeta marina (copepodite)	0.43	3.39					0.10	1.10	0.19	2.99	0.07	4.55	0.17	5.88			0.38	3.48	1.50	2.22		
Paraeuchaeta hebes			0.81	1.10	0.49	1.52													0.75	1.11		
Euchaeta spp.	0.22	1.69	0.81	1.10	1.23	3.79	0.10	1.10	0.88	13.43	0.14	9.09	0.09	1.96	0.10	5.00	0.19	1.74				
Amallotrix sp.					0.98	1.52																
Scaphocalanus sp.	0.22	1.69			0.25	0.76																
Spinocalanidae																			0.75	1.11		
Scottocalanus cf					0.25	0.76																
Rhincalanus sp.					0.25	0.76																
Subeucalanus cf.																	0.09	0.87				
Calanoides carinatus	0.22	1.69													0.83	5.00						
Calanus finmarchicus	0.22	1.69	0.81	1.10	0.25	0.76																
Calanus helgolandicus			0.81	1.10					0.10	1.49	0.07	2.27	0.09	3.92	0.10	5.00	0.09	0.87	0.75	1.11		
Calanus sp.	0.22	1.69			0.25	0.76			0.10	1.49							0.09	0.87				

	AH		CB		BG		CM		LD		MP		HB		HH		NE		LC		LC_{bottom}	
Stomach contents	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**% N**	**% F**	**%N**	**%F**
Mesocalanus tenuicornis					0.25	0.76	0.52	5.49			0.25	11.36	0.13	3.92	1.46	15.00	0.29	2.61				
Mesocalanus tenuicornis cf.															0.21	10.00	0.47	3.48				
Nannocalanus minor	1.94	11.86			0.25	0.76	1.56	13.19	3.70	32.84	12.36	63.64	5.83	54.90	11.75	80.00	4.42	13.91	1.50	2.22		
Neocalanus gracilis					0.25	0.76	0.10	1.10	0.10	1.49			0.04	1.96	0.31	10.00						
Neocalanus robustior							0.10	1.10														
Calanidae			0.81	1.10			0.10	1.10	0.78	5.97	0.14	9.09	0.22	9.80	0.83	15.00	0.28	2.61				
Calocalanus pavo							0.10	1.10	0.19	2.99	0.46	6.82	0.13	3.92			0.09	0.87				
Calocalanus styliremis							0.10	1.10	0.10	1.49	0.07	4.55										
Calocalanus tenuiculus							0.21	2.20														
Calocalanus spp.	1.51	5.08			0.25	0.76	1.66	14.29	1.27	13.43			0.43	7.84			0.19	1.74				
Paracalanus nanus	0.22	1.69			0.25	0.76	0.42	3.30	0.49	2.99												
Paracalanus parvus							0.52	5.49	0.10	1.49												
Paracalanus spp.	1.51	5.08	0.81	1.10	0.49	1.52	3.43	19.78	1.07	11.94	0.25	13.64	0.52	5.88			0.09	0.87				
Paracalanidae	0.22	1.69					0.83	7.69	1.07	8.96	0.07	2.27	8.17	3.92			0.19	1.74				
Nauplius Calanoida (nauplius)	0.22	1.69											0.26	7.84								
Calanoida (copepodite)					0.25	0.76																
Calanoida	5.60	23.73	10.48	14.29	4.18	9.85	4.99	39.56	4.28	35.82	0.81	27.27	1.12	25.49	0.31	15.00	1.60	13.04	3.76	5.56		
Copepoda: Non-Calanoida																						
Oithona atlantica									0.10	1.49												
Oithona brevicornis	0.22	1.69																				
Oithona plumifera							0.10	1.10	0.10	1.49												
Oithona setigera							0.10	1.10														
Oithona spp.	1.94	10.17			0.74	2.27	0.62	5.49	0.88	8.96			0.13	3.92			0.09	0.87				
Clytemnestra sp.							0.10	1.10														
Microsetella norvegica							0.10	1.10														
Microsetella rosea	1.29	8.47					0.21	2.20	0.10	1.49	0.04	2.27										
Euterpina acutifrons									0.10	1.49												
Agetus typicus	0.22	1.69											0.04	1.96								
Corycaeus cf. *clausi*													0.09	3.92								
Corycaeus crassiusculus									0.10	1.49												
Corycaeus spp.	0.43	3.39					1.14	10.99	0.19	2.99	0.11	6.82	0.13	1.96			0.47	3.48				
Corycaeidae	1.08	6.78					0.10	1.10	0.68	10.45			0.30	7.84	0.10	5.00	0.19	1.74				
Dithrychocorycaeus sp.									0.49	5.97			0.13	3.92								
Farranula carinata							0.10	1.10	0.19	2.99			0.13	1.96			0.09	0.87				
Farranula rostrata					0.49	1.52	0.83	7.69	2.33	14.93	0.25	13.64	1.21	13.73	0.31	5.00	0.09	0.87	0.75	1.11		

	AH		CB		BG		CM		LD		MP		HB		HH		NE		LC		LC_{bottom}	
Stomach contents	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**% N**	**% F**	**%N**	**%F**
Farranula spp.	0.65	3.39					0.21	2.20	0.97	7.46			0.35	11.76			0.19	1.74				
Oncaea conifera	1.51	10.17			1.47	4.55	1.14	6.59	0.97	11.94							0.28	2.61				
Oncaea cf *clevei*													0.04	1.96								
Oncaea curta	0.43	1.69																				
Oncaea englishi	0.43	3.39																				
Oncaea media	1.94	11.86	1.61	2.20	0.49	1.52	0.62	5.49	1.07	13.43	0.28	13.64	0.48	15.69	0.10	5.00	0.09	0.87				
Oncaea mediterranea	2.80	10.17	0.81	1.10	2.95	6.82	0.83	7.69	0.78	7.46			0.04	1.96	0.10	5.00	0.38	3.48				
Oncaea scottodicarlo	0.22	1.69							0.19	2.99	0.07	4.55	0.04	1.96								
Oncaea similis	0.22	1.69																				
Oncaea tenuimana	0.43	3.39																				
Oncaea venusta					1.23	2.27	0.62	6.59					0.09	1.96								
Oncaea spp.	15.52	45.76	1.61	2.20	2.95	9.09	1.66	14.29	1.36	16.42	0.21	11.36	0.61	15.69			0.47	3.48				
Triconia minuta	0.43	1.69					0.21	2.20														
Triconia dentipes	0.22	1.69																				
Oncaeidae									0.10	1.49							0.09	0.87				
Oncaeidae (copepodite)	0.22	1.69																				
Onychocorycaeus giesbrechti							0.10	1.10														
Onychocorycaeus latus							0.10	1.10					0.09	3.92								
Onychocorycaeus cf. *ovalis*													0.04	1.96								
Onychocorycaeus spp.					0.49	0.76							0.22	5.88								
Urocorycaeus furcifer	0.86	5.08							0.10	1.49												
Lubbokia squillimana							0.10	1.10	0.10	1.49			0.09	1.96								
Non-Calanoida	0.43	3.39																				
Copepoda	4.53	23.73	4.84	6.59	3.69	10.61	1.04	9.89	0.58	7.46	0.11	6.82	0.39	7.84							1.85	2.22
Copepod remains			2.42	2.20			0.31	3.30	0.19	2.99	0.07	4.55					1.03	6.09				
Copepodite					0.25	0.76	0.31	3.30			0.07	4.55										
Ostracoda								0.00														
Conchoecia curta					0.98	3.03	0.94	8.79	0.88	7.46	0.04	2.27										
Conchoecia hadonii / obtusata									0.10	1.49												
Conchoecia obtusata	2.37	11.86	4.03	5.49	1.47	3.03	2.91	21.98	5.06	29.85	0.28	13.64	0.30	5.88			0.75	5.22	1.50	2.22		
Conchoecia spp.	2.59	10.17	0.81	1.10	2.70	6.82	2.29	16.48	1.56	14.93	0.53	25.00	0.09	3.92	0.21	10.00	0.56	4.35				
Cypridina mediterranea		1.69																	1.50	2.22		
Philomedes globosa	0.43	1.69																				
Ostracoda	6.03	25.42	1.61	2.20	5.16	12.12	2.08	16.48	2.72	23.88			0.82	11.76	0.21	5.00	2.26	16.52	2.26	3.33		
Ostracoda remains			1.61	2.20																		

	AH		CB		BG		CM		LD		MP		HB		HH		NE		LC		LC_{bottom}	
Stomach contents	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**% N**	**% F**	**%N**	**%F**
Cladocera								0.00	0.10	1.49												
Evadne nordmanni											0.07	4.55										
Evadne spynifera							0.52	5.49					0.09	1.96								
Penilia avirostris							0.21	2.20														
Pseudoevadne tergestina													0.04	1.96								
Cladocera							0.10	1.10			0.07	4.55	0.04	1.96								
Amphipoda																						
Anchylomera blossevillei															0.10	5.00						
Anchylomera blossevillei cf.															0.10	5.00						
Primno macropa																					1.85	2.22
Phronima cf. *elongata*	0.22	1.69			0.25	0.76			0.10	1.49	0.04	2.27										
Phronimida											0.04	2.27					0.19	1.74				
Lysianassidae							0.10	1.10														
Lestrigonus schizogeneios							0.10	1.10			0.35	11.36	0.13	3.92	0.10	5.00						
Vibilia armata							0.31	3.30					0.04	1.96	0.42	15.00						
Hyperiidea							0.31	3.30			0.21	11.36										
Amphipoda	0.65	3.39			0.49	1.52	0.73	7.69			0.39	20.45	0.17	7.84	0.52	20.00	0.38	3.48	1.50	2.22		
Amphipoda remains	0.22	1.69											0.04	1.96								
Euphausiacea								0.00														
Euphausia krohnii (furcilia)											0.28	2.27	0.04	1.96	0.10	5.00	0.28	2.61				
Euphasia krohnii (juvenile)							0.21	1.10	0.39	2.99	0.04	2.27	0.09	3.92			0.66	3.48				
Euphausia krohnii													0.13	5.88	0.31	5.00	2.16	12.17	3.01	3.33		
Meganyctiphanes norvegica (juvenile)									0.10	1.49					0.10	5.00	0.09	0.87				
Meganyctyphanes norvegica					0.49	1.52	0.42	3.30	0.49	5.97	0.11	4.55	0.09	1.96	0.21	5.00	1.32	9.57			22.22	26.67
Nematoscelis megalops					0.25	0.76	0.10	1.10	0.49	5.97							1.50	8.70	3.01	3.33	3.70	4.44
Stylocheiron sp.					0.25	0.76											0.09	0.87				
Euphausiacea (metanauplius)	0.22	1.69																				
Euphausiacea (zoea)									0.10	1.49					0.10	5.00	0.09	0.87				
Euphausiacea (furcilia)	0.22	1.69	0.81	1.10	2.46	6.06	1.35	10.99	1.85	20.90	0.77	15.91	0.26	7.84	2.39	40.00	4.14	20.87				
Euphausiacea (juvenile)							0.21	2.20	0.39	5.97			0.17	3.92	1.98	10.00	4.79	14.78	0.75	1.11		
Euphausiacea	0.22	1.69			0.74	2.27	1.46	14.29	2.72	24.84	0.49	20.45	0.73	21.57	2.08	30.00	8.46	46.96	10.53	12.22	3.70	4.44
Mysidacea																						
Boreomysis arctica																	0.28	0.87			9.26	8.89
Lophogaster typicus																	0.09	0.87				
Mysidacea															0.10	5.00	0.09	0.87			1.85	2.22

	AH		CB		BG		CM		LD		MP		HB		HH		NE		LC		LC_{bottom}	
Stomach contents	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**% N**	**% F**	**%N**	**%F**
Decapoda																						
Crangonidae																					1.85	2.22
Gennadas elegans																					11.11	13.33
Pasiphaea multidentata																					1.85	2.22
Pasiphaea spp.																					1.85	2.22
Sergestes arcticus																					1.85	2.22
Sergestes corniculum																					3.70	4.44
Natantia																					5.56	6.67
Polycheles typhlops																					1.85	2.22
Eriphia verrucosa (metazoea)													0.04	1.96	0.10	5.00						
Necora puber (zoea)							0.21	2.20					0.43	3.92								
Brachyura (protozoea)															0.52	10.00						
Penaeida (protozoea)															0.21	5.00						
Metazoea															0.31	5.00						
Megalopa							0.21	2.20					0.17	5.88	0.21	10.00						
Decapoda																	0.47	4.35				
Nauplius	0.43	1.69			0.49	1.52			0.10	1.49												
Crustacean remains	0.86	5.08					0.83	8.79	0.19	2.99			0.09	3.92	0.10	5.00	0.18	1.74	1.50	2.22		
Faecal pellets	0.43	3.39							0.10	1.49												
Polychaeta (larva)	0.65	5.08			0.49	1.52	0.10	1.10	0.19	2.99	0.07	4.55	0.04	1.96			0.19	1.74				
Gastropoda																						
Atlanta sp.	0.22	1.69			0.49	1.52	0.52	4.40	0.29	4.48	0.77	18.18	0.65	11.76								
Limacina inflata							0.31	2.20			3.23	6.82			0.21	5.00						
Cavolinia inflexa							0.31	3.30			0.39	4.55										
Cavolinia tridentata													0.04	1.96								
Cavolinia sp.													0.09	3.92								
Clio pyramidata							0.10	1.10														
Creseis sp.							3.23	8.79	0.19	2.99	0.07	2.27										
Pteropoda							1.25	5.49			0.14	9.09	0.22	5.88	0.10	5.00						
Gastropoda							1.35	10.99	0.10	1.49												
Bivalvia							0.10	1.10														
Cephalopoda																					3.70	4.44
Chaetognatha																						
Sagitta spp	0.22	1.69							0.68	5.97												
Chaetognatha	1.29	10.17			0.98	3.03	0.73	7.69	1.95	26.87	0.60	34.09	0.91	41.18			3.38	21.74	0.75	1.11		

	AH		CB		BG		CM		LD		MP		HB		HH		NE		LC		LC_{bottom}	
Stomach contents	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**% N**	**% F**	**%N**	**%F**
Tunicata																						
Oikopleura spp.	2.59	11.86			0.74	2.27	10.82	25.27	2.04	14.93	31.87	20.45	0.04	1.96			0.28	1.74				
Appendicularia	1.29	8.47			0.98	3.03	6.97	20.88	1.17	8.96	5.58	31.82	0.43	3.92			2.16	5.22				
Salpa spp.											0.59	13.64					0.19	1.74				
Salpidae	0.22	1.69			0.49	1.52	4.47	12.09	0.19	2.99												
Siphonophora							0.10	1.10													1.85	2.22
Cnidaria							0.42	3.30														
Marine snow / discarded house											0.07	4.55										
Gelatinous remains	0.22	1.69					0.42	3.30			0.60	25.00	0.04	1.96								
Nematoda	0.22	1.69																				
Osteichthyes																						
Benthosema glaciale					0.25	0.76																
Lampanyctus crocodilus																					3.70	4.44
Notoscopelus bolini																					1.85	2.22
Myctophidae					0.98	3.03			0.39	5.97	0.21	4.55	0.22	7.84	0.21	10.00	0.28	2.61			1.85	2.22
Cyclothone braueri															0.10	5.00	0.09	0.87				
Stomiiforme											0.07	4.55					0.09	0.87				
Myctophiforme / Stomiiforme							0.21	2.20			0.32	13.64	0.26	9.80	0.31	5.00	0.09	0.87	3.76	5.56		
Mesopelagic fish					0.98	3.03	0.31	3.30	0.39	5.97			0.17	7.84	1.25	40.00	0.56	5.22	1.50	2.22	7.41	8.89
Fish larvae											0.07	2.27										
Scale	0.22	1.69			1.47	3.79	1.77	9.89	1.75	19.40	0.49	13.64	0.56	15.69			0.56	3.48	4.51	5.56		
Egg	0.43	3.39					0.31	3.30			0.11	6.82	0.13	3.92			0.09	0.87				
Pollen Pinaceae	0.22	1.69			0.98	3.03	0.31	2.20	0.10	1.49	0.07	4.55	0.04	1.96								
Insect wing													0.04	1.96								
Tintinnoinea					0.49	0.76																
Laackmanniella sp.							0.10	1.10														
Tintinnoinea							0.21	2.20														
Foraminifera								0.00	0.10	1.49											3.70	4.44
Globorotalia sp.							0.62	4.40														
Undetermined	1.29	10.17			0.98	3.03	0.52	3.30	0.49	7.46			0.22	7.84			0.09	0.87				
Undet. Organic matter / particulate matter	2.16	16.95	0.81	1.10	7.86	24.24	2.50	26.37	0.58	8.96	0.35	22.73	0.61	27.45	0.21	10.00	0.94	8.70	41.35	60.0		
Total food items	**464**		**124**		**407**		**962**		**1028**		**2849**		**2314**		**962**		**1064**		**133**		**54**	

Annex I

TROPHIC STRUCTURE BASED ON ISOTOPE ANALYSIS

This section presents the results of combined carbon ($^{13}\delta$C) and nitrogen ($^{15}\delta$N) stable isotope analyses (Table A.1) on the 18 most abundant western Mediterranean mesopelagic fishes and associated potential prey groups in order to examine niche partitioning within this group by contributing with complementary information to the stomach content analyses presented in **Chapter 4**. The determination of the trophic positions of migratory species becomes of special interest when it comes to describe the role of mesopelagic fish as vectors of matter transfer from the euphotic zone to demersal and benthopelagic species.

