

The role of food availability in regulating the feeding dynamics and reproductive cycles of bathyal benthopelagic fish in the northwest Mediterranean slope

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Abstract

Trophic dynamics and reproductive cycles of fish from the middle slope of the northwest Mediterranean Sea were investigated seasonally from ~ 500 to 1000 m depth. Gut contents of seven species were analyzed, and weights of gonads and livers and C:N ratios of muscle were measured. Additionally, food availability was estimated through analyses of densities of potential prey and of indicators of organic matter sources. The trophic structure of the community was a function of the size of predators and of the height in the near-bottom water column where megafauna fed. We established three trophic groups: benthic feeders, non-migrator macroplankton feeders, and small-sized fish, the latter feeding on macroplankton and hyperbenthos. Seasonal changes in fullness (*F*) were coupled to cycles of secondary production and to the availability of different food sources. The *F* of benthic feeders was related to total organic carbon percentage in the sediments, to surface production 2 months before the sampling, and to the abundance of benthic and hyperbenthic prey. For plankton consumers, lipids in the sediments and the abundance of *Pasiphaea* spp. explained trends in *F*. Temporal trends in gonadosomatic index (GSI) correlated to food derived from the winter bloom of surface production. Also, increased feeding intensity preceded increases in GSI, but different energy use strategies were deduced from the almost species-specific relationships between the GSI and different biological indices. Species from the same trophic guild reproduced at different times of the year, pointing to the existence of species-specific life strategies that enhance reproductive success.

The Deep-Sea floor is the largest ecosystem on Earth and it supports one of the highest levels of biodiversity (Snelgrove and Smith 2002). Deep-sea fauna rely almost exclusively (with the exceptions of hydrothermal vents and cold seeps) on allochthonous food sources generated in the euphotic zone, which are mainly transported vertically but can also be advected (Miquel et al. 1994). Only a small percentage of this food reaches the deep-sea bottom, resulting on a strong food limitation that shapes deep-sea biota and communities. In order to overcome this food limitation, organisms develop strategies that contribute to partitioning their food sources. Depth, food size, and the vertical distribution of food in the sediment or in the water column close to the seafloor are the main contributors to food partitioning among macro- and megafauna (Carrasón and Cartes 2002; Papiol et al. 2013).

The deep sea is not uniformly food poor, and food availability in deep-sea habitats varies regionally (e.g., between the Atlantic [Laborde et al. 1999] and the Mediterranean [Miquel et al. 1994]). The spatial differences in productivity regimes and the associated food inputs enhance proliferation of different meiofauna and macrofauna groups, resulting in distinct trophic webs where dominant megafauna consume particulate matter (e.g., echinoderms in the deep Atlantic [Iken et al. 2001]) or pelagic and benthic organisms (e.g., decapod crustaceans in the deep Mediterranean [Fanelli and Cartes 2008]). In the middle slope of the Catalan Sea (northwest Mediterranean), benthopelagic fish and decapod crustaceans (Stefanescu et al. 1994; Papiol et al.

2012) that primarily feed on macrofauna dominate the megafauna communities.

The amount of food reaching bathyal depths also varies seasonally, mainly depending on hydrographic conditions, on variations in primary production from the photic zone, and on changes in advective fluxes. In the continental slope of the northwest Mediterranean sea, the vertical flux of particles originating near the surface is greatest after the late-winter surface blooms when the water column is thermally homogeneous. Low particle flux is associated with periods of stratification that usually extend from May to November (Miquel et al. 1994). Additionally, advective currents through submarine canyons in the study area play a role in food availability, either by carrying organic matter across the slope or by resuspending organic matter (OM) from the upper sediment layers. These advective fluxes also vary seasonally (Buscail et al. 1990), and they are greatest in spring and least in summer. The changes in OM inputs cause fluctuations in the abundance of macrofauna relying on this food source (Cartes et al. 2002), which presumably ultimately lead to seasonal changes in dietary composition of deep-sea fish (Madurell and Cartes 2005; Fanelli and Cartes 2010). Yet, the link between food inputs and trophic responses at a community level still remains unclear.

In a fairly constant physical environment such as the Deep Sea, the temporal oscillations in food availability have also been proposed as driver of the reproductive cycles of deep-sea species (Gage and Tyler 1991). The relationship between reproductive cycles or recruitment patterns and cyclic food availability has been established for some macrofaunal groups (Cartes 1998; Cartes et al. 2002) and

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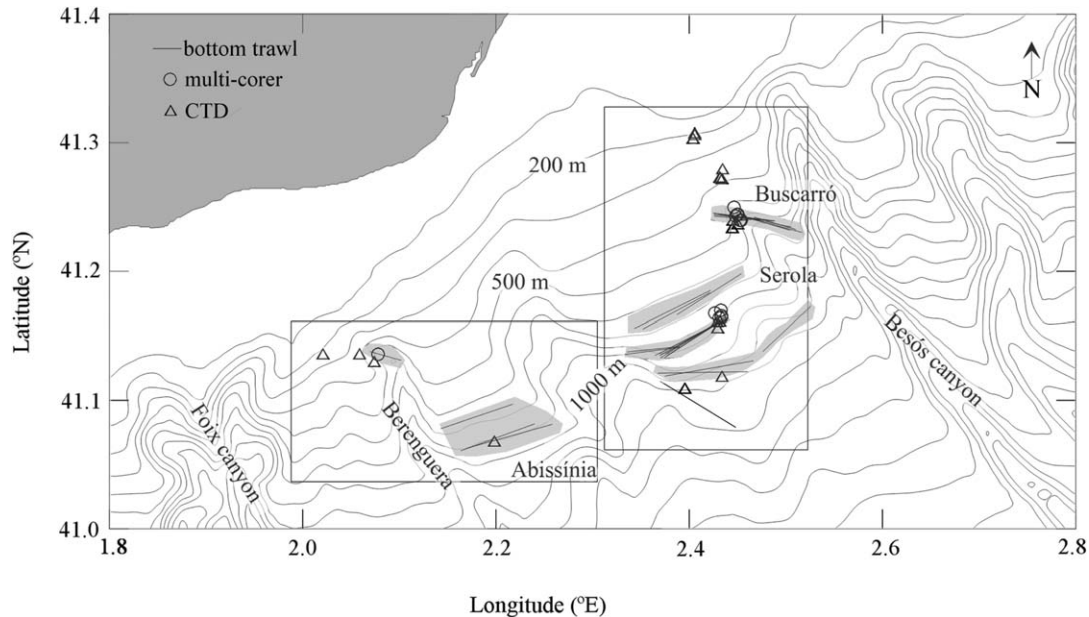


Fig. 1. Study area and sampling points with gear used specified.

for detritivorous megafaunal species (Ginger et al. 2001; Hudson et al. 2004). Yet, few studies have tackled this issue in deep-water predators. Fanelli and Cartes (2008) and Cartes et al. (2008) have related the reproductive cycles of bathyal benthopelagic shrimps to changes in their feeding habits, showing greater consumption of energy-rich prey during gonad development in prereproductive periods. Biological cycles of benthopelagic shrimps have also been related to vertical fluxes of organic matter reaching bathyal depths (Puig et al. 2001; Cartes et al. 2008), and delayed responses to blooms in surface production have been observed. Nevertheless, this issue remains largely unexplored in deep-sea benthopelagic fish (Drazen 2002a; Kemp et al. 2008).

In slope fauna both seasonal and continuous reproductive patterns have been found, specifically among fish (Drazen 2002a; Rotllant et al. 2002). Studies on the reproductive cycles of fish have usually focused on single species or species from the same families, and reproduction studies for species from different families have barely been addressed. Besides, distinctive species-specific timing of reproductive cycles is usual among coexisting slope fish (Massutí et al. 1995; Rotllant et al. 2002). If food availability was the most important factor controlling reproduction at bathyal depths, synchronized reproductive cycles might be expected in cohabiting species with contemporary inputs of organic matter. Therefore, the species-specific timing observed suggests that seasonal availability of food does not affect all species' reproductive processes equally.

In our study of middle-slope fish we aimed to: (1) identify the trophic and reproductive seasonal patterns of the whole fish assemblage, (2) determine the coupling between the trophic dynamics and the reproductive cycles, (3) determine the main food sources driving the trophodynamics and reproductive cycles.

The integration of simultaneous information on feeding and reproduction together with environmental data has seldom been performed and can provide useful insights for the comprehension of deep-sea ecosystem functioning.

Methods

Data describing diets and gut fullness of seven species of dominant benthopelagic fish of the middle slope of the Catalan Sea (Balearic Basin, northwest Mediterranean Sea; Fig. 1) were obtained using bottom trawls and analyzed on a seasonal basis over a 1 yr period.

Study area and sampling strategy—Five multidisciplinary surveys were performed between February 2007 and February 2008 on a seasonal basis (February, April, June–July, and October) at depths ranging from 423 to 1175 m within the project Identification of Biomarkers of Anthropogenic Impact in Marine Communities: An Ecosystem Approach (BIOMARE). Since our goal was to identify the main feeding and reproductive patterns within the middle-slope assemblage, distributed between ~ 600 and 1200 m (Stefanescu et al. 1994), three stations in an open slope area were sampled along the isobaths close to 650, 800, and 1000 m (mean depth span per trawl = 26.5 ± 24.4 m). In addition, samplings were performed on the southern wall of the Besòs submarine canyon, locally called El Buscarró, at ~ 600 m depth, because species can perform seasonal feeding and reproductive migrations inside canyons (Stefanescu et al. 1994). Some trawls in El Buscarró spanned > 100 m depth due to the impossibility to trawl along isobaths inside this canyon. Further sampling was performed in June–July at 650 and 800 m depth over a neighboring area 20 km south of Serola. Two different fishing gears were used for sampling. Nineteen hauls were performed using a semi-balloon otter trawl

Table 1. Fish species examined in the present study. Length (TL = total length; PAL = preanal length) and mean weight ranges, number of specimens dissected for measuring biological parameters, number of females dissected for measuring biological parameters, number of stomach contents analyzed that contained food (number of intestine contents analyzed in brackets), number of everted stomachs by season (win = winter; spr = spring; sum = summer; aut = autumn). Trophic guilds (*mM* = migrator macroplankton feeders; *nmM* = non-migrator macroplankton feeders; *B* = benthos feeders) from the literature are also indicated.

Species	Length (mm)	Mean weight (g)	Season	Dissected	Females	Diet	Everted	Trophic guild	Source
<i>Galeus melastomus</i>	300–605 TL	297±179	win spr sum aut	50 11 42 55	14 8 16 25	41 11 40 54	0 0 0 0	<i>mM</i>	Fanelli et al. 2007
<i>Hymenocephalus italicus</i>	30–50 PAL	7±2	win spr sum aut	39 18 91 22	19 12 52 10	19 8 60 20	8 1 13 3	<i>nmM</i>	Macpherson 1979; Fanelli and Cartes 2010
<i>Lampanyctus crocodilus</i>	100–180 TL	20±10	win spr sum aut	51 42 130 40	28 21 38 5	31 20 76 20	0 0 1 0	<i>mM</i>	Fanelli et al. 2014
<i>Micromesistius poutassou</i>	200–350 TL	120±57	win spr sum aut	48 17 15 31	19 12 9 24	12 12 10 18	0 2 1 1	<i>nmM</i>	Macpherson 1981
<i>Nezumia aequalis</i>	22–58 PAL	27±14	win spr sum aut	42 13 45 21	20 8 16 19	28 10 31 19	4 0 2 3	<i>B</i>	Macpherson 1979; Fanelli and Cartes 2010
<i>Phycis blennioides</i>	150–400 TL	147±96	win spr sum aut	32 28 41 36	8 13 20 15	5(22) 4(17) 14(23) 12(34)	21 12 27 24	<i>B</i>	Macpherson 1981
<i>Trachyrhynchus scabrus</i>	95–150 PAL	71±53	win spr sum aut	21 18 33 89	12 8 12 43	6(20) 4(12) 2(32) 7(85)	12 12 31 80	<i>B</i>	Macpherson 1979
Total				1121	506	594(245)			

(OTSB-14; Merrett and Marshall 1981) from the oceanographic vessel *García del Cid*. Twelve hauls were carried out on board the commercial vessel *Stella Maris III* with a commercial trawl (CT) gear locally called *bou* (Cartes et al. 2009). Details of the OTSB-14 samplings can be found in Papiol et al. (2012). The CT was only used at two stations (at ~ 600 m and ~ 800 m), and those tows were performed on dates close to OTSB-14 tows.

All hauls were carried out during daytime (06:58–20:55 h Greenwich Mean Time), and the duration of each haul (time on bottom) ranged between 45 and 90 min at a towing speed of around 1.34 m s⁻¹.

Samples of surface sediment (0–2 cm depth) for analysis of particulate organic matter (POM_{sed}) were collected with a multicorer from the 600 and 800 m stations throughout the sampling period. Sediment was immediately frozen at –20°C for later analysis.

Analyses of stomach contents and biological parameters—All benthopelagic megafauna collected was identified to species level, counted, measured, and weighed. Specimens were immediately frozen at –20°C for future analyses.

At the laboratory, we analyzed the biological parameters of 1121 fish belonging to the seven species dominating the slope assemblage (Table 1). Only adult individuals were selected for analysis. All individuals (ind.) were sexed and weighed (± 0.001 g), and total length (mm) (preanal length in the case of macrourids) was recorded. Stomach content, liver, and gonads were removed and weighed (± 0.001 g). The food items were identified to species or to the lowest feasible taxonomic level, and their weight after drying with blotting paper was recorded to the nearest 0.1 mg. Although analysis of gut contents has limitations (Fanelli and Cartes 2008) that are especially intense for the deep sea (e.g., hydrostatic decompression regularly results in regurgitation of prey in some deep-sea fish), they provide a great deal of information, e.g., on gut fullness variation or on specification of prey. Intestine contents were analyzed for species in which a significant number of stomachs were everted (i.e., *Trachyrhynchus scabrus* and *Phycis blennoides*). Prey in intestines were more broken and digested than those in stomachs, but hard parts (otoliths, mandibles, etc.) allowed prey identification, sometimes to species level. Due to greater digestion, prey remains in intestines were strongly mixed, and direct prey weighing was impossible in practice. For this reason, for intestines the percentage volume of each prey was estimated by the subjective points method (Swynnerton and Worthington 1940).

White muscle tissue from fishes was sampled for analyses of organic carbon and nitrogen. Samples were dried to constant weight at 60°C, then ground to fine powder. Total organic carbon (TOC) and total nitrogen were determined from the CO₂ and N₂ produced by combustion using a Thermo Electron Flash EA 1112 elemental analyzer.

Analyses of sediment organics—TOC of sediments (TOC_{sed}) was determined by the same procedure employed for muscle tissue.

Total lipid content of sediments was also analyzed following a modified Bligh and Dyer method (Alfaro et al.

2006). Lipids are a proxy for organic matter quality and phytodetritus is lipid rich (Fabiano et al. 2001); thus, lipids were used as indicators of fresh phytodetritus inputs.

In addition, phytoplankton pigments in sediments were analyzed as indicators of OM from surface primary production in the area (Vidussi et al. 2000) by means of reverse-phase high-pressure liquid chromatography.

For all sedimentary analyses, three replicates were analyzed for each sample at each collection station in each season. Details of the analyses of sediment organics can be found in Papiol et al. (2013).

Environmental variables—Casts were performed at each station with a Sea-Bird Electronics 32 Carousel water sampler equipped with a conductivity, depth, and temperature sonde (CTD) coupled with a fluorometer. Locations were the same as those where biological and POM_{sed} samples were collected. Values of fluorescence were recorded for each CTD profile at 5 m above the sea bottom (5 mab).

Phytoplankton pigment concentration (PPC; mg chlorophyll *a* m⁻³), obtained from <http://disc.sci.gsfc.nasa.gov/giovanni> (accessed 15 September 2010), was used as a rough index of surface primary production in the area. We used monthly average PPC values at the bottom trawl positions coincident with sampling, and 1, 2, and 3 months before sampling.

Data analysis—Two-way permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) with a crossed design considering sampling sites and surveys as factors was used to test spatial and temporal differences among environmental variables and sediment organics.

Abundances of the megafauna species analyzed and of potential prey species were standardized to a common swept area of 0.01 km² (ind. 0.01 km⁻²), according to the values proposed by Cartes et al. (2009).

Trophic data: The dietary composition was analyzed for the 594 individuals with stomachs containing food (Table 1). Intestine contents of 96 individuals of *P. blennoides* and of 149 individuals of *T. scabrus* were also analyzed. The contribution of each prey item to the diets was standardized to total fish weight (prey item weight/total fish weight). Analysis of similarities (ANOSIM) between intestine and stomach contents of the same individuals was performed using Bray–Curtis similarity index after square-root transformation of diet proportions by weight. Given the significant differences found between contents of the two organs (ANOSIM $p < 0.05$), seasonal patterns were studied based only on intestine contents, because the low number of available stomach contents provided inadequate dietary description.