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

i. Methodology

Samples were dried at 60^{o}C and kept in a desiccator until preparation for the stable isotope analysis. Particulate organic matter (POM) was collected from year-round moored time-series sediment traps placed on the slope (800 m depth and 30 m above the ocean bottom). Only data taken a month before the summer and autumn surveys were considered to provide a better temporal matching with macrofauna. In large fish dorsal white muscle was extracted for isotope analysis. The whole body minus head and gut was processed for small fish samples. In case of zooplankton or small fish (*C. braueri* and *A. hemigymnus*) samples, several specimens were pooled to obtain the required sample volume.

Table A.1 Trophic level (TrL), δ13C'and δ15N (mean values ± s.d.) of mesopelagic fish and potential food resources sampled in 2009 and 2010. Size range (in mm) and number (n) of samples analyzed. B: fishes collected from bottom trawls. Data were expressed in δ notation as parts per thousand relative to global standard CO_2.

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

Analyses were performed by continuous flow isotope ratio mass spectrometry (CF-IRMS) using a Thermo Delta X-Plus mass spectrometer. The analytical precision based on the standard deviation of replicates of a standard reference was ≤0.25 ‰ for both $\delta^{13}C$ and $\delta^{15}N$.

ii. Analyses

As lipids are strongly ^{13}C depleted with respect proteins and carbohydrates (Sweeting *et al.*, 2006), differential lipid contents can bias the interpretation of $\delta^{13}C$ values. Lipids were not removed from zooplankton and fish samples to avoid loss of material and because lipid contents. Potential lipid bias was explored using the relationship between C:N ratios (i.e., lipid content) and $\delta^{13}C$. Migratory myctophids can have high lipid contents (Lea *et al.*, 2002; Hoffman and Sutton, 2010). Thus, $\delta^{13}C$ data was lipid normalized to avoid potential lipid bias according to Post *et al*. (2007) by applying the formula:

$$\delta^{13}C' = \delta^{13}C_{bulk} - 3.32 + 0.99 \times C{:}N_{bulk}$$

Where bulk values are those observed in the untreated samples, and $\delta^{13}C'$ is arithmetically corrected to lipid free $\delta^{13}C$.

Analyses of isotopic baselines were conducted to inform any spatio-temporal isotope correction and identify prey sources for later application to SIAR diet mixing models. Mixing trophodynamics were evaluated using the package 'SIAR' (Parnell and Jackson, 2013) for R v3.0.2 (R Development Core Team, 2013) by the calculation of community metrics according to Layman *et al*. (2007). Area of the smallest convex polygon containing all organism species is assumed as a measure of trophic diversity and the average Euclidean distance of each species to the $\delta^{13}C/\delta^{15}N$ centroid is taken as a measure of the degree of trophic diversity.

Trophic level (TrL) was calculated by the following 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 $\delta^{15}N$ of the species, $\delta^{15}N_{ref}$ 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. $\delta^{15}N_{ref}$ was estimated from plankton size-fraction between 50 and 200 μm (mean $\delta^{15}N$=3.96‰±0.40) because web baseline was mostly composed of early copepod stages - nauplii and copepodites - (Fernández de Puelles *et al.*, 2014) and other small zooplankton that are mostly filter feeders (λ=2). Mean isotopic discrimination factor of mesopelagic fishes was 3.15‰ (Sweeting *et al.*, 2007a; Sweeting *et al.*, 2007b).

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 PERMANOVA, followed by post-hoc pairwise tests. Significance was set at p=0.05 and *p*-values were obtained using 9999 permutations of the untransformed data.

Interspecific analysis focused on temporal and prey utilisation by mesopelagic fishes, using the Bayesian mixing model SIAR v4.1.3 (Stable Isotope Analysis in R) (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 mesopelagic fish species with contrasting TrLs (*C. braueri versus L. dofleini*), representing different species of the same genera (*H. benoiti vs. H. hygomii,* or *L. pusillus vs. L. crocodilus*), inhabiting different locations in the water column (mesopelagic *L. crocodilus vs. L. crocodilus* from the bottom), or which might compete for food resources (i.e. the most common and abundant migratory myctophids, *C. maderensis* and *N. elongatus*, *vs*. the most common and abundant non-migratory stomiiforms, *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 from own observations. Models included 4 prey groups: *i)* mesozooplankton, *ii)* adult euphausiids (Williams *et al.*, 2001; Sutton, 2005; Pakhomov *et al.*, 2006; Champalbert *et al.*, 2008; Bernal *et al.*, 2013 Bernal, own observations), *iii)* other small mesopelagic fish (Roe and Badcock, 1984; Podrazhanskaya, 1993; Sutton, 2005), and *iv)* POM; particulate matter identified as marine snow or detritus (Palma, 1990; Miller *et al.*, 2012; Bernal, own observations). Due to seasonal differences in the potential prey organisms, mixing models were run separately for late autumn and summer. These four groups cover all major diet sources, except for gelatinous plankton.

No trophic enrichment factors exists specifically for mesopelagic fishes (Galvan *et al.*, 2012) and therefore date derived from the literature (and standard deviations). Sensitivity of analysis was assessed by running five mixing models with trophic enrichment factors previously used in other studies dealing with fish muscle (Pinnegar and Polunin, 1999; Vander Zanden and Rasmussen, 2001; Trueman *et al.*, 2005; Sweeting *et al.*, 2007a; Sweeting *et al.*, 2007b; Caut *et al.*, 2009) (Table A.2).

To investigate variability in $\delta^{13}C$ associated with varying lipid content, C:N and non-lipid normalized $\delta^{13}C$ data were explored. Analysis was conducted for the most frequently sampled fish species *L. crocodilus, C. maderensis* and *N. elongatus*, whose sampling included 61%, 30% and 47% of body length range respectively and encompassed all the size range reported for these species in the water column in the western Mediterranean (Goodyear *et al.*, 1972b; Olivar *et al.*, 2012).

Table A.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 preys: POM: particulate organic matter; Zoopl: mesozooplankton; Euf: *Meganyctiphanes norvegica* adults; Fish: fishes (see text). B: bottom. Values ≥0.30 are in bold.

iii. Results

The mesozooplankton and macrozooplankton fractions along with the adult stages of euphausiids exhibited lower $\delta^{13}C$ in summer (mean $\delta^{13}C$= –20.69±0.40) than in late autumn (mean $\delta^{13}C$ = –19.45±0.42) ($F_{1.45}$=55.59, p<0.001), but there was no influence of locations, bathymetric strata or size fraction. $\delta^{15}N$ was independent of season, location or bathymetric strata, but differed among plankton size fractions ($F_{2.45}$=66.25, p<0.001); $\delta^{15}N$ values were higher in progressively larger organisms (mesozooplankton < macrozooplankton < adult euphausiids).

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 TrLs (assuming trophic discrimination of nitrogen of 3.15‰) (Fig. A.1). $\delta^{13}C$ reflected basal patterns being lower in summer than late autumn (F=35.498 p<0.001). $\delta^{15}N$ and TrL were similar between seasons, locations and strata (all paired t-test p>0.05), therefore data were pooled to obtain a mean TrL value per species.

TrLs of the mesopelagic fish fell between 2.9 for *C. braueri* to 4.0 for *L. dofleini* (Fig. A.2) and were significantly different (ANOVA, $F_{18,132}$=7.972 p<0.001), with differences between species of lower levels (*C. braueri* and *A. hemigymnus*) and those of the upper levels *L. dofleini*, *N. elongatus*, bottom dwelling stages of *L. crocodilus*, and *Diaphus holti*).

SIAR mixing model

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

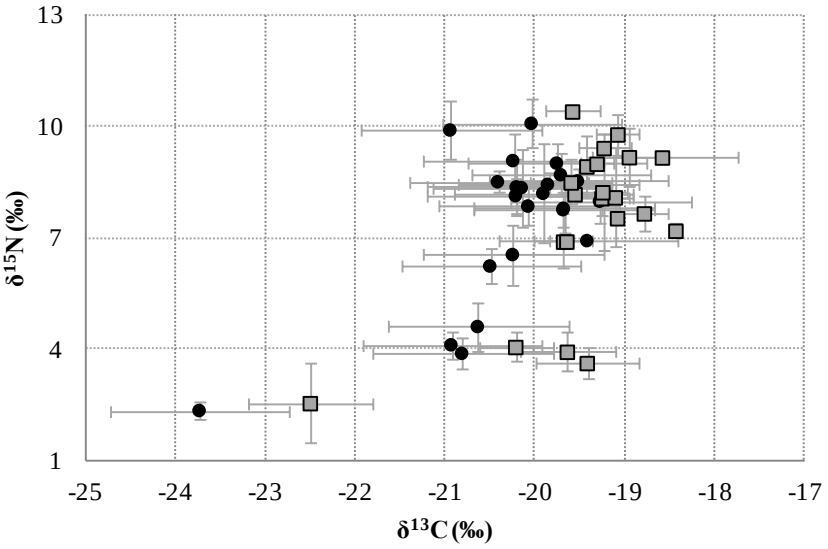


Fig. A.1 Scatterplot of the δ15N and δ13C values (mean±s.d.) of POM, zooplankton (micro-, meso-, and macrozooplankton and adults of *M. norvegica*) and mesopelagic fishes from the Balearic Sea collected during July 2010 (black circles) and December 2009 (grey squares) surveys.

deviations) when using Sweeting *et al*. (2007a; 2007b) and Vander Zanden and Rasmussen (2001), except for the large benthic *L. crocodilus*, for which Caut *et al*. (2009) appeared most appropriate because of larger trophic carbon discrimination (Fig. A.3).

Considering that prey items are categorized by major functional groups, the use of mixing models determines fish predator species as showing preference for *i)* mesozooplankton/POM; *ii)* mesozooplankton/euphausiids, and *iii)* euphausiids and fish. The lowest δ^{15}N was observed in *C. braueri* for which SIAR derived a diet based heavily on mesozooplankton and POM. On the opposite side, *L. dofleini*, with the highest TrL estimates, showed substantial contributions of larger prey, particularly fishes and euphausiids. In the middle of the trophic continuum, the two *Hygophum* species showed fairly similar contributions of the several potential prey, although *H. benoiti* had slightly higher contribution of

mesozooplankton and *H. hygomii* higher contribution of fish. *L. pusillus* diet included all dietary endpoints except POM.

SIAR provided low resolution of diet resource estimations for some fish species (*A. hemigymnus*, *N. elongatus* and *C. maderensis*) attending to the functional prey groups considered. Late autumn individuals of *A. hemigymnus* seem to rely less on POM than those collected in summer, although seasonal differences were slightly perceptible in this fish species. High seasonal variation was evident in *N. elongatus*, which showed a relatively higher fish contribution in late autumn and zooplankton' in summer. *C. maderensis* and *N. elongatus* also exhibited diet overlap in summer against diet segregation in autumn, when *C. maderensis* seem to depend on smaller prey types than *N. elongatus*.

The *L. crocodilus* from pelagic hauls showed clear preference for mesozooplankton, with a small contribution of euphausiids and fish. In contrast, bottom-dwelling and non-migratory individuals of *L. crocodilus* exhibited high piscivory in summer and late autumn and minor mesozooplankton contributions.

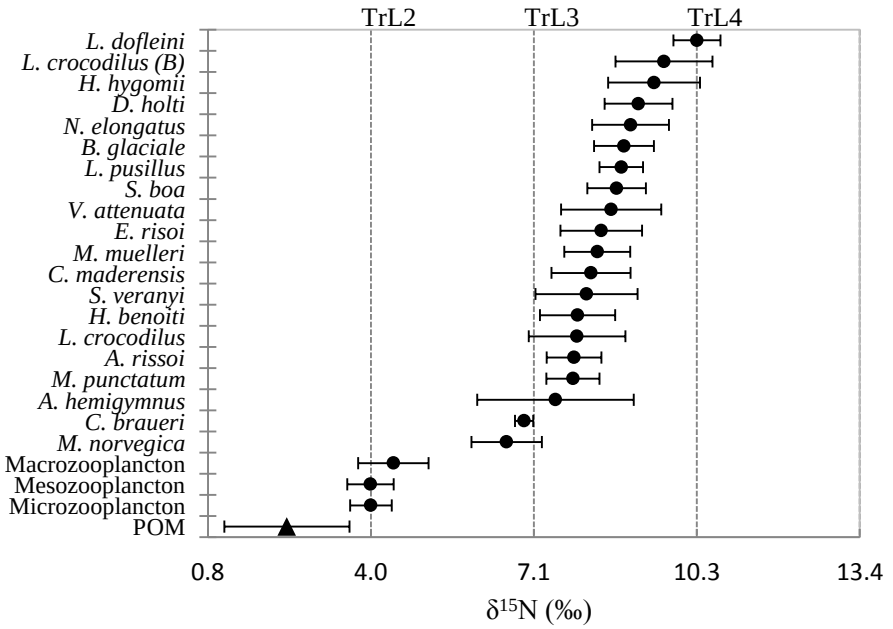


Fig. A.2 δ15N (mean ± s.d.) values and estimated trophic level (TrL) of mesopelagic fishes and potential prey organisms.