Stomach fullness (F) was used to measure feeding intensity and was calculated as:

$$\%F = 100(\text{stomach content weight/predator weight}) \quad (1)$$

Overall diet of species: Diet was analyzed for each species in each haul of each survey. We analyzed between 8 and 26 individuals per haul for all species, each haul constituting a sample. We considered minimum sample sizes of 8 individuals as the minimum representative for

diet analysis, as reflected by cumulative curves. For the 25% of hauls analyzed with low numbers of individuals (< 8), specimens from different hauls within the same survey were pooled to attain minimum sample sizes of 8 individuals. Previous to this, and in order to check if lumping of fishes sampled at different depth strata could be a source of error, we checked for bathymetric differences in the diet composition of each species by means of a two-way permutational univariate ANOVA (Anderson 2001) with a crossed design considering both survey and depth stratum (600 = canyon, 650, 800, and 1000 isobaths) as factors. The interaction between the two factors was never significant, and we proceeded. Finally, for each diet category (prey type) in each sample, prey biomass was standardized to an equivalent for 10 individuals by the formula: $10(\text{average prey mass}/\text{individual})$.

To identify overall trophic interactions among fish species, a hierarchical cluster analysis (average grouping method) was performed on the square-root-transformed biomass data using the Bray–Curtis similarity index. A similarity profile (SIMPROF) test was applied to obtain statistical significance of the groups obtained by cluster analysis (Clarke and Warwick 2001). To illustrate observed similarities or dissimilarities (distances) between the groups resulting from the cluster analyses, multidimensional scaling (MDS) analysis was carried out on the same data matrix. The groups obtained were compared with postulated trophic groups based on the literature (Table 1). Similarity percentage analysis (SIMPER) was performed to identify characteristic prey for each group obtained by cluster analysis and to calculate the degree of similarity and dissimilarity among diets of the different groups of species.

Seasonal intraspecific variations: Seasonal variations in diets of each species were measured by applying a one-way PERMANOVA to square-root-transformed proportional biomass data of individual stomach contents based on the Bray–Curtis similarity index. Additionally, temporal trends in prey consumption (biomass) were studied by merging all replicates from the same season.

Two-way permutational univariate ANOVA with a crossed design considering both sex and survey as factors was used to test sexual differences in fullness. Pair-wise tests were performed when the interaction between the two factors was significant in order to allocate the source of variation.

Biological indices: Two indices were calculated for describing the overall physiological condition of the fish. The hepatosomatic index (HSI) was calculated as a proxy for energy reserves stored in the liver: $\%HSI = 100(\text{liver weight}/\text{body weight})$. The gonadosomatic index (GSI) is often considered as a surrogate for reproductive effort, and it was calculated as: $\%GSI = 100(\text{gonad weight}/\text{body weight})$. All weights were wet weights. Taking into consideration that females allocate more energy to reproduction than males (Tsikliras et al. 2010) and that the liver plays an important role in energy storage, HSI and GSI were only estimated for adult females.

In addition, C:N ratios were calculated for muscle tissue, since C:N is a relatively good surrogate for tissue

lipid content (i.e., samples containing more lipids have higher C:N; Tieszen et al. 1983).

One-way permutational univariate ANOVAs based on Euclidean distances were adopted to test seasonal changes in fullness, C:N, GSI, and HSI after arcsine transformation in order to approximate normality. Significance was set at $p = 0.05$; p -values were obtained using 9999 permutations of residuals under a reduced model.

Drivers of fullness and reproductive cycle: A draftsman plot (Clarke and Warwick 2001) was applied to environmental variables to identify whether any of them were strongly correlated, thus providing redundant information. Redundant variables ($r > 0.70$) were discarded, simplifying the matrix for later Generalized Linear Models (GLMs).

In order to identify which variables explained patterns of fullness and GSI, values of fullness and GSI were compared with independent explanatory variables by means of GLMs after normalizing the dependent variables by arcsine transformation. Inherent interindividual variations of fullness values within a sample prevent the analysis of fullness per individual; thus, in GLMs for fullness we used mean values of fullness per haul and species. GLMs for fullness were calculated on each of the trophic groups obtained by cluster analyses for two main reasons: (1) avoiding eventual low sample sizes resulting from analyzing data per haul and species and (2) detecting the main food inputs driving fullness patterns on each of the trophic guilds identified by cluster analysis as a whole. GLMs for GSI were calculated on each species using GSI per individual (not mean per haul) in order to increase sample size. The distribution family used was Gaussian with identity link. The models were computed by adding single terms based on minimizing Akaike's Information Criterion and only including variables that were significant ($p < 0.05$). Both in the fullness and the GSI models we included environmental variables: (1) surface phytoplankton pigment concentration (PPC) simultaneous with and 1, 2, and 3 months before the sampling, (2) fluorescence_{5mab}, (3) sediment TOC_{sed}%, (4) lipids_{sed}, and (5) the sum of sediment chlorophylls (chlorophylls and their degradation products). Standardized abundances of potential prey of megafaunal fish caught with the OTSB-14 (i.e., sergestid and pasipheid shrimps, brachyuran crabs, benthic shrimps including *Calocaris macandreae* and *Alpheus glaber*, nekto-benthic decapods—mainly pandalids and *Acanthephyra eximia*, mesopelagic myctophids, and benthopelagic fish, euphausiids, mysids, isopods, and polychaetes) were also included in the model, as were predator density, mean length and W (biomass/abundance), or individual length and W (in the case of models for GSI). For the models of GSI, fullness, C:N ratio, and HSI were also included, as they are indicators of energy consumption and storage in the organism. Considering fullness is an immediate reflection of what is consumed, we compared fullness with variables sampled simultaneously. Given that gonad growth has some delay in relation to the food ingested or the energy stored in the organism, values of GSI were compared with variables sampled simultaneously and those from the previous sampling. For previous biological indices, mean values per sampling station were used

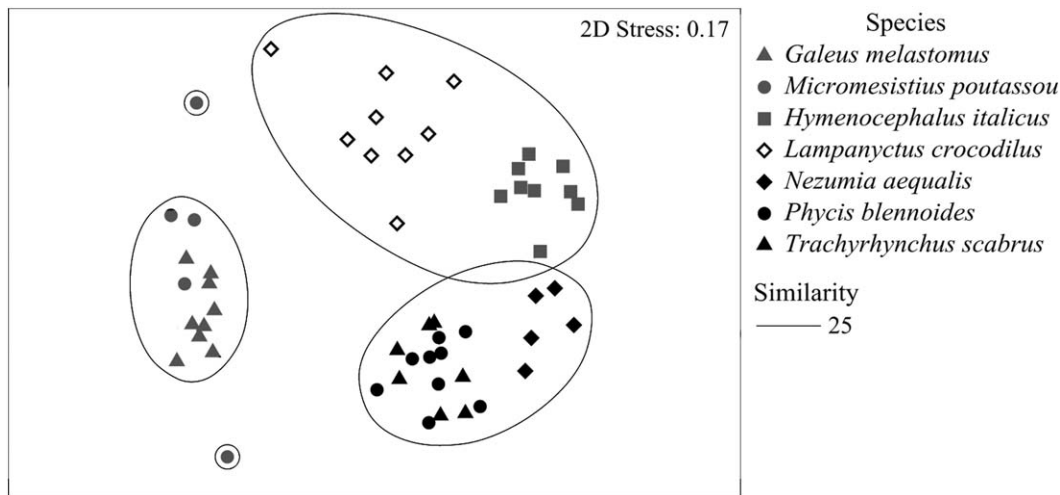


Fig. 2. MDS ordination plot of stomach contents of the different fish species per haul, based on Bray–Curtis resemblance matrix of square-root-transformed prey weight data. Data points are identified by species and by trophic guilds from the literature (Table 1): black = benthos feeders; dark gray = non-migrator macroplankton feeders; white = migrator macroplankton feeders. Circles indicate cluster overlay at 25% of similarity.

because we considered the animals sampled in each season as a cohort from the same population.

All statistical analyses were performed using PRIMER6 and PERMANOVA+ (Clarke and Warwick 2001; Anderson 2001), R 2.14.2 (<http://www.r-project.org/>), and STATISTICA 6 (StatSoft Inc.) software.

Results

Organic matter sources—At 600 m TOC% in sediments was highest in February and lowest in June. Total lipids in sediments at 600 m were highest in February and April and lowest in June–July and October. Both patterns of TOC% and lipid content were inverse at 800 m.

At 600 m concentration of chlorophylls and degradation products in sediments were lowest in February and greater during the rest of the year, with maxima in April and October. At 800 m, minimum concentration was in April, and it increased afterwards.

Values and seasonal trends in fluorescence measured 5 mab were similar at all depths sampled. Maximum fluorescence was observed in February relative to the low values of both April and June–July. Surface PPC followed the same temporal sequence at all sites: minimum values in June–July, and maximum values in January and April.

More detailed results on organic matter variables are detailed in Papiol et al. (2012, 2013).

Overall diet of species and resource partitioning—The MDS analysis performed on dietary data shows that both the position of predators along a benthos–plankton gradient, i.e., the vertical distribution of their prey in the near-bottom water column, and the size of predators are the main structuring factors (Fig. 2). Combining the results of cluster analysis with the MDS ordination we distinguish three groups at 25% similarity. Benthos feeders (*B*; i.e., *Phycis blennoides*, *Nezumia aequalis*, and *T. scabrus*) cluster together at the right side of the MDS, while non-migrator

macroplankton feeders (*nmM*; *Micromesistius poutassou* and *Galeus melastomus*) cluster on the other side. The small macrourid *Hymenocephalus italicus*, classified a priori as *nmM*, groups with the migrator–macroplankton feeder (*mM*) *Lampanyctus crocodilus*, constituting a group that comprises the small-sized fish species. SIMPROF test shows that the groups obtained by cluster analysis differ significantly in their multivariate structure ($p < 0.01$).

SIMPER reveals that dissimilarities between all groups are $> 75\%$. Greatest dissimilarity is between benthic feeders and *nmM* (90.26%). For benthic feeders, 55.14% of the diet was accumulated by the crab *Monodaeus couchii*, the benthic shrimp *Calocaris macandreae*, the hyperbenthic isopods *Munnopsurus atlanticus* and *Natatolana borealis*, and the mysid *Boreomysis arctica*. For the *nmM*, 55.81% of the diet was characterized by pasipheid shrimps and mesopelagic myctophids. Main prey (53.73% of the diet) of small fish species (i.e., *L. crocodilus* and *H. italicus*) were hyperbenthic mysids (*B. arctica*) and isopods (*Natatolana borealis*).

Seasonal trends in diets—Statistical significance estimates for seasonal variations in diets are in Table 2.

Benthos feeders: The most abundant prey in the diet of *Nezumia aequalis* were benthic polychaetes and hyperbenthic gammaridean amphipods (suborder Gammaridea), especially *Rhachotropis* spp. (Fig. 3). Main prey of *Phycis blennoides* were the brachyuran crab *Monodaeus couchii*, the hyperbenthic isopods *Natatolana borealis* and *Munnopsurus atlanticus*, and gammaridean amphipods (Fig. 3). Most abundant prey in the diet of *T. scabrus* were benthic polychaetes, *Monodaeus couchii*, and *Natatolana borealis*.

Consumption of polychaetes was rather constant throughout the year in all benthos feeders. Diets of both macrourids *Nezumia aequalis* (pseudo- $F_{3,87} = 2.61$; $p < 0.001$) and *T. scabrus* (based on intestine contents; pseudo- $F_{3,148} = 2.43$; $p < 0.05$) changed seasonally, but that of *Phycis blennoides* did not. Diets of both *Nezumia aequalis*

Table 2. Results of PERMANOVA pair-wise tests comparing diet composition of each fish species in consecutive seasons (win = winter; spr = spring; sum = summer; aut = autumn) based on the Bray–Curtis similarity matrix and on 9999 permutations. t -values and p -level significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) indicated. ns = not significant.

Species	win–spr	spr–sum	sum–aut	aut–win
<i>Nezumia aequalis</i>	1.75*	1.42*	ns	1.65*
<i>Phycis blennoides</i>	ns	ns	ns	ns
<i>T. scabrus</i>	ns	1.57*	ns	ns
<i>H. italicus</i>	2.21*	1.72*	ns	ns
<i>L. crocodilus</i>	ns	1.46*	ns	1.56*
<i>G. melastomus</i>	1.60*	ns	2.19***	1.60*
<i>Micromesistius poutassou</i>	ns	ns	1.82*	2.28**

and *T. scabrus* changed from April to June–July, mostly associated to an increased consumption of hyperbenthic, rather than benthic, prey in June–July (greater proportions of gammaridean amphipods in *Nezumia aequalis* and of isopods, namely *Munnopsurus atlanticus*, in *T. scabrus*). Diet of *Nezumia aequalis* also changed from February to April and from October to February. *Calocaris macandreae* was important prey in February and April and *B. arctica* in

October. Diet of *T. scabrus* did not change across any other seasons.

Non-migrator macroplankton feeders: The most abundant prey in the diet of *G. melastomus* were the mesopelagic shrimp *Pasiphaea multidentata*, *Monodaeus couchii*, and cephalopods (Fig. 4). In contrast, the diet of *Micromesistius poutassou* (Fig. 4) mainly comprised mesopelagic fish (namely myctophids) and pasipheid and sergestid shrimps.

The diet of both *nmM* changed seasonally (*G. melastomus* pseudo- $F_{3,145} = 3.07$; $p < 0.001$ and *Micromesistius poutassou* pseudo- $F_{3,51} = 2.62$; $p < 0.001$), and for both species the diet in October differed from that in the previous and consecutive seasons. In October both species mostly consumed mesopelagic crustaceans: *Pasiphaea multidentata* was dominant prey of *G. melastomus* and sergestids dominated in the diet of *Micromesistius poutassou*. The diet of *G. melastomus* both in February and June–July consisted of similar proportions of the dominant prey mentioned above. In contrast, cephalopods were dominant prey in April. In *Micromesistius poutassou*, mesopelagic fish were important prey from February to June–July, as were pasipheids in April and June–July.

Small-sized fish: Hyperbenthic prey, namely *B. arctica*, gammaridean amphipods, and *Natolana borealis*, were

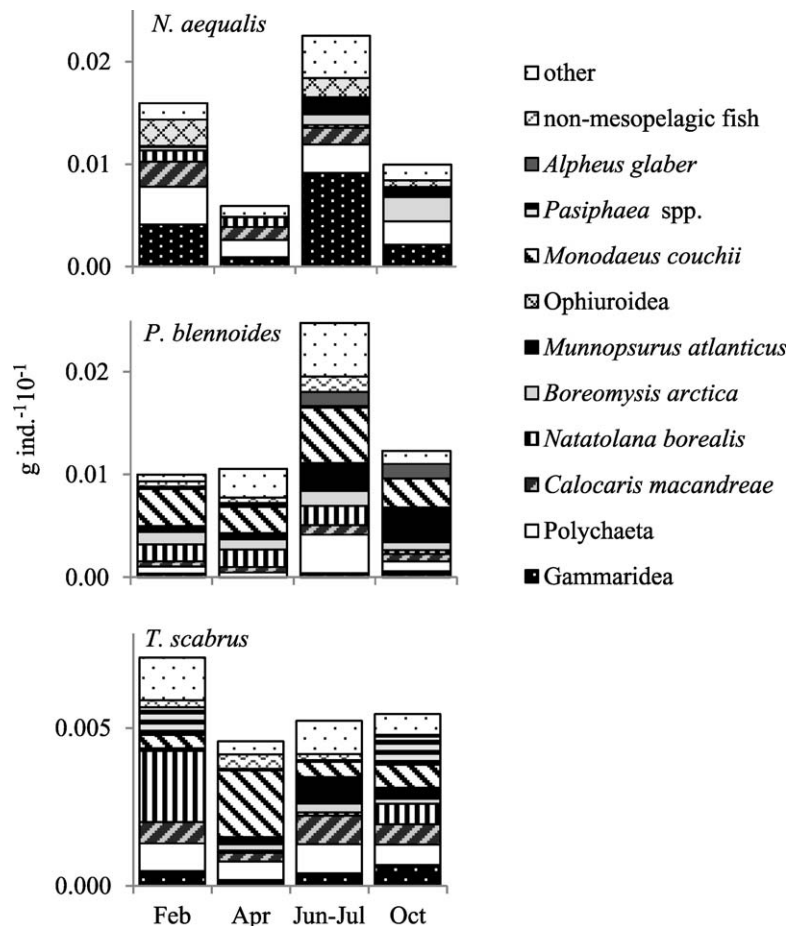


Fig. 3. Seasonal changes in stomach (*Nezumia aequalis*) or intestine (*Phycis blennoides* and *Trachyrhynchus scabrus*) content (by weight) of benthos feeder fishes.