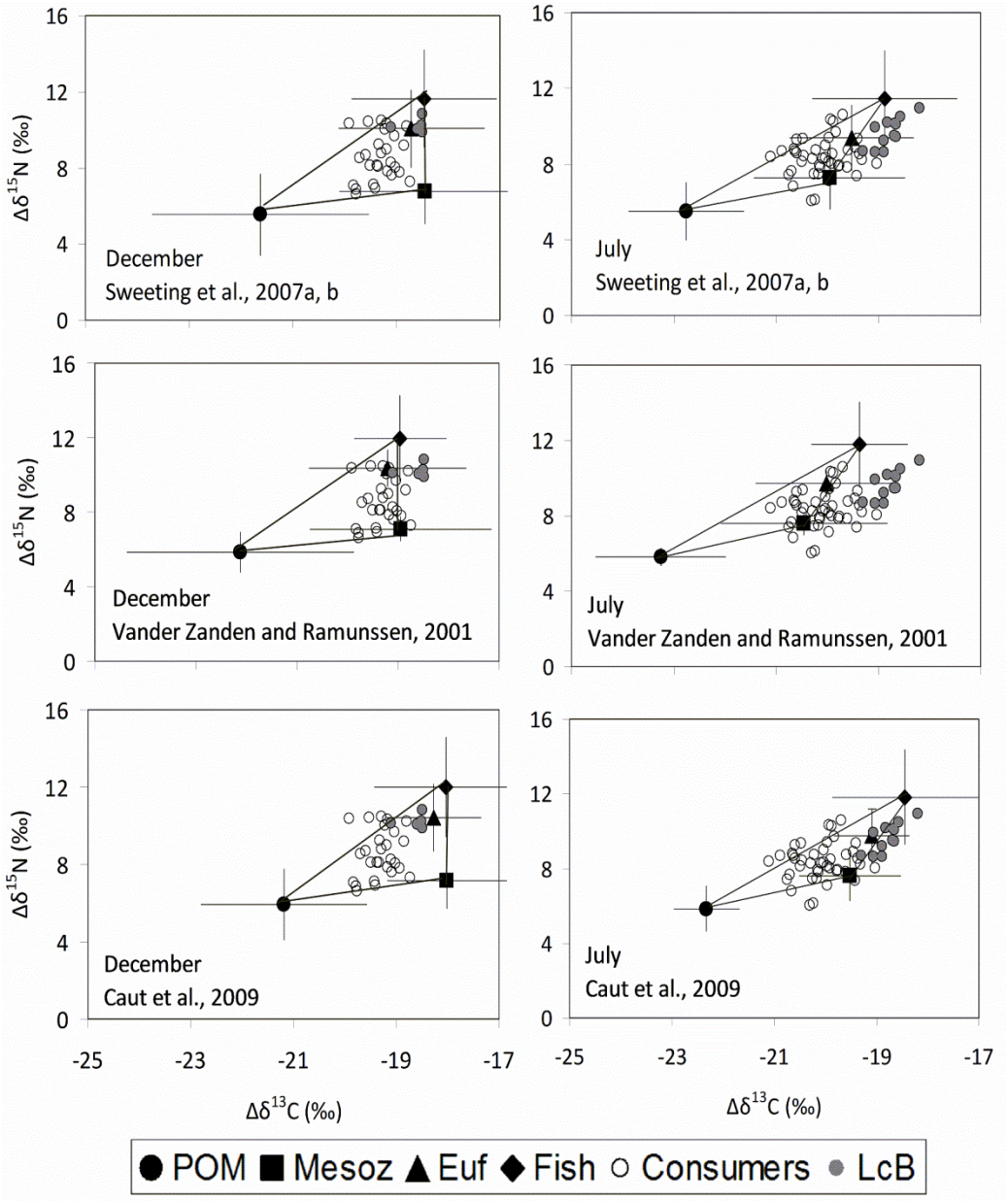


Fig. A.3 Stable isotope ratios of mesopelagic fishes (circles) and feasible diet contribution of potential prey organisms (black symbols) according to SIAR. Bars denote standard deviations. Points in grey correspond to *Lampanyctus crocodilus* specimens from bottom trawls.

Three species had body size ranges and samples sizes that were amenable to exploration of size influences on intra-population trophodynamics (Fig. A.4).

L. crocodilus δ^{15}N and δ^{13}C both exhibited strong positive relationship with SL (R=0.863 and R=0.923 respectively; 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 the three species, however it was only significant for *L. crocodilus* and *C. maderensis*, (R=0.892, p<0.001 and R=0.798, p<0.05, respectively). C:N (lipid content) decreased with increasing size for *L. crocodilus*, whilst *C. maderensis* showed the opposite trend.

iv. Discussion

The analysis revealed seasonal differences in δ^{13}C of plankton and mesopelagic fishes, but little variation over the spatial scale sampled. The observed seasonality in δ^{13}C would be consistent with a higher fraction of diatoms mixing period in late autumn (Fry and Wainright, 1991; Miller *et al.*, 2008) than during the stratification in summer, when phytoplankton is dominated by dinoflagellates (Estrada *et al.*, 1999).

By contrast, δ^{15}N variation was minimal at spatial and temporal scales. δ^{15}N was low in mesozooplankton and sinking POM and consistent with previous values reported in the Mediterranean (Koppelmann *et al.*, 2009; Fanelli *et al.*, 2011) and oceanic waters from other oligotrophic regions such as the NW Atlantic (Fry and Quinones, 1994). Large-sized zooplankton such as euphausiids, (particularly *M. norvegica*) has higher δ^{15}N values. Mean isotopic value of mesozooplankton coincide with those of copepods historically collected from the same region (Fanelli *et al.*, 2009), reinforcing the conclusion that copepods were the main constituents of the mesozooplankton (>70%) in summer and late autumn across series of years (Fernández de Puelles *et al.*, 2014).

According to Layman *et al*. (2007), the measurement of trophic diversity for the whole community showed higher values for the autumn than summer (Valls *et al.*, 2014).

Trophic structure of mesopelagic fishes

The analysis suggests tight trophic interactions between mesozooplankton, euphausiids and mesopelagic fishes constituting an important links between primary production and the nektonic community (Yoon *et al.*, 2007; Miller *et al.*, 2010; Letessier *et al.*, 2012). The analysis included 5 stomiiforms, 1 aulopiform and 12 myctophiforms (Table A.1), which are the most abundant and frequent mesopelagic fish species in the Mediterranean Sea (Goodyear *et al.*, 1972b; Olivar *et al.*, 2012) and their distributions coincided with the main scattering layers detected by echosounders (Peña *et al.*, 2014).

TrL estimations of Myctophidae here are similar to other reported elsewhere, and determine myctophids as secondary and tertiary consumers of the pelagic food web (Cherel *et al.*, 2010; Choy *et al.*, 2012; Flynn and Kloser, 2012). Previous TrL estimations for Stomiiforms include large dragonfishes (*Chauliodus sloani*, *Stomias boa* and *Idiacanthus* spp.) which were estimated at 3-3.4 (Choy *et al.*, 2012). Here, the lowest TrL 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 an intermediate TrL of 3.5, similarly to the TrL estimates by Choy *et al*. (2012), although it is interesting to remark the smaller size of Mediterranean specimens -80-125 mm SL in the Mediterranean *vs.* 126-168 in the Mid-Atlantic Ridge (Choy *et al.*, 2012).

According to the presented results, the mesopelagic fishes in the Mediterranean show a trophic continuum of δ15N that can be related to behavioural patterns of

in some species. *C. braueri* and *A. hemigymnus* had the lowest δ^{15}N/TrLs, reflecting lower energetic requirements. The fact that these two stomiiforms are non-migratory species along to the more stable environment that they occupy at intermediate depths (mainly at the 400-600 m DSL) (Badcock and Merret, 1976; Andersen and Sardou, 1992; Ross *et al.*, 2010; Olivar *et al.*, 2012), implies a low energy-demanding behaviour. In contrast, the upper TrLs were represented by migratory myctophids such as *L. dofleini* or *H. hygomii*, which experience a changeable environment.

The relatively high δ^{13}C and δ^{15}N of the bottom-dwelling *L. crocodilus* indicate that they have an isotopic niche closer to other benthic organisms (Valls *et al.*, 2014) than to the younger migratory stages of the own species. Comparisons of δ^{15}N among a non-migratory and two migratory myctophids in the western Pacific showed lower values in those ones that feed in the upper water column than in those feeding in deeper layers (Sugisaki and Tsuda, 1995). Conversely, for the gonostomatid, near-bottom specimens collected in a previous study (Fanelli *et al.*, 2009) showed isotope signatures similar to those observed in the *C. braueri* individualsfrom the DSL of this study, pointing out to an analogous dependence on pelagic prey. Interpretation of dietary differences however, requires some caution due to potential confounding effects of depth on dietary isotope basal signatures.

Trophic patterns and niche segregation

The use of stable isotope mixing Bayesian models to ascertain diet is subjected to a number of weaknesses, such as the number and type of food sources included in the analysis and those associated to trophic discrimination factors (TDFs) (Galvan *et al.*, 2012). TDFs are not available for any of the species studied here or their close relatives. This study used Vander Zanden and Rasmussen (2001), Sweeting *et al*. (2007a; 2007b) and Caut *et al*., (2009)

values. Previous studies 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 juveniles of sardine and anchovy in the northwestern Mediterranean (Costalago *et al.*, 2012). These TDFs are similar to those of aquatic organisms reviewed elsewhere (Vander Zanden and Rasmussen, 2001; Post, 2002; Sweeting *et al.*, 2007a; Sweeting *et al.*, 2007b; Caut *et al.*, 2009) and have extensively been applied for the pelagic marine environment (Bode *et al.*, 2007; Miller *et al.*, 2010; Olson *et al.*, 2010).

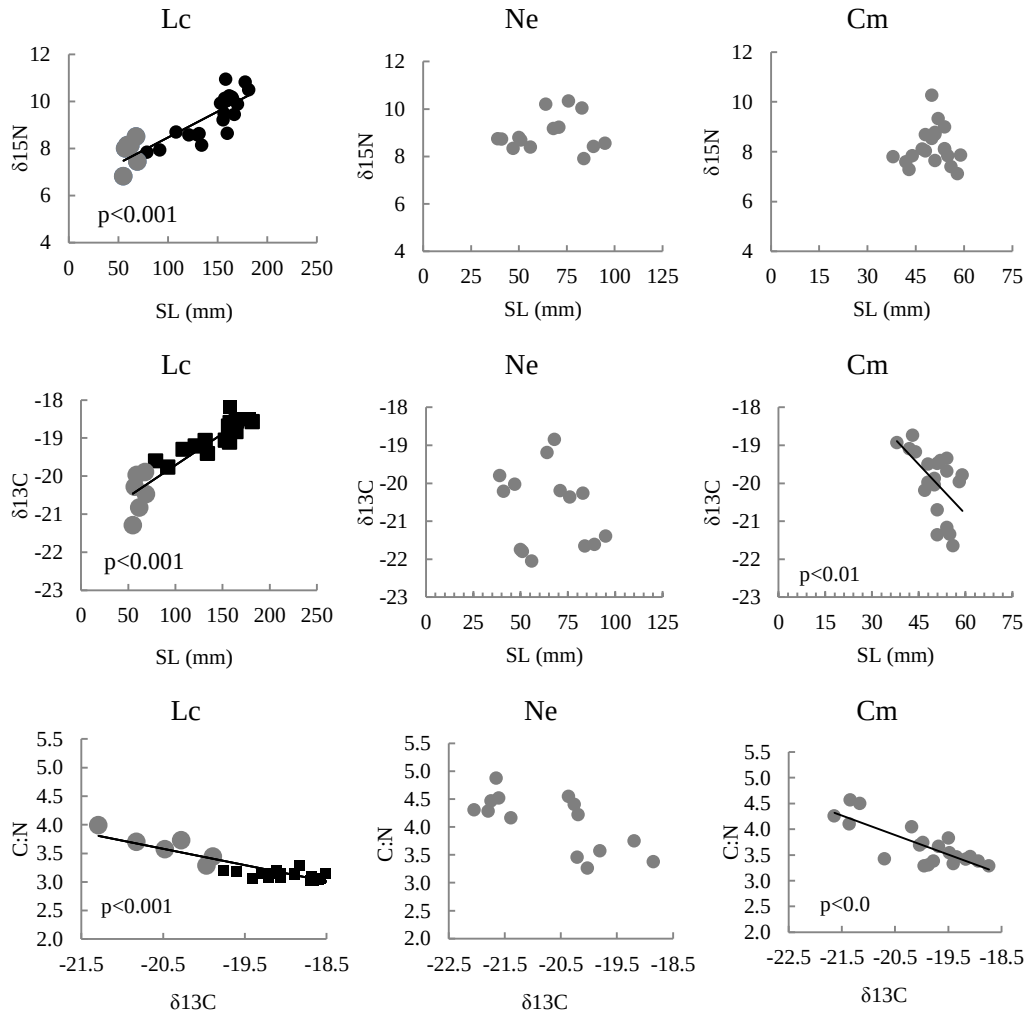


Fig. A.4 Relationship between body size (SL, mm) and δ15N (‰), body size and δ13C (‰), and C:N ratio and δ13C (‰) of *L. crocodilus* (Lc), *N. elongatus* (Ne) and *C. maderensis* (Cm). Black squares represent non-migratory stages of *L. crocodilus*.

Therefore, the applied TDFs appear suitable for the mesopelagic species of this study since maximize the number of individuals occurring in the "prey polygon". Additionally, dietary endpoints appearing within the polygon for one of the cruises provided estimations consistent with pre-existing stomach contents of *C. braueri* (Palma, 1990), *L. pusillus* (Bernal *et al.*, 2013), *L. crocodilus* from bottom trawls (Stefanescu and Cartes, 1992) and some reported in Chapter 4. SIAR 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), although the reliability of the estimated contributions of each dietary endpoint is subject to great uncertainty. The accuracy of these types of models is strongly dependent on the available information from stomach content analysis. It would be interesting for further investigations to separately analyse some taxonomic groups within the mesoozooplankton such as copepods.

Table A.2 and Fig. A.3 show how most of the studied mesopelagic fishes had feasible prey combinations that encompassed several trophic levels by consuming a mixture of mesozooplankton, euphausiids and, to a lesser extent, potential POM or small fish. POM was particularly important for *C. braueri*, whilst small mesopelagic fishes seem to be representative in *L. dofleini* and the bottom-dwelling *L. crocodilus*. The most common and abundant myctophid in the water column, *C. maderensis*, seems to have a diet mainly based on zooplankton, therefore sharing most prey types with many 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 (Hulley, 1990; Pakhomov *et al.*, 1996a; Gaskett *et al.*, 2001; Pusch *et al.*, 2004; Petursdottir *et al.*, 2008; Shreeve *et al.*, 2009; Bernal *et al.*, 2013). 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 matter from all types of marine organisms (e.g. bacteria, phytoplankton, zooplankton) (Miller *et al.*, 2012).

Niche segregation was observed between congeners, e.g. for *H. benoiti* and *H. hygomii*, which basing on SIAR models, differed in fish/mesozooplankton utilisation. Similarly, for migratory stages of *Lampanyctus* spp., *L. crocodilus* diet was more dependent on mesozooplankton and POM than *L. pusillus* that had higher contributions of euphausiids and mesozooplankton. Stomach content analysis for *L. pusillus* (Chapter 3) support this interpretation by showing that copepods and euphausiids were the main prey items of adults stages (Bernal et al., 2013).