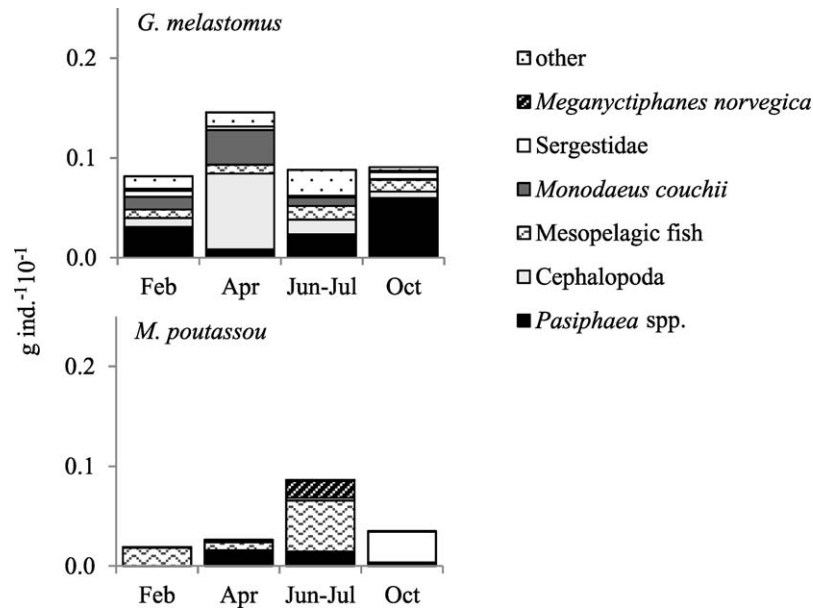


Fig. 4. Seasonal changes in stomach content (by weight) of non-migrator macroplankton feeder fishes.

the main prey of *H. italicus* (Fig. 5). In contrast, main prey of *L. crocodilus* were sergestid shrimps, euphausiids, and *B. arctica* (Fig. 5).

For both species diet changed seasonally (*H. italicus* pseudo- $F_{3,108} = 2.65$; $p < 0.01$ and *L. crocodilus* pseudo- $F_{3,146} = 1.75$; $p < 0.05$), changing from April to June–July in both cases, as observed in benthos feeders. We observed that both species increased the consumption of natatorial prey in June–July, e.g., euphausiids and *B. arctica*. In addition, *H. italicus* consumed the shrimp *Processa canaliculata* (category “other” in Fig. 5), and *L. crocodilus*

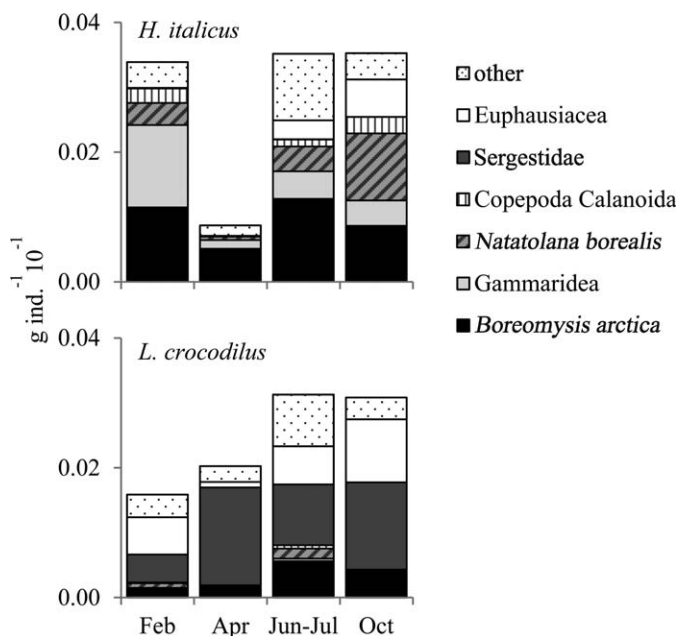


Fig. 5. Seasonal changes in stomach content (by weight) of small-sized fish species.

ate sergestids. Greater consumption of more natatorial prey (i.e., *Meganyctiphanes norvegica*, sergestids) persisted to October in both species.

Seasonal trends in fullness (F)—The interaction between the two factors (sex and survey) of the two-way ANOVA performed on fullness of each species was never significant ($p > 0.05$). Likewise, no significant pair-wise comparisons were found. Thus, *F* of males and females were analyzed jointly.

F of the benthos feeders *Nezumia aequalis* and *Phycis blennoides* was greatest in June–July (Fig. 6). High *F* persisted to October in *Nezumia aequalis* but not in *Phycis blennoides*. *F* of *T. scabrus* was greatest in February, and low the rest of the year, changes not being significant.

G. melastomus and *Micromesistius poutassou* both presented greatest *F* in June–July (Fig. 6). Relatively high *F* was maintained in October, and significantly lowest *F* occurred in February. *F* of *G. melastomus* was the greatest of all species.

H. italicus and *L. crocodilus* followed a similar temporal pattern of increasing *F* from April to October (Fig. 6); minimum *F* in April was significant in *H. italicus*. In February *F* was high in *H. italicus* but low in *L. crocodilus*.

Seasonal trends in C:N ratio of muscle—The muscle C:N of *Nezumia aequalis* was significantly greater in October than in February and April (Table 3). For this macrourid, we did not have results in June–July. Both *Phycis blennoides* and *T. scabrus* had greatest C:N in April, followed by significant minima in June–July. *G. melastomus* had greatest C:N in June–July and lowest C:N in April, the decrease from February to April being the only significant change. C:N of *Micromesistius poutassou* remained relatively high from February to June–July, and it was significantly lower in October. C:N of *H. italicus* remained constant throughout the whole period. In

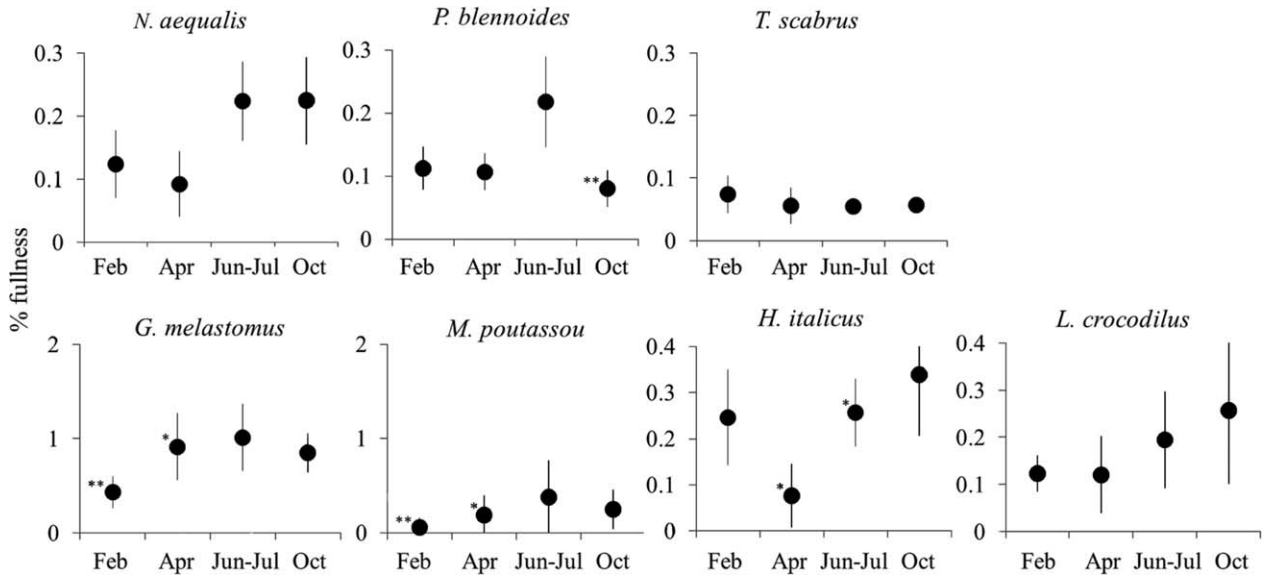


Fig. 6. Seasonal trends in %fullness (F) of the different fish species. Vertical bars indicate 95% confidence intervals. Asterisks indicate significant differences with preceding sampling: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

contrast, *L. crocodilus* C:N increased from February to June–July, and then decreased significantly such that C:N was similar in October and February.

Seasonal trends in biological indices—Among species feeding upon similar trophic resources, GSI showed different temporal patterns (Fig. 7). The most common pattern observed was of greatest (or almost for *Nezumia aequalis*) GSI in February. *G. melastomus* and *Phycis blennoides* had greatest GSI in October. Most species presented marked seasonal peaks in GSI, but the two macrourids, *H. italicus* and *Nezumia aequalis*, had high GSI values during various seasons, suggesting continuous reproduction.

Out of the seven species studied, five presented a trend of high HSI preceding maximum GSI (Fig. 7). In *L. crocodilus* and *T. scabrus* the pattern was the opposite. The HSI peaked seasonally in species in which GSI did so. In contrast, HSI varied little in species presenting prolonged high GSI (i.e., *Nezumia aequalis* and *H. italicus*).