Size-based feeding

Dietary shifts are commonly observed within the benthic and coastal systems (Galvan *et al.*, 2010). Three fish species could be assessed to determine TrL shifts with growth, although only *L. crocodilus* exhibited a pronounced positive increase of the TrL with size. However, the vertical distribution of *L. crocodilus* is associated to size cohorts, thus it is important to warn that ontogenetic charges of TrL might be confounded by basal changes in the isotope signature with depth. Assuming as minimal the potential confounding influences of depth, isotope analysis suggests that the small individuals of *L. crocodilus* are more strongly dependent on the pelagic environment than the larger bottom-dwelling individuals, the latter with higher δ^{13}C. Additionally, tendency towards higher δ^{15}N with increasing body size also indicates that larger and non-migratory specimens of *L. crocodilus* are dependent upon prey with higher trophic levels, such as fish or others. Concurrent stomach content analysis in adults from the

bottom trawls indicated that, after decapods, euphausiids and fishes were their main prey items (Chapter 4). Such patterns are also consistent with previous hypothesis on migratory behaviour of this species (Olivar *et al.*, 2012).

On the other hand, it is likely that size trends in $\delta^{13}C$ are a function of size specific variation in lipid content. Small migrating individuals of *L. crocodilus* exhibited higher lipid content (C:N ratios) in muscle tissue than non-migrating larger individuals, which is probably derived from their energy requirements and the different type of lipids stored. C:N of the largest individuals was low (3.1), which is indicative of absence or very limited lipid content in the tissue (Sweeting *et al.*, 2006).

No size trends were observed with $\delta^{13}C$ and $\delta^{15}N$ in *N. elongatus* despite high $\delta^{13}C$ variability, suggesting wider inter-individual contribution to total niche width (Bearhop *et al.*, 2004) than in the two other species. *C. maderensis* exhibited negative correlations of $\delta^{13}C$ with bogy size and $\delta^{13}C$ with C:N owing to the lower energy storage in the smallest individuals. Differences in general life strategies were thus observed between these vertical migratory species with overlapping distributions.

Chapter 5

GENERAL DISCUSSION

The general discussion of this thesis follows the same order as the previous chapters. This thesis aimed to provide integrated information on important ecological traits of mesopelagic fishes from the western Mediterranean (WM) that either were poorly studied or were analysed by first time; particularly those aspects concerning species identification, species diversity, vertical distribution in the pelagic environment and trophic ecology. Target species were the most common and abundant Mediterranean mesopelagic fishes that belong to the orders Myctophiformes and Stomiiformes.

Two main parts compose the axis of this work: *i)* Chapter 1 corroborated by DNA barcoding the identity of mesopelagic larvae, which was previously based only on morphological characters. It was also emphasized the importance of using genetic procedures in studies that attempt to determine species diversity in a region, where certain pelagic fishes are usually difficult to ascribe to a sole species. *ii)* The following chapters were focused on ecological patterns; on one hand, discerning the vertical distribution of most mesopelagic fish species throughout the water column and, on the other hand, discerning their diet, feeding patterns and trophic position.

At the end of this general discussion, the trophic results based on numerical abundance and prey composition are contrasted with carbon weight estimations of stomach contents. Carbon weight was obtained through conversion of the prey body width and length using regression formulae based in prey dimensions from the literature listed in Table 5.2. The importance of potential prey resources was assessed by this alternative method. Results from both prey composition and carbon content were then discussed.

5.1 Fish identification

DNA Barcoding proved species identity of larval stages of mesopelagic fishes, since their identity had never been confirmed by genetic techniques (Chapter 1).

Larval descriptions of Myctophiformes and Stomiiformes species have been mostly based on morphological and pigmentation patterns (e.g. Moser and Ahlstrom, 1974). Application of the Forensically Informative Nucleotide Sequencing (FINS) methodology on DNA segments >565 bp of the cytochrome oxidase subunit I (COI) for most mesopelagic species, or alternatively, segments of 375 bp of the 12S rRNA mitochondrial DNA for *Cyclothone* spp., allowed the successful identification of larvae of most Mediterranean mesopelagic species.

Larvae of midwater fishes do not resemble adult specimens. Some of the earliest stages of myctophids and gonostomatids are very similar morphologically and, consequently, difficult to discern at species level, especially between congeners. Moreover, larval specimens are fragile and pigmentation is usually lost during sampling resulting in often misidentification or unrecognition of those specimens. In this study, DNA barcoding constituted a valuable tool to quickly identify cryptic species. Applied to an extensive geographical region with high species diversity and rapid speciation events, the morphological identification of species in combination with DNA barcoding appears as an essential method to detect how much biodiversity holds a particular region, since it has been found that cryptic lineages in the open ocean split in absence of geographical barriers (Miya and Nishida, 1997). It means that speciation events might be happening more often than expected and generate recent species with slight morphological dissimilitude. On the contrary, the Mediterranean holds low mesopelagic fish richness. The fishes studied here have one or two species per genus, which turns this region suitable for easier larval identification by genetic methods. According to this, larval stages of the congeners *L. pusillus* and *L. crocodilus*, *H. benoiti* and *H. hygomii* and the cryptic species *N. elongatus* and *N. bolini*, were clearly discriminated with DNA barcoding using a primer combination described by Ward *et al.* (2005) that was able of unambiguously identifying a vast majority of fish species.

DNA barcoding would be also useful for studies related to the distribution and biomass of specific larvae, in which fish larval species are usually mistaken by others with a similar appearance. The main trouble of applying DNA barcoding is that, whereas the visual identification of the larval specimens is time-consuming, genetic procedures are costly and cannot be applied to routine identifications. The confirmation of morphological identification by genetic procedures is crucial to guarantee that the morphological features used in the classification of species are valid.

Phylogenetic relationships of myctophiforms and stomiiforms still require additional sequencing of mitochondrial or nuclear *loci* to be fully resolved. Despite the phylogeny of Mediterranean myctophiforms and stomiiforms was beyond the objective of the present study, it was attempted to provide an insight to the resolution of 'minor groups' (genera, tribes) under the discrimination threshold of one genetic marker (COI). Poulsen *et al*. (2013), using mitogenomic sequences and gene order rearrangement, provided valuable results that confirmed the classical classification based on osteological and photophore features of some 'major groups' of myctophids. The results presented in Chapter 1 recognized two wide clusters corresponding to the accepted monophyletic orders Myctophiformes and Stomiiformes (e.g. Paxton, 1972; Fink, 1984; Poulsen *et al.*, 2013) by using Maximum likelihood and Bayesian inference. The Bayesian tree was also able to recover the families Myctophidae, Phosichthyidae and Sternoptychidae, the subfamily Lampanyctinae, and even some tribes accepted in the current phylogeny. Poulsen *et al.* (2013) resolved most tribes, but found low supportive values for the subfamilies Lampanyctinae and Myctophinae, in line with the present study. Moreover, the authors (*op*. *cit*., 2013) omitted many myctophid species (the order Myctophiformes includes ca. 250 spp.) of cosmopolitan or broad distribution, some of them reported in the present thesis (e.g. *Hygophum hygomii*, *H. benoiti*, *Lampanyctus pusillus*).

5.2 Ecological traits of mesopelagic fishes in the western Mediterranean

The oligotrophic condition of the Mediterranean Sea determines that the inhabitant fauna experiences particular biological phenomena. One of these events consists in the smaller body size ("dwarfism") of animals with respect organisms of colder, more productive and larger oceans. The particular characteristics of dwarfism in fishes involve, apart from a smaller body size, faster growth and earlier sexual maturity (Sonin *et al.*, 2007). Por (1989) proposed that dwarfism in the oligotrophic Mediterranean responded to a special expression of *r*-strategy, which is in line with several traits of many species of myctophids and stomiiforms elsewhere that have a short life span and early maturity – with smaller size at maturity than in productive regions (Hulley, 1984).

Physical conditions, orography and seasonality address to complex processes in the western Mediterranean such as the convergence of huge water masses (described in the Introduction section). For this thesis, samples were collected during two seasonal periods, summer and autumn, with contrasting environmental and hydrodynamic conditions, and over the shelf and slope of Mallorca Island. However, further detail about the physical state of the region is not discussed here, as negligible effects upon the vertical distribution and feeding dynamics of myctophids and stomiiforms were detected. For none of the target species seasonality exerted high influence in determining vertical distributions or leaded to differential interspecific feeding regimes, at least, referring to their diet composition. The strong thermocline set during the summer does not restrict the displacements of juvenile and adult stages to the upper depth levels (Chapter 2). Light intensity is the unique physical agent that appears to schedule myctophid migratory activity in the western Mediterranean. The effect of light intensity in the diel vertical migrations of adult myctophids is

broadly accepted (Clarke, 1970; Blaxter, 1974; Young, 1983; Benoit-Bird and Au, 2004; Benoit-Bird *et al.*, 2009), although vertical distribution of mesopelagic larvae is more related to the thermocline (Woodhead and Woodhead, 1955; Blaxter, 1973; Sabatés *et al.*, 2003; Sabatés, 2004; Olivar *et al.*, 2014). Moreover, the bathymetric profile might be incorrectly attributed as a proxy in determining the structure of the vertical distribution of fishes instead of ascribing to ontogenetic responses the vertical structure of fish populations. This trend was clearly observed throughout a wide range of body sizes in *L. crocodilus* and reported in previous studies (Stefanescu and Cartes, 1992; Olivar *et al.* 2012) and this study (Chapter 2).

Vertical distributions of adult myctophids are tightly related to migratory routines and imply specific physiological traits. Body condition in WM midwater fishes showed a strong correlation with their migratory dynamics. All myctophids (Chapter 1b) presented faster increase in weight than in length, whilst the gonostomatid *C. braueri* exhibited the opposite trend. Lipid content and relatively stronger body construction in myctophids than gonostomatids reflected a more active lifestyle for diel migrant myctophids. On the other hand, *C. braueri* remained in a lethargic state near the DSL during the whole day. A comparative study of the life histories of meso- and bathypelagic species (Childress *et al.*, 1980) indicated that the epipelagic sardines and bathypelagic fish species have higher rates of growth than myctophids and other mesopelagic migrants, which alternatively give priority to energy storage with high lipid levels, for using in their migratory activity, at the expense of mass growth.

5.2.1 Abundance and vertical distribution of mesopelagic fishes

The vertical distribution of the most abundant and frequent mesopelagic fish was studied (Chapter 2) by means of a combination of different fishing gears and acoustic methods that use multifrequency echosounders. Acoustic

techniques allowed detecting fish aggregations. They may be eventually applied to assess abundance of fish species at discrete horizontal layers by following the acoustic backscatter from these fishes and sorting out the species from catches concurrently performed. The study of vertical distribution patterns was important to understand the migratory activity of myctophids, since revealed the presence of active and non-migratory individuals in the Mediterranean as well as the occurrence of mixed aggregations of fish species at precise depth levels.

Mesopelagic fishes represent a diverse group of vertebrates, with ca. 250 species of myctophiforms (Catul *et al.,* 2010) and ca. 400 species of stomiiforms (Eschmeyer, 1998) reported worldwide. In the western Mediterranean, these two diverse orders account with few species, comparing to the large Atlantic and Pacific oceans, of which this study reported 14 myctophiforms and 8 stomiiforms, representing the most abundant species captured in the WM. Low diversity of deep-sea fishes is a particular phenomenon in the Mediterranean related to its geological history and semi-enclosed condition that restrict the genetic fluxes, leaving consequently unsaturated niches.

Mesopelagic fishes are the most numerous and abundant fish group in all world's oceans (Nelson, 2006), even when their abundances are commonly subestimated as a consequence of net avoidance (Kaartvedt *et al.*, 2012; Davison *et al.*, 2013). More than 99% of fish appearing in the midwater trawl captures performed in this study during late autumn and summer belonged to meso- and bathypelagic species, and the only noticeably contribution of other fishes correspond to a few pelagic species such as *Trachurus* spp. Estimations of mesopelagic fish abundances should be considered attending to the specific developmental stage as they can have different vertical distributions in relation with development. Many mesopelagic fishes from this study showed that migratory behaviours, shifts in ecological niches and feeding patterns responded to ontogenetic factors. For the most accurate sampling of all the developmental

stages, the combination of different fishing nets is mandatory. To obtain coverage of the largest possible age-cohorts, several fishing gears were used in this study, considering the variability of the efficiency of each of them in catching different fish sizes. The most abundant myctophid species collected in this study coincided, in terms of relative abundance, with those captured with small nets in previous reports (Tåning, 1918; Jespersen and Tåning, 1926; Goodyear *et al.*, 1972a; Andersen and Sardou, 1992; Alemany, 1997; Alemany *et al.*, 2006). The use of large pelagic trawls revealed novel information on the most abundant myctophid species, showing higher captures of large species (*C. maderensis*, *N. elongatus*, *L. crocodilus* and *H. benoiti*) than the biomass numbers provided by small nets. The echosounders at 18 and 38 kHz showed the strongest scattering mainly assigned to myctophids and bristlemouths, respectively (Peña *et al.*, 2014). Acoustic techniques also reflected the night-time ascension of juvenile and adult myctophids to the upper layers, demonstrating that Mediterranean myctophids perform DVMs in line with the common pattern observed in myctophid species from all oceans (Gartner *et al.*, 2008; Sutton *et al.*, 2008). The explanation of this migratory behaviour seems to respond to a trophic reason, since this is the time lapse in which zooplankton is more concentrated in the surface. Fish occurrences associated with sound scattering layers were usually deeper at daytime. Moreover, the occurrence of the oldest myctophid individuals with increasing depth was evidenced by bottom-trawls in the present and other several studies (Barham, 1963; 1966). An unbalanced size frequency distribution of the species at different depths points out that younger individuals are more active migrants than the oldest ones. The occurrence of old and non-migratory stages near the seabed has been commonly reported for some myctophid species (Stefanescu and Cartes, 1992; Kaartvedt *et al.*, 2009; Staby *et al.*, 2011). This study found large adults of *N. elongatus*, *L. crocodilus* and *B. glaciale* near the bottom at night, indicating that they are non-migrants or do not have regular migratory activity. The migratory pattern was

clearly distinct between myctophids (mostly migrants) and the stomiiforms *A. hemigymnus* and *Cyclothone* (weak or non-migratory activity).

Differential distributions between day- and night-time are also reflected on the density of fish concentrations. Myctophids were mainly concentrated in the surface at night to forage, but found less aggregated at the DSL or disperse at deeper layers during daytime. Daylight concentrations of myctophids for 400 DSL were never as high as those obtained for the night near-surface samples. Large dispersion of individuals has been generally observed in myctophids from different regions (Pearcy, 1983; Auster *et al.*, 1992). It is probably that by remaining less aggregated and at deeper and darker waters, mesopelagic fishes are less susceptible to predation during the light hours (Auster *et al.*, 1992).

Migratory fishes are more or less active swimmers in the night ascension to the surface, and seem to have a kind of "saltatory" swimming in the upwards way during which they may search intermittently for prey. For this study, the fullest stomachs with high incidence of fresh prey were those corresponding to the catches performed in the upper layers at night. This observation links feeding behaviour with the DVMs of myctophids and it is reinforced by the finding of a high incidence of empty stomachs during the day.

No inverse diel vertical migration (IDVM) was observed in any mesopelagic fish species from the Mediterranean, despite some authors recently detected this pattern in *B. glaciale* from cold waters (Kaartvedt *et al.*, 2009; Dypvik *et al.*, 2011). Although a few individuals of this species with digested remains were found near the bottom at daytime, the most part of the species population followed the general nocturnal ascension.

5.2.2 Developmental shifts

Ontogeny appears as a main vector in modelling midwater-fish behaviour and determines onset of the migratory routines, spatial distribution, diet composition

and prey selectivity. To determine the ontogenetic shift experienced by midwater fishes in feeding habits, the myctophid *Lampanyctus pusillus* was selected for this purpose (Chapter 3), because information on the biology of this common and widely distributed species in the Mediterranean was very scant. Similarly to what has been reported in other regions, myctophid larval stages in the WM occur in epipelagic layers (e.g. Sabatés, 2004; Sassa *et al.*, 2004; Sassa and Kawaguchi, 2006; Olivar *et al.*, 2014). The larvae of *L. pusillus* (present study) are restricted to the first 200 m of the water column, whereas juvenile and adults spread from the bottom to the upper layers depending on sun light. Therefore, *L. pusillus* larvae are adapted to feed in the epipelagic zone during daylight hours, whilst feeding activity in adults takes place at night in the layers where potential prey concentrates, i.e., the near-surface layer and DSL. Some late postflexion stages fed nocturnally, suggesting that *L. pusillus* must shift its diurnal activity by this time. In other myctophids, nocturnal feeding begins after transformation (Sassa, 2010), which is associated with changes in the visual system (Evan and Browman 2004 in Sassa 2010). A differential use of food resources in myctophids coupled to spatial and temporal shifts from larval stages to adulthood, functions as a mechanism to avoid intraspecific competition (Werner and Gilliam, 1984) as well as implicates the most advantageous use of the environment according to the requirements and biological traits of each developmental stage. The peaks of feeding activity in *L. pusillus* larvae occurred from the

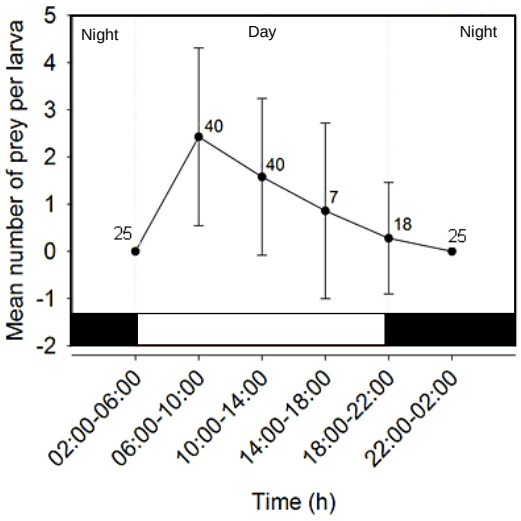


Fig. 5.1 Mean number of prey per larval gut as a function of local time (h). Values above the data points indicate the number of examined larvae per time period.

dawn to the midday (Fig. 5.1) in epipelagic layers (c.a. 60 m; Fig. 5.2), and oppositely to the usual pattern observed in adults. By remaining near the surface, larvae maximise prey captures as they are visual feeders that lack an adapted retinacular system with rods and the migration of the retinomotor pigment required for low light intensities (Sabatés *et al.*, 2003; Conley and Hopkins, 2004; Morote *et al.*, 2011).

The vertical distribution of larvae of mesopelagic fishes in the Mediterranean during day- and night-time indicates that myctophids and the most abundant stomiiforms (i.e. *C. braueri* and *Vinciguerria attenuata*) mainly spread through the first 100 m of the water column during both the stratified and mixing periods. An exception to this statement, were larvae of the sternoptychids *A. hemigymnus* and *Maurolicus muelleri* that were mainly distributed from 100 to 200 m depth, probably as result of a better visual adaptation to dark environments (Sabatés, 2004; Olivar *et al.*, 2010; Olivar *et al.*, 2014). In fact, the largest postlarvae were distributed in deeper depths at night than the earliest stages (Sabatés, 2004; this study; Olivar *et al.*, 2014).

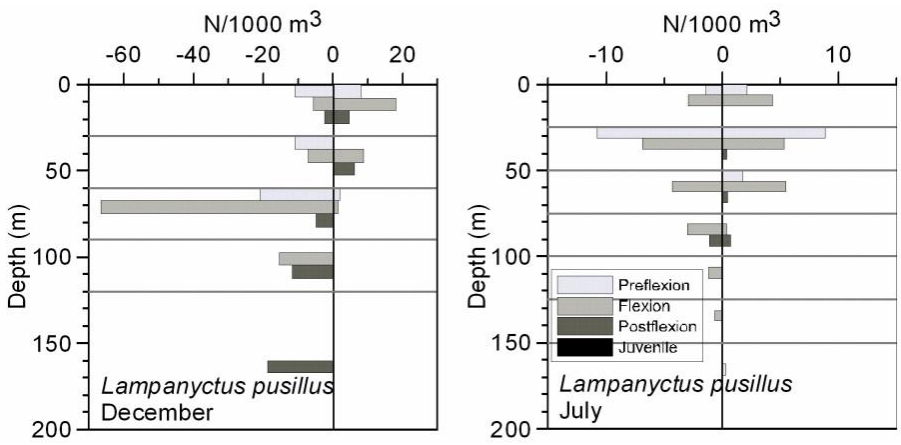


Fig. 5.2 Vertical distribution of developmental stages of *L. pusillus* during the day (right bars) and night (left bars) in the first 200 m of the water column. The values correspond to the mean abundance at all stations for each depth stratum and developmental stage. From Olivar *et al*. (2014).

Larvae of mesopelagic fishes feed on small organisms compared to mature adults, incorporating larger organisms to the diet as they grow (Chapter 3). This shift might be primarily explained by the increase of mouth width and an improvement of the swimming and visual skills with development. As a case in point of dietary shift, the initial preflexion and flexion larval stages of *L. pusillus* focused diet consumption mainly on items <200 µm prey width (small prey) (Fig. 5.3). Postflexion stages incorporated larger prey than the earliest larvae as a consequence of mouth enlargement. Juvenile and adult specimens preferred preying on large organisms (>400 µm prey width) that supply their higher energetic requirements (Chapter 3 and 4). Mouth size showed positive correlation with body length (SL) in *L. pusillus* (Fig. 5.4); mouth enlargement was slightly similar to body growth, whilst mouth width grew more slowly.

Larval feeding patterns agreed with previous observations in the Mediterranean and other regions (Sabatés *et al.*, 2003; Sassa and Kawaguchi, 2004; 2005): *L. pusillus* larvae were diurnal feeders, except for some postflexion individuals,

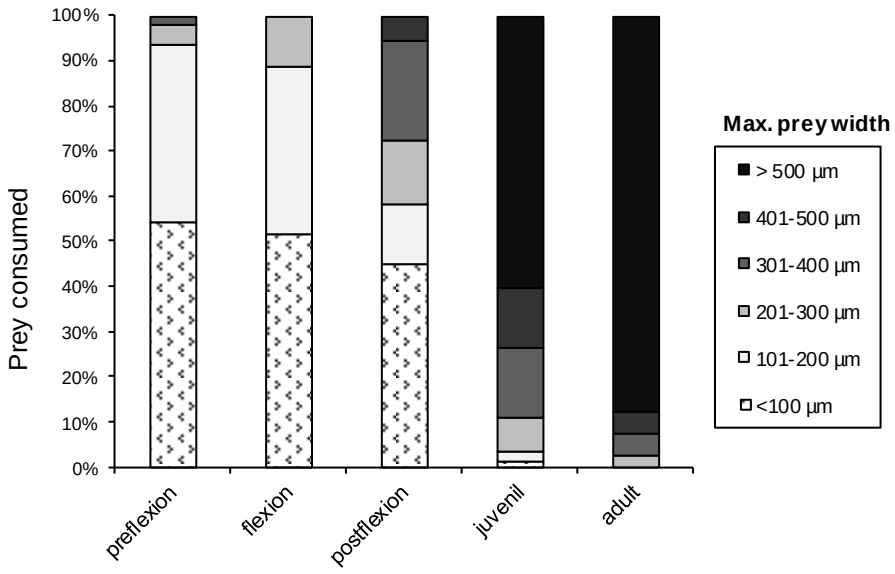


Fig. 5.3 Percentage of prey consumed of each width range plotted against developmental stages of *L. pusillus*, showing an increase in the consumption of larger prey items throughout development.

with an eventual increase in feeding incidence with growth, which is a common feature in fish larvae (Arthur, 1976; Anderson, 1994; Sabatés *et al.*, 2003). Most Mediterranean larvae feed primarily on nauplii and copepodites, specially preflexion and flexion stages. Moreover, preflexion larvae seemed to have considerable lower feeding incidence than postflexion in agreement with other studies (Arthur, 1976; Hunter, 1981) and with the fact that a more complex structure of the alimentary canal implicates higher feeding incidence. Thus, the morphology of the alimentary system has an essential influence on food retention or feeding success (Conley and Hopkins, 2004) and can determine food composition.

In feeding studies the degree of specialization on prey size is measured by calculation of the trophic niche (TN) breadth defined by Pearre (1986). A decrease of the TN breadth (standard deviation of the logarithm of mean prey width) with development indicates that diet becomes more specialized with growth. An increasing trend or constant niche, on the contrary, indicates generalized feeding. Scharf *et al.* (2000) affirmed that TN breadths generally tend to narrow for the largest predators, which may be a common event in

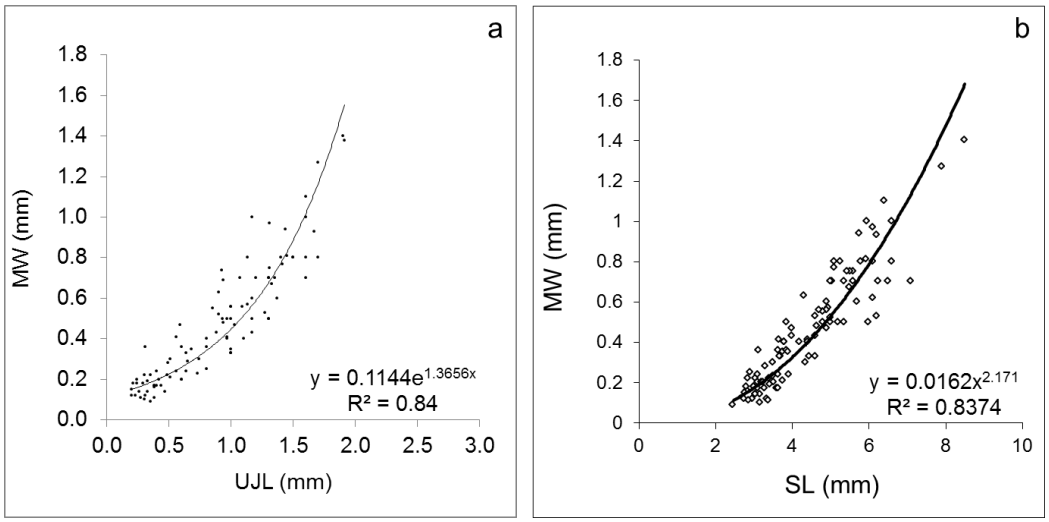


Fig. 5.4 Relationship between (**a**) larval mouth width (MW) and upper jaw length (UJL), and (**b**) mouth width and larval length (SL) in *L. pusillus.*

animals. For some pelagic fish species, TN breadth remained constant throughout larval development (Pearre, 1986; Sabatés and Saiz, 2000; Morote *et al.*, 2008b; Morote *et al.*, 2010; Bernal *et al.*, 2013), whilst for other fish, variations in larval trophic niche breadths were found (Morote *et al.*, 2008a; Morote *et al.*, 2011; Murphy *et al.*, 2012; Llopiz, 2013). In general, juvenile and adult stages of Mediterranean mesopelagic fishes did not show significant trends of TN breadth with development, although the species *L. crocodilus* exhibited a negative slope with growth. This result may show that large *L. crocodilus* are restricted to a lower number of feeding resources, tending to have a diet focused on large food items that constitute a more nutritive intake than that from younger stages.

5.3 Trophic ecology of the mesopelagic fish assemblage and its importance for the food web in the western Mediterranean

Mesopelagic fishes play an important role in the marine ecosystems due to their high abundance and being concurrently predators and prey in the pelagic food web. The integrated analysis of the feeding patterns of the mesopelagic fish assemblage (Chapter 4) is essential to approach the functioning of the ecosystem and subsequent fisheries management, even though the studied species are not of commercial interest at present.

The results presented here showed that adults of Mediterranean midwater fishes were mainly zooplanktivorous (meso- and macrozooplankton). In general, their diets constituted a combination of different proportions of copepods, euphausiids and larvaceans, followed by other taxa of lesser numerical importance. The ingested mesozooplankton (0.2-20 mm) was compounded by diverse species of copepods, ostracods, gastropods, larvaceans and chaetognaths. Macrozooplankton (20-200 mm) comprised mainly euphausiids, decapods and amphipods. These prey organisms occupy different trophic

positions in the food web leading to trophic cascades. For instance, calanoids are predominantly herbivorous and omnivorous, whereas the non-calanoid genera *Oithona*, *Oncaea*, *Corycaeus* and chaetognaths have been defined as carnivorous (Pearre, 1980; Kouwenberg, 1994). Euphausiids are essentially omnivorous (Barange *et al.*, 1991), except for *Nematoscelis megalops*, which has been described as carnivorous (Andersen *et al.*, 2001). Moreover, some Mediterranean species of copepods (e.g. *Pleuromamma gracilis, Euchaeta marina, Candacia armata*), ostracods (Riandey *et al.*, 2005) and euphausiids (*Meganyctiphanes norvegica, Euphausia krohni*, *N. megalops*) (Banse, 1964; Mauchline, 1980; Barange *et al.*, 1991) perform circadian displacements to the surface at night, constituting available food supplies for mesopelagic fishes at different depth levels. The genus *Pleuromamma* was an important prey item for all the mesopelagic fishes from this study. Its migratory species *P. gracilis* and *P. abdominalis* (Riandey *et al.*, 2005; Brugnano *et al.*, 2012) are quite abundant in the upper layers and frequently found in the night catches performed at ca. 200 m depth (Fernández de Puelles *et al.*, 2014), which coincides with the surface-ascent of migratory myctophids at sunset and night. Many common prey (e.g. the calanoids *Pleuromamma* spp., *Nannocalanus minor*, the ostracod *Conchoecia*) are bioluminescent organisms, aspect that likely favors their capture by visually-oriented predators (Hopkins and Baird, 1977), for instance, the sternoptychid *A. hemigymnus* and most myctophids from the present thesis.

The dominance of crustaceans in the stomachs of most Mediterranean midwater fishes is consistent with previous studies in other regions (e.g. Kozlov, 1995; Pakhomov *et al.*, 1996b; Gaskett *et al.*, 2001). The broad extent of prey taxa partly reveals feeding behaviour of these migratory species; predators displace to locations where potential prey items have high densities –at night in the surface (Clarke, 1978; Kinzer and Schulz, 1985; Watanabe *et al.*, 2002). High biomass of planktonic organisms concentrates in the surface layers of the

Mediterranean Sea and large oceans. Therefore, the epipelagic stratum is essential for trophic interactions between plankton and surface-migratory midwater fishes (Gray and Kingsford, 2003). A higher seasonal feeding on a specific prey type by some predator species is attributed to the fact that a food resource has an abundance peak for that seasonal period (Macpherson, 1981; Cartes, 1998). This is reflected in fish species that tend to be rather opportunist, such as some myctophids in the present study that reported different proportions of particular prey items between summer and late autumn (i.e. *C. maderensis*), despite these differences were not significant.