Table 3. C:N ratio of muscle tissue of the different fish species by season (win = winter; spr = spring; sum = summer; aut = autumn). Significant differences with contiguous season indicated with asterisks (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

	win	spr	sum	aut
<i>G. melastomus</i>	2.29*	2.13	2.38	2.23
<i>H. italicus</i>	3.92	4.04	3.90	3.83
<i>L. crocodilus</i>	2.70**	3.35***	3.86***	2.79
<i>Micromesistius poutassou</i>	2.34	2.51	2.26*	1.19
<i>Nezumia aequalis</i>	2.29	2.25	—	2.78*
<i>Phycis blennoides</i>	1.95	2.45**	1.85*	2.30
<i>T. scabrus</i>	2.94	3.24*	2.09*	2.95

Drivers of fullness (F)—The GLMs performed on F data for each trophic group identified by the hierarchical cluster analysis revealed that fullness of benthos feeders was negatively correlated to abundances of mysids and benthic polychaetes (Table 4). In contrast, benthos-feeders F was positively related to surface production 2 months before the sampling and to TOC% in sediments. GLM explained 49% of the total variation.

The GLM for the *nmM G. melastomus* and *Micromesistius poutassou* explained 65% of the total variation. The GLM revealed that F was greater in heavier animals (Table 4). Additionally, lipids in sediments were inversely correlated with F , while positive correlation was observed between F and the abundance of *Pasiphaea multidentata*.

For *H. italicus* and *L. crocodilus*, the GLM explained 80% of the total variation and included C:N ratio and lipids as explanatory variables, the former positively and the latter negatively correlated to F (Table 4).

Relationship between reproductive cycle and food availability—The GLMs performed on the GSI of females revealed that either length or weight were positively correlated with GSI in six of the seven species (not in *Micromesistius poutassou*; Table 5). No other variables had such a consistent influence on the models. Besides, indicators from surface production (i.e., surface production 1 and 2 months before the samplings and lipids in sediments) were positively or negatively correlated with GSI in six of the seven species. The variation explained by the GLMs performed on GSI ranged between 33% (*H. italicus*) and 74% (*L. crocodilus*).

Discussion

In the present study we integrated information of both the trophic ecology and the reproductive cycles of middle-

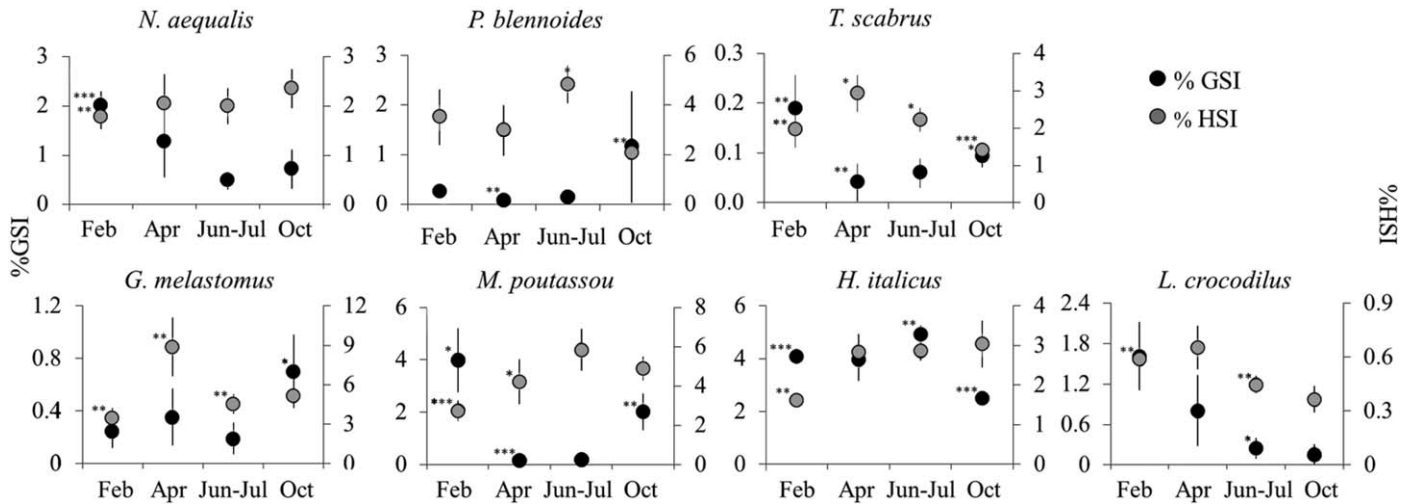


Fig. 7. Seasonal trends in gonadosomatic index (%GSI) and hepatosomatic index (%HSI) of females of the different fish species. Vertical bars indicate 95% confidence intervals. Asterisks indicate significant differences with preceding sampling: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 4. Generalized Linear Models performed on fullness of fish, considering the trophic groups obtained by cluster analyses. df = degrees of freedom; PPC_{2months} = phytoplankton pigment concentration at surface 2 months before the sampling; TOC_{sed}% = total organic carbon in sediments; AIC = Akaike's Information Criterion; Lipids_{sed} = lipids in sediment; Polychaetes = density of polychaetes; Mysids = density of mysids; *P. multidentata* = density of *Pasiphaea multidentata*; NULL = null model.

	% explained	F	Sign of correlation	p-value
Benthos feeders				
NULL				
PPC _{2months}	11.88	5.822	(+)	0.023
Polychaetes	14.00	6.860	(-)	0.015
Mysids	13.31	6.526	(-)	0.017
TOC _{sed} %	9.80	4.805	(+)	0.038
	48.99			
df 29				
AIC=-202.38				
Small fish species				
NULL				
Muscle C : N	59.32	16.496	(+)	0.001
Lipids _{sed} %	20.88	5.807	(-)	0.025
	80.21			
df 24				
AIC=-137.63				
Non-migrator macroplankton feeders				
NULL				
<i>P. multidentata</i>	32.44	15.045	(+)	0.001
Weight	16.69	7.742	(+)	0.013
Lipids _{sed}	16.38	7.596	(-)	0.014
	65.50			
df 19				
AIC=-90.94				

slope fish. We observed that seasonal food availability in the deep sea can be an important driver of feeding and reproductive patterns. Different food inputs drove the trophodynamics of deep-sea fish from different trophic guilds. Besides, in addition to own fish size, food availability, most commonly food derived from the winter surface bloom of production also explained patterns in gonad growth. We also detected a temporal segregation in the reproductive cycles of fish species feeding on similar resources. This was related to almost species-specific relationships between feeding and energy allocation and use.

Although we studied benthopelagic fish of the northwest Mediterranean, most of the genera here considered are widely distributed in the deep sea (e.g., *Trachyrhynchus* spp., *Nezumia* spp.), so our results may apply to a broader context beyond the Mediterranean.

Feeding guilds—As already reported for deep fish of the northwest Mediterranean, fish species partition their food according to size (Macpherson 1981; Carrassón and Cartes 2002) and by preying at different levels of the near-bottom water column. Deep-sea fish exploit a wide array of mesopelagic, hyperbenthic, and benthic prey as resources, but each species preferentially consumes prey comprised within one of these compartments. Hence, trophic guilds can be deduced, as shown by Carrassón and Cartes (2002) from diets below 1000 m. We obtained three trophic groups consisting of large benthic feeders, large macroplankton feeders, and small fish species, the latter mainly feeding on hyperbenthos and small zooplankton. In general, we obtained results for fish diets that agree with previous studies (see Table 1). The only exception was the macrourid *H. italicus*, sometimes considered as a non-migrator macroplankton feeder (*nmM*), which fed mainly on hyperbenthic fauna and only occasionally consumed macroplankton (i.e., the euphausiid *Meganyctiphanes norvegica*).

We observed that feeding intensity varied among feeding guilds, and *nmM*, especially sharks, fed more intensely than

Table 5. Generalized Linear Models performed on gonadosomatic index of the seven species of benthopelagic fish. Species: Naeq = *Nezumia aequalis*; Pble = *Phycis blennoides*; Tsca = *T. scabrus*; Hita = *H. italicus*; Lcro = *L. crocodilus*; Gmel = *G. melastomus*; Mpou = *Micromesistius poutassou*. % deviance explained and significances specified. Sign of the correlation indicated in parentheses. AIC = Akaike's Information Criterion. df = degrees of freedom. Factors: density = density of each fish species; PPC = phytoplankton pigment concentration at surface; sim = simultaneous to sampling; 1 survey = value from the preceding sampling survey; densities of different potential prey sampled with the bottom trawl also indicated.