In general, Mediterranean midwater fishes constitute an assemblage with a broad feeding spectrum. The degree of diet specialisation found in the Mediterranean fishes was low, except for the oldest stages of some myctophids (*B. glaciale*, *L. crocodilus*, *L. pusillus*, *N. elongatus*, *H. hygomii*). Hence, the whole group of mesopelagic fishes was rather typified by high prey diversity, and generally low degree of selectivity on one or two prey types, according to Chesson's index. For instance, the juvenile and adult stages of *L. dofleini*, *N. elongatus*, *L. crocodilus* and *L. pusillus* showed positive selection for euphausiids.

A new cluster analysis - along with SIMPER procedure - run on the whole dataset of mesopelagic fish from the western Mediterranean, suggests some trophic groups based on the dominance of certain prey types (Fig. 5.5). Three large main clusters are distinguished. One main group is constituted by summer and adult individuals of *L. crocodilus, N. elongatus, L. pusillus* plus benthic specimens of *L. crocodilus*. The diet composition of this cluster was represented by high proportion of euphausiids (72% contribution). A second grouping contains early stages of *A. hemigymnus* and *H. benoiti* with a total prey contribution of copepods, the 41% of which are non-calanoids. The third wide

cluster is a combination of myctophids from both seasons (*M. punctatum*, *H. benoiti*, *N. elongatus* from the autumn, *C. maderensis* from the summer, and *L. dofleini* from both seasons) and denotes a varied diet, where at least 7 different prey types contribute to the 60% of diet composition. *Cyclothone braueri* and adult *Hygophum* spp. constitute monotypic groups. *C. braueri* has the particularity of recording high percentage of vacuity, low number of ingested prey and notable preference for *Pleuromamma* spp., whilst the genus *Hygophum* is characterized by intense voracity and diversity of prey types.

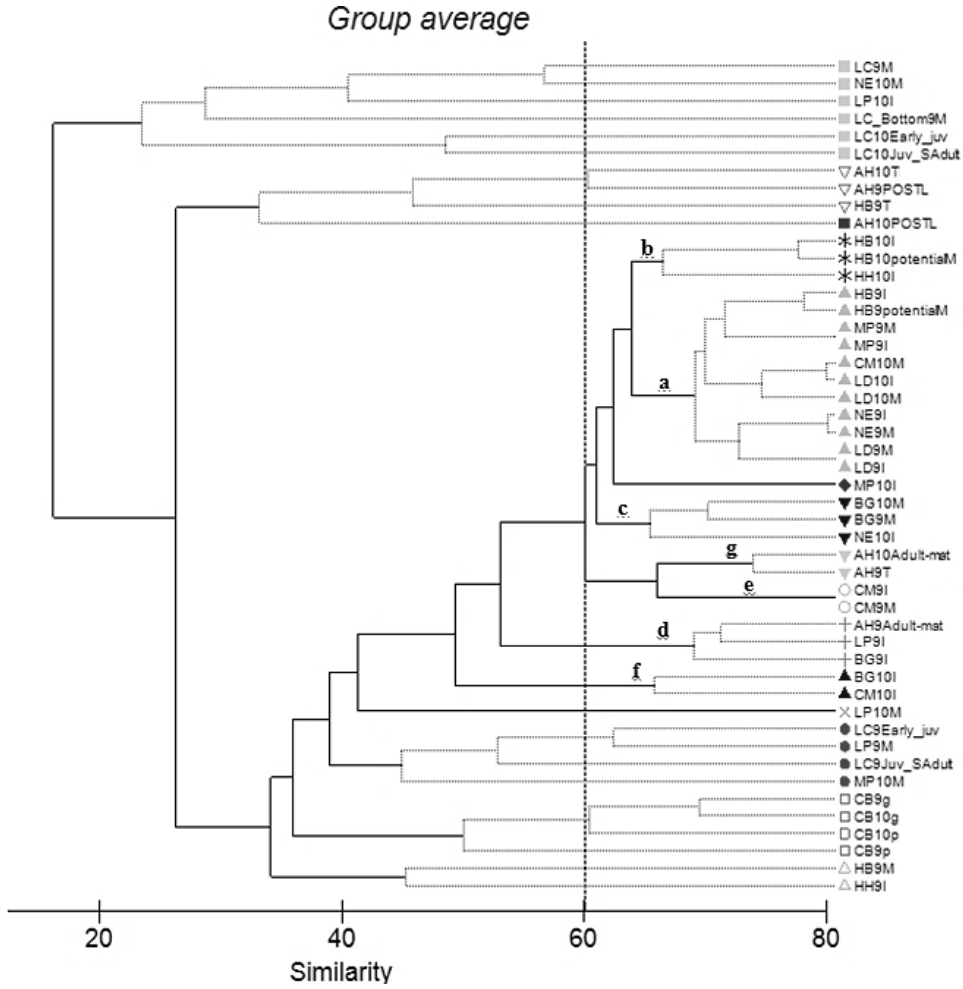


Fig. 5.5 Hierarchical clustering based on Bray-Curtis similarity for the diet composition of 11 Mediterranean mesopelagic fish species. The prey abundance matrix was averaged in relation to the following factors: species, maturity stage and seasonal survey. Grey lines show significant associations and letters in lower case indicate clusters with more than 60% similarity, showed in table 6.1.

The clusters with the highest average contributions (over 60%) are represented in Table 5.1 and are contained within the three main clusters.

Table 5.1 Similarity percentages within groups determined from cluster analysis + SIMPER for the diet composition of 11 species of mesopelagic fishes. Percentage contributions of prey types per species are also shown (cut-off 60% dietary contribution).

	Groups	a	b	c	d	e	f	g
	Average similarity within group	71.01	70.20	67.02	69.79	81.29	65.78	73.87
Percentage contributions of prey types	*Candacia* spp.			7.76	6.33	3.49		
	Clausocalanus spp.	5.00		6.06	7.14	4.28	9.99	7.32
	Centropagidae		6.17					
	Euchaeta spp.	4.22		5.30	7.63			
	Mesocalanus tenuicornis					2.99		
	Nannocalanus minor	6.03	8.40			3.31		
	Paracalanidae	4.30				5.22		6.44
	Pleuromamma spp.	8.76	17.49	11.13	11.31	5.84	13.15	6.16
	Temora stylifera							
	Unknown Calanoida	5.03		6.54		5.01		6.44
	Oncaea spp	4.78		6.59	7.96	4.78	11.88	9.79
	Corycaeidae	4.78	5.44			3.25		6.44
	Unknown Copepoda				7.98			6.44
	Adult euphausiids	5.57						
	Furcilia or juvenile euphausiids		8.89	6.75			11.88	
	Amphipoda		5.67					
	Conchoecia spp	5.29		7.06	8.06	5.45	13.15	8.71
	Creseis sp					4.02		
	Gastropoda (mainly *Limacina* sp.)					3.31		
	Chaetognaths	4.65						
	Larvaceans					5.92		6.44
	Other gelatinous					3.49		
	Pisces		5.94	5.30				

The cluster analysis shows high similarity among the diet compositions of the studied midwater fishes. However, an explicit index is needed to ascertain the degree of diet overlap. The measurement of "overlap" in comparative ecological studies is usually tested using the Morisita-Horn index (Horn, 1966), which is shown for this general discussion between pairs of species (Table 5.2). This index is defined as follows:

$$\mathrm{CH} = 2\left(\sum p_{ij} p_{ik}\right) \Big/ \sum p_{ij}^{2} + \sum p_{ik}^{2}$$

Where p_{ij} is the proportion of the prey *i* of the total food items consumed by the species *j*, and p_{ik} is the proportion of the prey *i* in the total food items consumed by the species *k.*

The Horn index was calculated for the overall diet of each species, without differentiating between the two seasonal periods (summer and late autumn). In general terms, diet overlap ranges in different degrees among several species in agreement with the previous analyses of this thesis (i.e. CCA in Chapter 4 and Cluster analysis). Moreover, the Horn index provides complementary information about the degree of dissimilarity between pairs of diets. Only the utmost values were taken into account for comments. Thus, *C. braueri* showed the most dissimilar diet with respect those of the other mesopelagic fishes. A very low prey number for the gonostomatid might imply that rare prey types and common prey are equally shared with other fish species. Specifically, the diet of *C. braueri* is the most divergent with respect the myctophid diets, especially those with the highest trophic values (i.e. *N. elongatus*, *L. pusillus*, *Hygophum* spp. and *B. glaciale*). However, among myctophids, the lowest values of the Horn index, which means higher dissimilarity between pairs of diets, were precisely for those species with the highest trophic values and selective strategies towards large prey types. This points out that, apart from the selected prey organisms, the rest of the dietary items consumed by myctophids with the highest trophic values, were diverse and thus less shared with other fish species.

Diet overlap was usual among the most opportunistic species (i.e. *Myctophum punctatum*, *C. maderensis* and *B. glaciale*). Prey-type contributions varied among mesopelagic species, and the type of prey consumed was mainly determined by prey body size and the developmental stage of predator. Prey selectivity was therefore more oriented to reduce within species diet overlapping among different developmental stages - which have contemporarily different needs in feeding in line to physiological stages – than to minimise interspecific

diet overlap. Myctophids, sternoptychids and gonostomatids are fishes of small size with a short lifespan, characteristics that usually encourage an opportunistic behaviour. However, the investigation of prey selection demonstrated that most species were not completely opportunistic feeders, showing mixed feeding strategies.

Diet overlap among Mediterranean midwater fishes was supported by a continuum of TrL values revealed by isotope analysis. On the basis of combined analysis of carbon (δ13C) and nitrogen (δ15N) stable isotopes for the 18 most abundant mesopelagic fish species, the trophic position of these species was calculated and ranged from 2.9 to 4. Certain degree selectivity could be addressing these slight differences of the TrL continuum among fish species. The less active non-migratory stomiiforms, *Cyclothone* spp. and *A. hemigymnus*, occupy the lowest TrL. These two species are adapted to a more stable deep-environment (below 400 m) and have low energy-demanding strategies. In contrast, the highest trophic positions of the assemblage were represented by myctophids, for which we observed a daily migratory behavior (*L. dofleini* or *N.*

Table 5.2
Horn index for the study of diet overlap among 11 mesopelagic fish species. Values>0.5 indicate overlap.

	N. elongatus	*M. punctatum*	*L. pusillus*	*L. crocodilus*	*L. dofleini*	*H. hygomii*	*H. benoiti*	*C. braueri*	*C. maderensis*	*B. glaciale*	*A. hemigymnus*
N. elongatus		0.7027	0.0180	0.3368	0.1098	0.0945	0.1167	0.1763	0.5856	0.2139	0.7753
M. punctatum	0.7027		0.7529	0.8488	0.6658	0.7336	0.7511	0.7879	0.2623	0.7508	0.8203
L. pusillus	0.0180	0.7529		0.3497	0.1169	0.0872	0.1048	0.1409	0.6145	0.1958	0.7240
L. crocodilus	0.3368	0.8488	0.3497		0.4035	0.5550	0.5838	0.4748	0.6659	0.4436	0.7815
L. dofleini	0.1098	0.6658	0.1169	0.4035		0.2229	0.2072	0.0974	0.4050	0.0799	0.5171
H. hygomii	0.0945	0.7336	0.0872	0.5550	0.2229		0.0237	0.2342	0.7069	0.3441	0.8514
H. benoiti	0.1167	0.7511	0.1048	0.5838	0.2072	0.0237		0.2256	0.6682	0.3378	0.8129
C. braueri	0.1763	0.7879	0.1409	0.4748	0.0974	0.2342	0.2256		0.5357	0.1025	0.5214
C. maderensis	0.5856	0.2623	0.6145	0.6659	0.4050	0.7069	0.6682	0.5357		0.4448	0.4840
B. glaciale	0.2139	0.7508	0.1958	0.4436	0.0799	0.3441	0.3378	0.1025	0.4448		0.4016
A. hemigymnus	0.7753	0.8203	0.7240	0.7815	0.5171	0.8514	0.8129	0.5214	0.4840	0.4016	

elongatus), and by bottom dwellers (*L. crocodilus*) (Valls *et al.*, 2014) that fed on large zooplankton and micronekton near the benthic boundary layer.

Of particular interest is to mention that, although Mediterranean myctophids are not usually piscivorous, some specimens had fish remains or an entire body of a small mesopelagic fish in their stomachs. Intraguild competition could be an explanation for exceptional situations in which midwater fishes prey on similar fish (Polis, 1981). Remaining at particular locations with low concentration of zooplankton or micronekton (the vertical space before reaching the surface or near the bottom) could promote fish-feeding behaviours. On the other hand, feeding on fish could mean a surplus ingestion linked to particular events of high energetic requirement such as a peak of spawning.

Midwater fishes might exert a notorious feeding pressure on zooplankton in the western Mediterranean. However, fish-diet overlapping might indicate that the abundance of prey organisms does not constitute a strong limiting factor in the Mediterranean western basin. Differences among fish species were addressed by dissimilar levels of voracity, contrasting patterns of vertical migration between myctophids and stomiiforms and unlike requirements by developmental stages. High overlap in diet composition is opposed to the general assumption of strong selective-feeding strategies in areas with low productivity. The imperceptible interspecific competition can be related to the fact that the mesopelagic fish community is not saturated with species in the western Mediterranean. Feeding strategies fluctuate from an opportunistic feeding to low/medium selectivity on particular prey types/sizes (Chapter 4). The plasticity to adapt feeding strategies in accordance with energy requirements plays a decisive role to raise myctophids and stomiiforms as a successful group in the ecosystem that reaches high biomass and numerical abundance.

5.4 Comparison of stomach content analysis with carbon weight estimations

The estimation of the carbon that can be transported by mesopelagic fishes throughout the water column was beyond the main scope of this thesis. Nevertheless, carbon content in fish stomachs was calculated through conversions of the prey dimensions into carbon weight (CW) and related to diet-composition-based results of the Chapters 3 and 4. In addition, the dietary contribution of potential prey, in terms of carbon weight, was discussed.

Stomach content examination is a very time-consuming technic that relies on the analysis of high number of specimens in order to properly reproduce several feeding situations. It requires taxonomic skills to identify a large set of prey organisms in diverse degrees of degradation. As each methodology, stomach content analysis has limitations and can bias prey composition by underestimating soft items when food in the stomachs is in advanced stage of digestion. In order to avoid misreporting, the analysis of stomach contents is often combined with stable isotope analysis. On the other hand, stomach content examination has the advantage of a meticulous characterization of the diet composition that other procedures, which are based on the measurement of wet/dry weight contents, cannot offer.

Nevertheless, Pillay (1952) remarked that the numerical percentage of food items constitutes a valuable procedure solely if the food is not fragmented during intake or if body dimensions do not differ excessively from one prey to another. Along these lines, the numerical percentage of prey items does not ascertain the amount of ingested biomass. For this purpose, the body dimensions of a great deal of prey found in fish stomachs (Appendix 5.1) were transformed to carbon weights. CW estimations allowed an approximation to the biomass ingested by an only fish. These estimated values of CW should be taken cautiously because in some cases we could be largely over- or subestimating the

actual value when most prey are in an advance stage of digestion. Consequently, only the stomachs filled with food above the 50% of their capacity were used to provide the potential bulk of carbon ingested.

Frequently, the organic matter held in fish stomachs is insufficient for weighting or materials other than zooplankton are present. In both cases, it is desirable to estimate individual weights from direct length/width measurements of specific zooplankters (Uye, 1982). Oppositely to the organisms ingested by large commercial fishes, preys of mesopelagic fishes are generally tiny items that would bias their individual contribution to total weight by trying to weight them individually. Berg (1979) alluded to the importance of considering those stomachs in which prey items are in a similar stage of digestion or dominated by recognizable items which, on the contrary, was very unusual in mesopelagic fish samples. Nevertheless, Arntz (1971) assumed that only the absolute weight of ingested food changes, whilst the weight proportions of various food components remain comparatively the same. If this is true, the proportions of weight within one food component may not be changed much by digestion either.

The carbon content for each prey was estimated by means of the algorithms presented in diverse literature and collected in Table 5.3. CW per stomach was obtained for the fish species *A. hemigymnus, B. glaciale, C. maderensis, H. benoiti, L. dofleini, L. pusillus, M. puntatum* and *N. elongatus*, and subsequently, plotted against standard length (SL) (Fig. 5.6). Individual prey measurements for the species *C. braueri, L. crocodilus* and *H. hygomii* were very scant due to a low prey number in the two former species, and the advanced stage of digestion of prey in a substantial number of stomachs in the three species. In addition, the amount of carbon that comes from the major ingested prey types was standardized by the number of stomachs per developmental stage, giving an approximation to the prey contribution by developmental stage.

Table 5.3
Length-weight relationships to calculate carbon content of different prey types extracted from literature. DW: Dry weight; CW: Carbon weight; µg: micrograms; mg: milligrams; µm: micrometres; mm: millimetres; PL: Prosome length; TL: Total Length; Vol: Volume.

Prey item	(DW/CW=a L_{mm}^{b})	a	b	C (%DW)	Source
Calanoida	$\ln(DW_{\mu g})=2.74\ \ln(PL_{\mu g})-16.41$	$7.47E^{-08}$	2.74	42.4	van der Lingen (2002)
Cyclopoida	$\ln(DW_{\mu g})=1.96\ \ln(PL_{\mu g})-11.64$	0.00001	1.96	42.4	van der Lingen (2002)
Harpacticoida	$\ln(DW_{\mu g})=1.96\ \ln(PL_{\mu g})-11.64$	0.00001	1.96	42.4	van der Lingen (2002)
Ostracoda	$DW_{\mu g}=3.946\ TL_{mm}^{2.436}$	3.946	2.436	42.4***	James (1987)
Cladocera	$DW_{\mu g}=3.946\ TL_{mm}^{2.436}$	3.946	2.436	42.4***	James (1987)
Euphausiacea	$DW_{mg}=0.0012\ TL_{mm}^{3.16}$	0.001	3.16	42.9*	van der Lingen (2002)
Mysidacea	$CW_{mg}=1.7287\ TL_{mm}^{3.08}$	1.729	3.08	42.9*	Uye (1982)
Decapoda (Natantia)	$\log(CW_{\mu g})=2.65\ \log(TL_{\mu m})$	2.168	2.65		Uye (1982)
Decapoda larvae	$DW_{\mu g}=3.946\ TL_{mm}^{2.436}$	3.946	2.436	38.6**	James (1987)
Amphipoda	$DW_{mg}=0.005\ TL_{mm}^{2.311}$	0.005	2.311	37	James (1987)
Crustacean eggs	$\ln(DW_{\mu g})=0.0143\ (Ø_{\mu m})-3.381$			40	van der Lingen (2002)
Larvacean (*Oikopleura dioca*)	$CW_{\mu g}=10^{-6.84}\ TL_{\mu m}^{2.59}$		2.59		Lopez-Urrutia (2003)
Salp	$CW_{\mu g}=0.0005\ TL_{\mu m}^{2.78}$	2.88	1.59	40	Cetta *et al*. (1986)
Chaetognatha (*Sagitta* spp.)	$DW_{mg}=0.00097\ TL_{\mu m}^{2.2365}$	0.00097	2.237	29	Sameoto (1971)
Atlanta/ *Limacina* sp.	$DW_{mg}=0.257\ L_{mm}^{2.141}$	0.257	2.141	42.4***	Gannefors *et al*. (2005)
Gastropoda/ Bivalvia	$DW_{\mu g}=47.386\ TL^{3.663}$	47.386	3.663	42.4***	James (1987)
Cavolinia inflexa	$\log CW_{\mu g}=\log(-0.833)+1.068\ \log DW_{\mu g}$	0.147	1.068	42.4***	Gorsky *et al*. (1988)
Polychaeta	$DW_{\mu g}=0.157\ (0.01\ TL_{mm}^{2.136})$	0.01	2.136	52	Alexandrov (2001)
C. braueri	$DW_{mg}=0.0001\ SL_{mm}^{3.582}$	0.0001	3.582	40	James (1987)
N. elongatus (27-30 mm)	DW=56 mg			40	This study
Fish eggs	$DW_{\mu g}=0.093 Vol_{mm}^{3}+0.0012$			46	van der Lingen (2002)
Foraminifera	$\log CW_{\mu g}=-0.460+0.866\ (\log Vol_{\mu m}^{3})$			40****	Chisholm & Roff (1990)

*Value from the isotope analysis of *Meganyctiphanes norvegica* (M. Valls, unpubl. data)
**Value from Lindsay (2003)
***van der Lingen (1988)
****Stratham (1967)

Size classes grouped fish into 5 mm (for *L. dofleini* and *A. hemigymnus*) and 10 mm SL-cohorts (the rest of species). Differences in the carbon content between *i)* fish species, and *ii)* size classes/developmental stages (immature *versus*

mature) were evaluated with one-way and multifactorial ANOVA tests using STATISTICA 7.

5.4.1 Carbon weight results and discussion

Mean carbon content per stomach increased linearly with gut fullness. ANOVA tests showed significant differences in mean CW among size-cohorts in most fish species, except for the stomiiform *A. hemigymnus* and the myctophids *M. punctatum* and *N. elongatus*. Main results are summarised in Table 5.4. However, non-significant differences were denoted between mature and immature stages for any midwater fish species (F=1.812; p=0.083).

Therefore, absolute data of total carbon weight per stomach showed an increasing non-linear tendency in all species, with the exception of *M. punctatum*, and weak coefficients of correlation for *C. maderensis* and *N. elongatus* (Fig. 5.6). Intercepts and positive slopes in the increase of CW with SL were varied among fish species; the highest regression coefficient by far was showed by *L. dofleini*, followed by *L. pusillus*. Considering the migratory and feeding behaviours of the mesopelagic fish community, all mesopelagic fish species have an important role in the bulk of matter transported through the water column in different ways. For instance, *i*) adult stages of *L. dofleini* ingest a great deal of prey biomass, indicating high voracity for this small-sized species; *ii) N. elongatus* and *M. puntatum* consume a significant bulk of carbon content (reaching near 100 mg CW) across all developmental stages; both are large-sized species, constituting potential vectors of transport; *iii*) *C. maderensis*

Table 5.4
Summary table of results for the ANOVA test performed to test differences among 5-6 size groups for each mesopelagic fish species. df, degrees of freedom.

	A. hemigymnus	*B. glaciale*	*C. maderensis*	*H. benoiti*	*L. dofleini*	*L. pusillus*	*M. punctatum*	*N. elongatus*
F	2.0985	4.286	6.14	4.2002	4.4672	2.9743	1.4641	1.9386
df (error)	36	79	76	24	40	61	24	91
df (size group)	5	6	5	2	4	4	5	6
p	0.08813	**0.00086**	**0.00008**	**0.02729**	**0.00445**	**0.02609**	0.23826	0.08289

is a medium-sized species less voracious than *L. dofleini* and also holding less carbon content than *N. elongatus* and *M. punctatum*. However, it constitutes the most abundant myctophid in the western Mediterranean, being also an important carrier for carbon transport.

CW contributions of prey to fish diets were analysed within the different developmental stages (Table 5.5) and deserve some comments. The sternoptychid *A. hemigymnus* showed that calanoid copepods were the most representative prey in both carbon content and numerical abundance in immature and mature individuals. Only the transforming stages of this species showed a major contribution of chaetognaths, which contrast with the %N and %F results, for which non-calanoid copepods were the main prey items. For myctophids, the major CW contributions to mature stages of *H. benoiti*, *B. glaciale*, *C. maderensis*, *L. pusillus*, *N. elongatus* and *M. punctatum* were due to euphausiids and, in some cases, small fishes. *N. elongatus* also showed important carbon inputs from decapods, although these large prey types were always fewer numerically than copepods.

Kiørboe (2013) affirmed that copepods and other crustaceans have a condensed body plan attractive to predators because of a high nutrient content compared to other zooplanktonic groups. Prey types such as small fishes, which occurred occasionally, but filled the fish stomachs at once, constitute a resource of low numerical representation, but important carbon contribution likely decreasing the potential predator daily rations.

The pacific myctophid *Stenobrachius leucopsarus* would hold 52 mg of food that could potentially transport to the deep layers, according to estimates using a daily food intake assessed at 0.8-1.5% of body weight for three species of medium-sized myctophids (Balanov and Gorbatenko (1995) in Radchenko, 2007). Applying the same daily food intake to Mediterranean myctophids, *C. maderensis* (50 mm SL, body weight=1.25 g) and *M. punctatum* (body weight=2

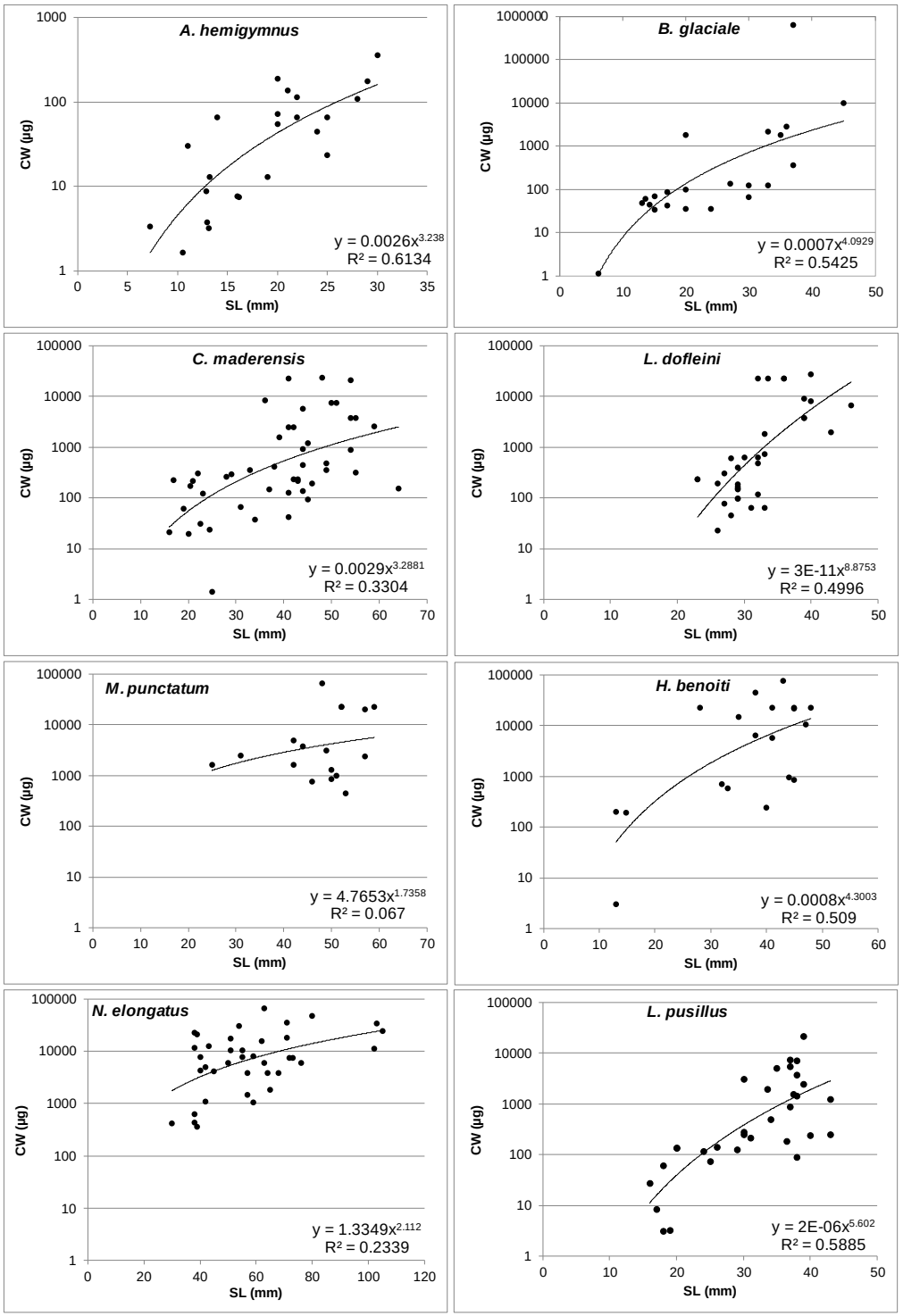


Fig 5.6 Nonlinear regression (curve fit) between carbon weight per stomach and standard length (SL) for 8 species of Mediterranean mesopelagic fishes. Only the fullest stomachs where considered (gut fullness >50%).

Table 5.5
Mean contribution of CW per prey type and by developmental stage for 8 mesopelagic species.

	Dev. Stage	Dominant CW contribution	CW (µg)/ind.	$\overline{CW}$ (µg)
A. hemigymnus	Transforming	Chaetognatha	10.7	82.5±62.4
	Immature	Calanoida	21.5	12.0±20.7
	Mature	Calanoida	25.2	66.1±81.3
H. benoiti	Transforming	Calanoida	45.2	
	Immature	Euphausiacea	969.7	4472±10471
	Mature	Pisces	3733.3	11477.4±19569.5
B. glaciale	Transforming	Calanoida	16.6	15.5±22
	Immature	Pisces/Calanoida	44.6/16.0	75.5±230
	Mature	Pisces/Euphausiacea	8101.0/200.0	14916.3±1600.9
C. maderensis	Immature	Gastropoda	297.8	441.8±2528.1
	Mature	Euphausiacea/Pisces	940.8/457.2	2234±5383.9
L. dofleini	Immature	Calanoida/Euphausiacea	80.456/37.8	158±178.3
	Mature	Pisces/Euphausiacea	2317.2/1033.2	5203±8491.1
L. pusillus	Immature	Euphausiacea/Calanoida	97.5/26.9	227.9±624
	Mature	Euphausiacea	639.4	1983.9±4602.1
M. punctatum	Immature	Euphausiacea/Pisces	612.6/4116.6	6127.8±16986.2
	Mature	Euphausiacea/Pisces	1332.8/1702.1	3408.8±7389.8
N. elongatus	Immature	Euphausiacea/Pisces	2675.5/849. 0	3363.8±6271
	Mature	Decapoda/Pisces	5820.9/2030.3	10722.8±15106.2

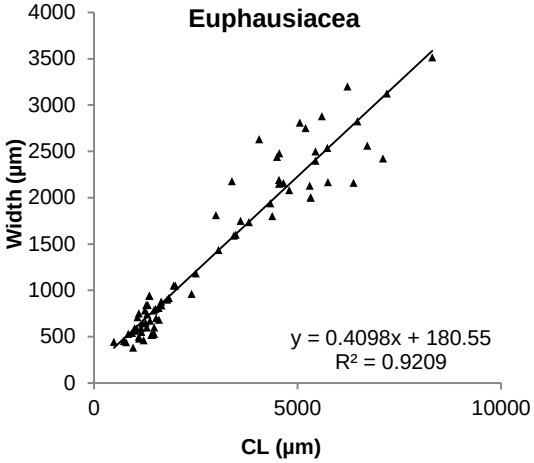
g) would hold 10-19 mg and 30-46 mg of carbon content, respectively. These estimated values are in the same order of magnitude than those from Radchenko (2007).