Factor	Species						
	Naeq	Pble	Tsca	Hita	Lcro	Gmel	Mpou
Length			41.21*** (+)	4.55* (+)	7.10*** (+)		
Weight	4.16* (+)	47.05*** (+)				56.27*** (+)	
Density	47.07*** (+)			4.48* (-)		7.81** (+)	
Fullness _{1survey}				15.00*** (-)			
HSI _{sim}					3.98** (+)		
HSI _{1 survey}	8.70*** (+)	4.69* (+)					6.65** (+)
C: N _{muscle}							43.97*** (-)
PPC _{1 month}		4.03* (-)	2.79* (+)			4.34* (-)	11.88*** (+)
PPC _{2 months}	4.77* (-)						
Lipids _{1survey}			2.65* (+)	9.24** (+)			
TOC _{1 survey}			22.04*** (-)				
<i>Meganyctiphanes norvegica</i> _{1survey}					63.14*** (+)		
% explained	64.70	55.77	68.70	33.22	74.23	68.16	62.50
AIC	-191.69	-209.18	-146.30	-316.42	-269.68	-188.77	-164.05
df	46	53	53	71	57	36	45

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

benthos feeders. Fish that feed on macroplankton are usually active swimmers that forage in the water column and have higher metabolic rates (Koslow 1996; Drazen 2002b). Greater feeding intensity may supply the necessary energy for maintaining higher metabolism. In agreement, high daily rations have been found previously in the sharks *G. melastomus* and *Etmopterus spinax* (Madurell and Cartes 2005) in the eastern Mediterranean. Mesopelagic prey are more energy rich than benthic prey (Madurell and Cartes 2005; Brey et al. 2010), which may contribute to satisfying the higher energy demands of swimming fish.

Seasonal trophic dynamics—Diet composition of benthos feeders (*Phycis blennoides* and *T. scabrus*) varied less seasonally than that of large macroplankton feeders (*G. melastomus* and *Micromesistius poutassou*). This has been reported previously to be a consequence of the lower variation in abundance and composition of benthic macrofauna compared with pelagic macroplankton (Cartes et al. 2010; Mamouridis et al. 2011). However, pronounced changes in the dietary composition of both benthos feeders and small-sized fish were observed from spring to summer. This pattern seems related with a greater contribution of hyperbenthos to their diets after spring, when it is more available (Cartes 1998), as previously reported in the eastern Mediterranean (Madurell and Cartes 2006). Still, species from each of the feeding guilds shifted diets to exploitation of distinct taxa depending of their swimming capacity, i.e., benthic feeders preyed upon less mobile hyperbenthos (i.e., gammaridean amphipods and isopods) than small-sized fishes (i.e., *B. arctica* and *Processa canaliculata*). Among nmM (*G. melastomus* and *Micromesistius poutassou*) main dietary changes were observed in

autumn, when this assemblage shifted diets to feed mainly on mesopelagic crustaceans.

In relation with what is discussed above, peaks in prey availability should explain greater feeding intensities (F) found in summer that usually persisted to autumn. However, the effect of the densities of prey on the F of fish was only partially reflected in our results. This is because the sampling gear used (i.e., OTSB-14 and *bou*) did not provide a complete quantification of all potential prey due, for example, to the small size of some prey (e.g., amphipods) or to their mesopelagic habits (e.g., sergestids). Instead, temporal trends in F were related with indicators of food sources of such prey, and the results concur with the existence of two main trophic webs based on benthic or pelagic production (Iken et al. 2001; Papiol et al. 2013). Among benthos feeders, feeding intensity was greater 2 months after the peak of surface production when there was greater TOC% in the sediments. Those likely support increases of some benthic and hyperbenthic taxa that quickly respond to nutrient inputs (Cartes et al. 2002; Fig. 8). That initial trophic step may in turn increase densities of carnivorous or omnivorous hyperbenthos (e.g., the isopod *Munnopsurus atlanticus*; the amphipod *Rhachotropis* spp.) in summer (Cartes et al. 2001). Those are important prey of fish such as *Nezumia aequalis* (Fig. 8). Thus, fresh inputs of organic matter to bathyal depths are quickly transformed (in ~ 2 months) into food for benthic-feeding fish by a variety of pathways through trophic webs. *T. scabrus* was the exception to this summer maxima of F , and the species did not exhibit any seasonal change in feeding intensity. F may not be properly estimated in *T. scabrus* because it was calculated on intestine contents, where prey of different feeding episodes are accumulated. Also, the large number of

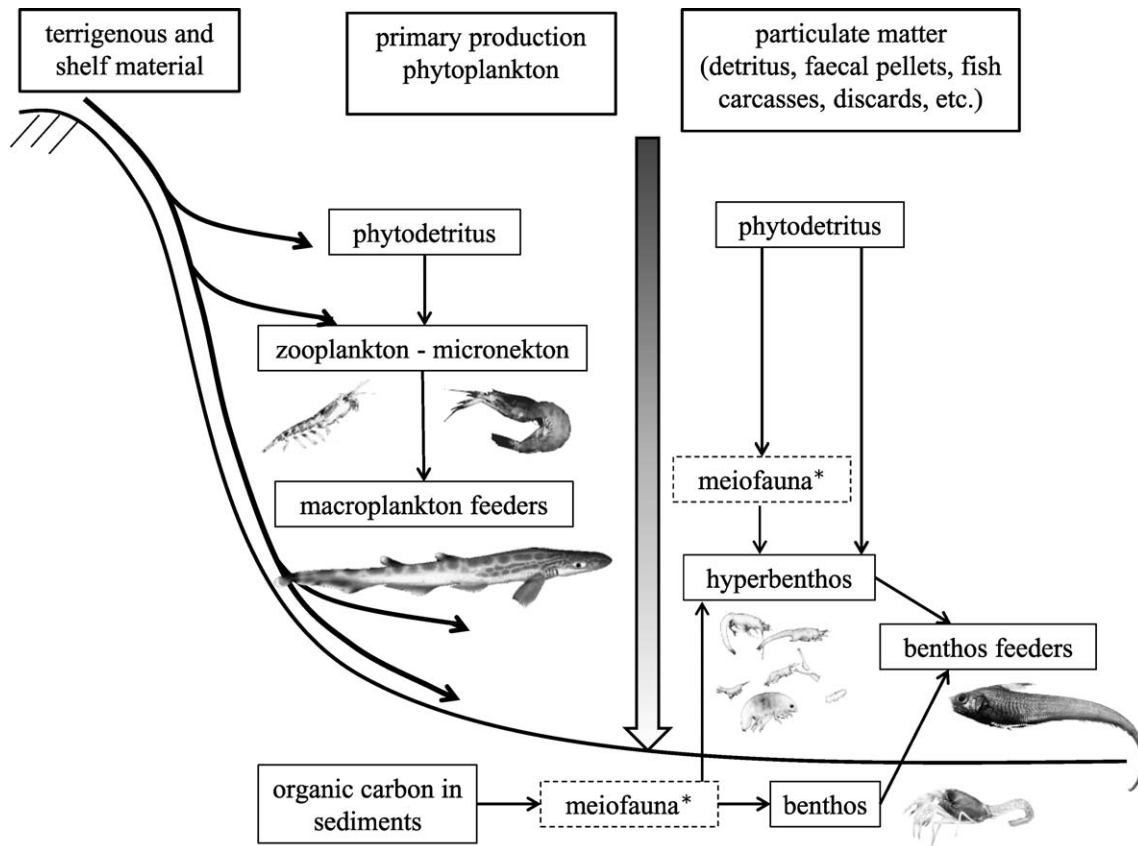


Fig. 8. Scheme of the main fluxes of organic matter to benthos and macroplankton feeder fish off the Catalan slope. An asterisk shows the fauna compartment not analyzed in the present study.

pyloric caeca of *T. scabrus* (unpubl., V. Papiol and J. E. Cartes) may have caused intestine contents that were rather liquefied and only hard parts of prey remained.

The *F* of small-sized fish and of non-migrator macroplankton feeders increased after lipid deposition in sediments. Lipids are easily degradable, highly energetic compounds abundant in phytodetritus (Fabiano et al. 2001), which constitutes the main food of macroplankton (Fanelli et al. 2011). Hence, we expected greater availability and subsequent consumption of planktonic prey after lipid deposition. This is consistent with the increase of gut *F* in: (1) macroplankton feeders, related with the abundance of the mesopelagic decapod *Pasiphaea multidentata*; and (2) small fish, related with greater consumption of mesopelagic crustaceans (Fig. 8). Ingestion of pelagic prey in small fish species favored a quick storage of energy in the muscle tissue, as deduced by the correlation between *F* and C : N of muscle (De Pedro et al. 2001). Exploitation of macroplankton when abundant was not only restricted to macroplankton feeders, and some species (e.g., *T. scabrus* or *H. italicus*) seasonally expanded their trophic niche to use this largely available food source. Generalized dependence of the middle-slope benthopelagic community of the Catalan Sea on mesopelagic prey after winter–spring was also detected through depletion of its $\delta^{13}\text{C}$ signature (Papiol et al. 2013).