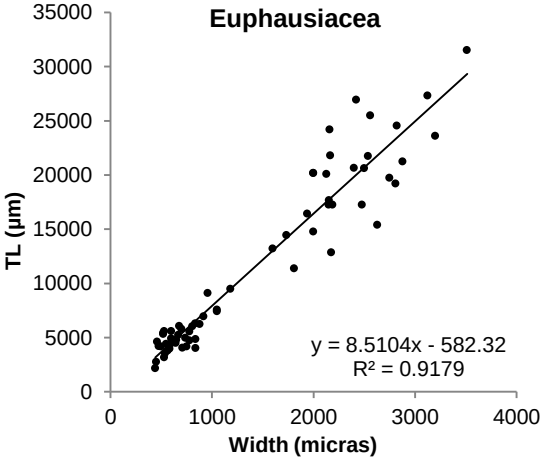
Allowing for the migratory and feeding patterns of the Mediterranean mesopelagic fishes, and combining with our estimations of carbon content, it can be presumed their relevance in the ecosystem functioning. Vertical migrations and prey consuming by mesopelagic fish contribute to the active flux of carbon to the depths, as fishes release part of the assimilated material by respiration and by excretion of the material in the stomachs (Hidaka, 2001). The role of this fish community has scarcely been considered in previous works about active flux and its importance is being acknowledged by recent

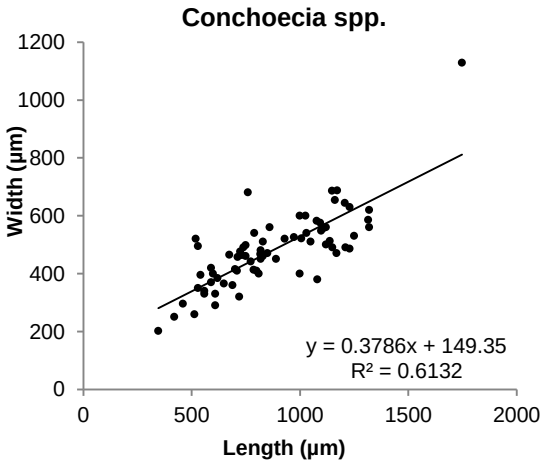
investigations on carbon fluxes through the water column (Hernández-León *et al*., 2010). The mesopelagic fish community exerts a top-down control in the vertical flux of carbon towards deep waters that is difficult to assess because, up to now, accurate estimates of the numerical abundances and information on the feeding rates (gut filling and gut evacuation rates), and the carbon content that the species and developmental stages can hold in the stomachs, is lacking. Further research in order to assess the active flux due to the mesopelagic fish community is needed, since its assessment will be of paramount importance to the knowledge of the biological pump in the ocean.

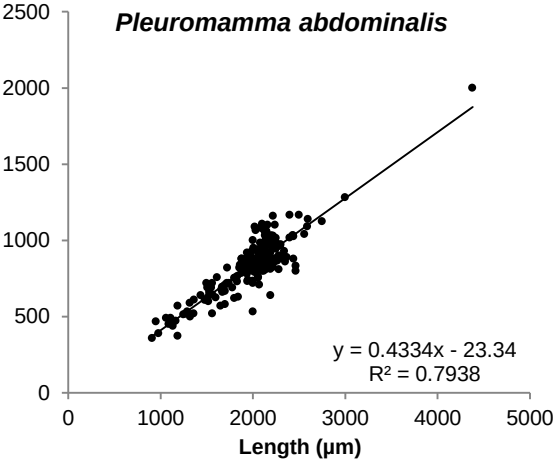
Appendix 5.1

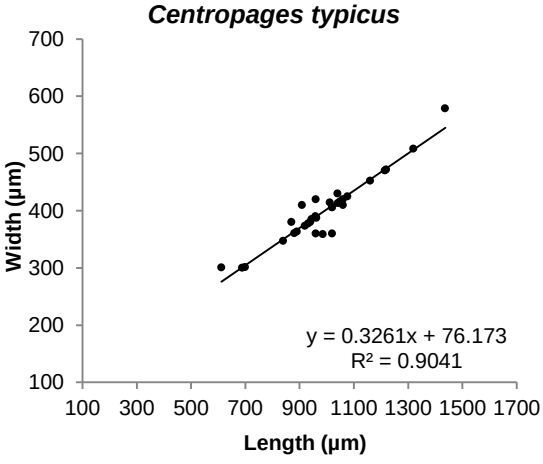
Body widths plotted against body lengths of different prey types identified during the analysis of stomachs contents.

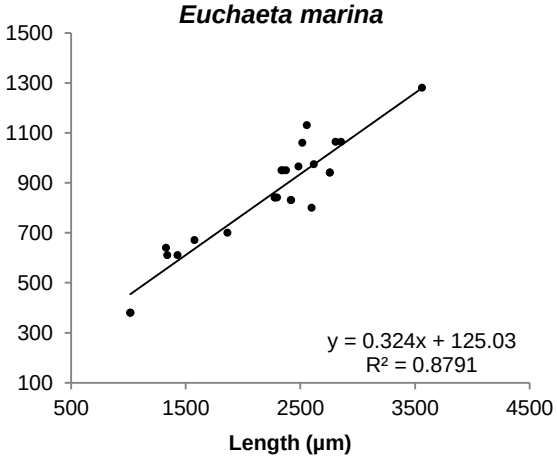


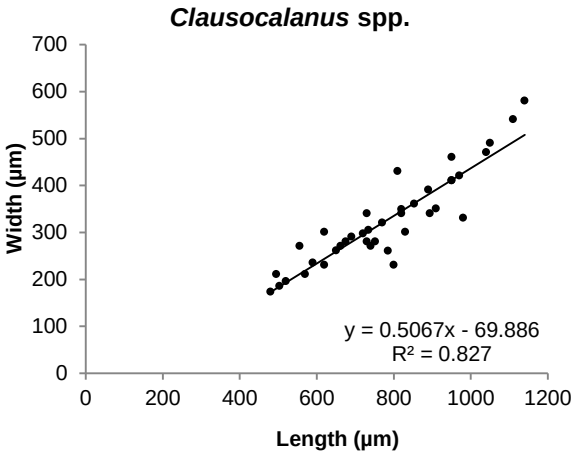


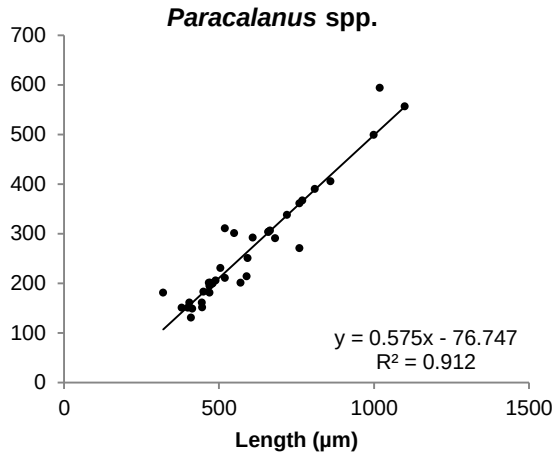


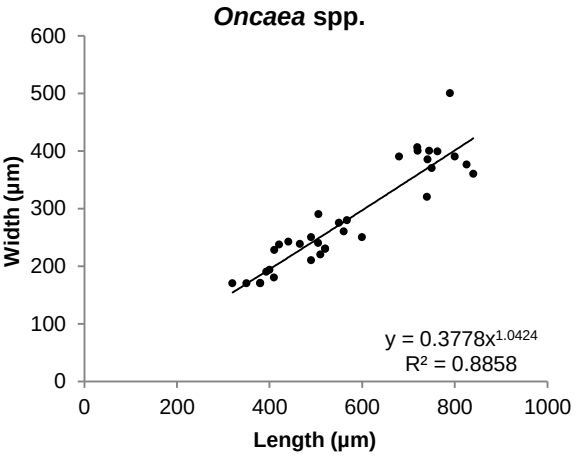


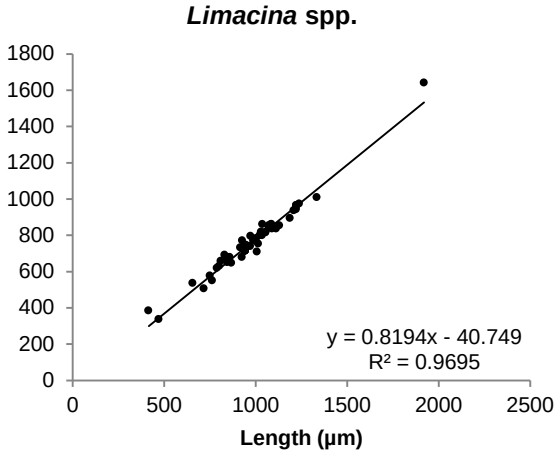


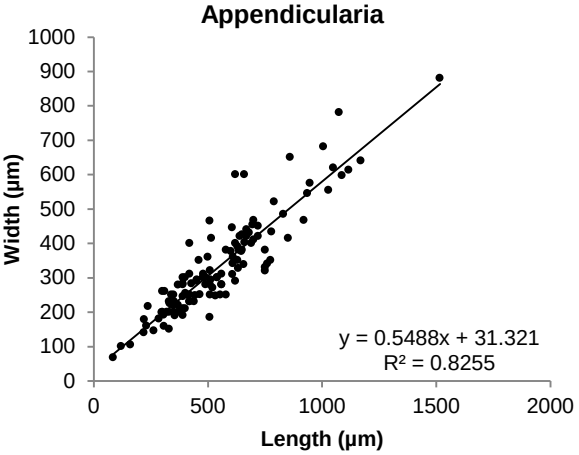



Clausocalanus spp.
Width (µm)
Length (µm)
y = 0.5067x - 69.886
R² = 0.827


Paracalanus spp.
Length (µm)
y = 0.575x - 76.747
R² = 0.912


Oncaea spp.
Width (µm)
Length (µm)
y = 0.3778x^1.0424
R² = 0.8858


Limacina spp.
Length (µm)
y = 0.8194x - 40.749
R² = 0.9695


Appendicularia
Width (µm)
Length (µm)
y = 0.5488x + 31.321
R² = 0.8255

CONCLUSIONS

1. The mesopelagic fish community of the western Mediterranean was similarly structured in autumn and summer, with the level of the water column being the most important factor differentiating the fish assemblages. The lack of dissimilarities in fish-species composition and vertical distribution between the two seasonal periods, points out that seasonality does not exert strong influence on the overall vertical migratory patterns.

2. Two migratory patterns were detected:

   i) One pattern corresponds to the species that do not migrate to the near surface, represented by the stomiiforms *Cyclothone braueri* and *Argyropelecus hemigymnus*. Both species remain in middle-depths below the 400 m-DSL, where the environment is more stable than in the surface.

   ii) The second pattern corresponds to the near-surface migrants that perform extensive diel vertical migrations (DVMs) during the night. Diel migrants are represented by most of the juvenile and adult stages of myctophids, although not all the specimens of some myctophid populations performed migrations or started their ascension at the same time. The oldest adult individuals of *Lampanyctus crocodilus* and *Notoscopelus elongatus* were exceptions to this general migratory pattern.

3. The two migratory behaviours can be reflected in fish body shape, since it has great relevance on the ability to move through the water. Therefore, the non-migratory gonostomatid *C. braueri* has a pronounced slender body shape advantageous for its more passive foraging strategy, in contrast with migratory myctophids, that have strong muscles and osteological development.

4. Myctophid larvae inhabit the first 200 m and feed during daytime in the epipelagic layer, showing that they are visual feeders. Juvenile and adult myctophids migrate to the surface to feed at night, demonstrating that DVMs were related to foraging and feeding. Uniquely, the non-migratory and adult individuals of *L. crocodilus* and the stomiiforms *A. hemigymnus* and *C. braueri* showed active feeding at daytime.

5. Myctophiforms and stomiiforms are primarily zooplanktivorous, preying mainly on the most abundant prey categories (80% surface and migratory copepods) with a high ingestion of particular copepod species, such as *Pleuromamma gracilis* and *P. abdominalis*, which are frequent species in epipelagic waters at night.

6. Most mesopelagic fish species showed a decrease in the frequency of ingestion of small prey with ontogenetic development in favour of larger and more nutritive prey consumption (i.e. euphausiids) by the oldest fish stages. However, the trophic niche breadths of mesopelagic fishes did not show significant trends, indicating that, in most cases, they incorporated large prey at the time that kept feeding on available and common zooplankton taxa of diverse sizes.

7. Feeding strategies in Mediterranean midwater fishes varied according to the species and developmental stages, from widely diverse diets to mixed strategies that are in between of generalist and selective behaviours. Selective strategies were particularly true for the most developed stages of some mesopelagic species. In this regard;

   i) The stomiiforms *A. hemigymnus* and *C. braueri* were found to be rather opportunists, with dominance in their stomachs of conspicuous and

available prey at the DSL (i.e. the copepod *Pleuromamma* and the ostracod *Conchoecia*).

ii) The myctophids *Ceratoscopelus maderensis*, *Myctophum punctatum* and *Lobianchia dofleini* are active predators that feed upon a wide assortment of unrelated taxa and appear to have mixed strategies partly affected by seasonal variations in zooplankton biomass and composition. The high prey diversity in the stomach contents of these three species suggests a tendency towards generalist behaviours.

iii) The two *Hygophum* congeners shared similar spatial distribution and foraged upon the same diet categories. However, the frequency of consumption of similar prey taxa diverged between them. Thus, differences in the consumption of prey taxa may lead to reduce potential competition between congeners.

iv) The outstanding number of prey items in *M. punctatum* indicates that its foraging strategy consists in ingesting the maximum number of unrelated organisms.

v) The largest specimens of adult *N. elongatus* and bottom-dwelling *L. crocodilus* were specialised on the consumption of large euphausiids.

8. Stomach content analysis revealed strong diet overlap within the mesopelagic fish assemblage. Then, potential species competition could be reduced by differential voracity, prey selectivity and spatial distribution through the water column.

9. Indeed, a lack of strong competition pressure among Mediterranean mesopelagic fishes can be deduced from the observation of generalist/mixed feeding strategies in most fishes and could be related to the existence of

"vacant niches", since the Mediterranean mesopelagic fish assemblage is not saturated (that is, with low number of species compared to other mesopelagic fish communities from large oceans of low/mid latitudes).

10. Trophic level (TrL) values of mesopelagic fishes spanned from 2.9 to 4.0. TrL values also show overlapping, with the most noticeable niche segregation observed between the non-migratory and small stomiiforms *C. braueri* and *A. hemigymnus*, and the migratory myctophids *L. dofleini* and *N. elongatus*, the latter whose migrant behaviour implicates higher energy requirements.

11. An ability to prey on diverse taxa of zooplankton would be advantageous for mesopelagic fishes in a changeable and oligotrophic ecosystem. This versatility partly results in high numerical abundance of midwater fishes in the pelagic environment.

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