In addition to prey availability, seasonal changes in feeding patterns of megafauna may also be driven by the

need to cover higher energy requirements in prereproductive periods, as discussed below.

Relationship between trophic dynamics and reproduction—We observed both patterns of continuous and seasonal reproduction among middle-slope fish. The two smaller macrourids, *Nezumia aequalis* and *H. italicus*, presented a continuous (or almost continuous) pattern of reproduction that is consistent with previous observations (Massutí et al. 1995; D’Onghia et al. 1996). Protracted spawning is considered to be an adaptive strategy aimed at ensuring that at least some of the larvae will encounter favorable conditions for survival and growth (Sherman et al. 1984). It could be one factor contributing to the great abundances of *Nezumia* spp. and *H. italicus* over Mediterranean continental margins (Stefanescu et al. 1994; Madurell et al. 2004; Papiol et al. 2012). The rest of species reproduced seasonally, and in general our results agree with patterns of reproduction previously described in the northwest Mediterranean (*T. scabrus*: Massutí et al. 1995; Fernández-Arcaya et al. 2012; *Phycis blennoides*: Rotllant et al. 2002; *Micromesistius poutassou*: Sbrana et al. 1998). The only exception was the catshark, *G. melastomus*, which presented a seasonal peak of gonad maturation in October that contrasts with the pattern of continuous mating and egg deposition previously described in the Mediterranean Sea and the Atlantic (Costa et al. 2005; Rey et al. 2005).

Seasonality in reproduction responds to natural fluctuations in environmental factors (Gage and Tyler 1991), and the main seasonal fluctuations in the area are associated with pulses of food reaching bathyal depths. Coherently, GSI of fish from the present study was associated with the availability of trophic resources to some extent. Among most mid-slope fish (excluding *Nezumia aequalis*) GSI was partially controlled by indicators of the vertical flux from surface production, implying rapid transfer and use of vertical production to deep-sea predators of different trophic guilds, probably through diel vertical migrations of macroplankton (e.g., pasipheid shrimps; Cartes 1993). This is further evidence of the important role of organic flux from the photic zone as driver of changes in bathyal and abyssal faunal communities (Drzen 2008; Stowasser et al. 2009). The influence of surface production on GSI was observed not only among pelagic feeders, but also in some benthos and hyperbenthos feeders (*T. scabrus*, *H. italicus*) that shifted their diets and consumed macroplankton in autumn (October), prior to the beginning of their GSI increases in winter (February). Phytodetritus inputs may therefore favor gonad growth, most likely by enhancing the proliferation of highly energetic mesopelagic prey that most fish consumed before gonad maturation. This is parallel to the pattern observed in deep-sea shrimps, which shift to more energetic diets in prereproductive periods in order to match the energy demands for gonadal development (Cartes et al. 2008; Fanelli and Cartes 2008, 2010).

The temporal patterns in GSI differed among species within the same feeding guild, pointing to successive and nonoverlapping spawning. Thus, even though the seasonality in food inputs exerts some control on reproductive cycles of deep-sea species, other factors contribute to regulation of the timing of reproduction. This is in agreement with the idea that temporal (or spatial) segregation in reproduction is an adaptive strategy for avoiding interspecific competition among offspring (Tsikliras et al. 2010).

In all the species, notwithstanding their pattern of reproduction, the main increase in feeding intensity preceded their increases in GSI, pointing to food consumption, and thus food availability (see above), as the trigger for reproduction. Similarly, demersal fish in the Ionian Sea eat more in prereproductive periods coupled to greater prey availability (Madurell and Cartes 2005). In the same sense, high feeding intensity through protracted seasons in species with prolonged spawning periods (i.e., *Nezumia aequalis* and *H. italicus*) likely provided the necessary energy for continuous gonad maturation. Therefore, species with continuous reproduction presumably relied continuously on food available in the environment for gonad maturation (Murua and Saborido-Rey 2003). These species made limited use of their body reserves, as suggested by the low seasonal oscillations of the HSI and the C:N ratio of muscle (Drzen 2002b). In contrast, species with short spawning seasons largely depended on their body reserves for gonad maturation, and presented corresponding large oscillations in HSI and C:N from muscle. In some species (e.g., the gadids *Micromesistius poutassou* and *Phycis blennoides*) we observed opposing trends between HSI and GSI that reflect the importance of the liver in providing

energy for gonad development. Furthermore, the increase in HSI preceded gonad growth, suggesting that the energy stored in the liver was later used for gonad development, as already observed in other gadids (e.g., the Atlantic cod (*Gadus morhua*); Marshall et al. 1999). In other species (i.e., *L. crocodilus* and *T. scabrus*), however, we unexpectedly observed peaks of HSI during and after greatest GSI. This temporal scheme does not agree with previously published patterns of energy use and storage, and further studies on these species throughout their whole depth distribution (~300–2200 m for *L. crocodilus* and ~300–1600 m for *T. scabrus*; Fanelli et al. 2013) might help us understand the variety of population maintenance strategies (e.g., seasonal vertical migrations for reproduction; Fernández-Arcaya et al. 2012) employed by fish living under restricted food availability.

Combinations of population density and size also played an important role in the reproductive cycles of some species, especially those with protracted spawning. The relationships between GSI and population density and size suggest that large reproducing adults of species with a bigger–deeper distribution (e.g., *Nezumia aequalis* and *G. melastomus*) aggregate at specific depths (Massuti et al. 1995; Morales-Nin et al. 2003). These species have largest reproducers (with high fecundity) below 1000 m, at depths free of any trawling activity. Larger and older fish females often produce higher-quality offspring that grow faster and are more resistant to starvation (Green 2008). In contrast, species with a uniform distribution of body size along the bathymetric gradient (i.e., *H. italicus*; Massuti et al. 1995) do not seem to aggregate for reproduction.

We observed in bathyal fish a common trend of reproduction between late autumn and spring–early summer that did not exactly follow the main pattern of spring–summer spawning found mainly on Mediterranean fish from the continental shelf zones (Tsikliras et al. 2010). The early stages of shelf fish generally feed on matter from the photic zone and/or the deep chlorophyll maximum (Sabatés et al. 2007). Little is known about the ecology of early stages of bathyal fish. They typically produce buoyant pelagic eggs (Gage and Tyler 1991), and recruitment of juveniles at the benthic boundary layer often occurs some months after the spawning period (Massuti et al. 1995). The early spawning of bathyal fish may be timed to match both the peak in surface production and the formation of the thermocline. Larvae hatching in the winter–spring period in shallower layers of the water column most probably find maximum abundance of particulate organic matter from surface production in that period (Miquel et al. 1994). Then, early downward vertical migration of small individuals can allow them to reach depths below the thermocline before it is formed (May–June; Papiol et al. 2012), thus avoiding the difficulty of transiting such a barrier. In-depth studies of early stages of bathyal fish species would be required in order to clarify this issue and how this can affect the reproductive cycles of mid-slope fish.

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References

- ALFARO, A. C., F. THOMAS, L. SERGENT, AND M. DUXBURY. 2006. Identification of trophic interactions within an estuarine food web northern New Zealand using fatty acid biomarkers and stable isotopes. *Estuar. Coast. Shelf Sci.* **70**: 271–286, doi:10.1016/j.ecss.2006.06.017
- ANDERSON, M. J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* **26**: 32–46.
- BREY, T., C. MÜLLER-WIEGMANN, Z. M. C. ZITTIER, AND W. HAGEN. 2010. Body composition in aquatic organisms—a global data bank of relationships between mass, elemental composition and energy content. *J. Sea Res.* **64**: 334–340, doi:10.1016/j.seares.2010.05.002
- BUSCAIL, R., R. POCKLINGTON, R. DAUMAS, AND L. GUIDI. 1990. Fluxes and budget of organic matter in the benthic boundary layer over the northwestern Mediterranean margin. *Cont. Shelf Res.* **10**: 1089–1112, doi:10.1016/0278-4343(90)90076-X
- CARRASSÓN, M., AND J. E. CARTES. 2002. Trophic relationships in a Mediterranean deep-sea fish community: Partition of food resources, dietary overlap and connections within the benthic boundary layer. *Mar. Ecol. Prog. Ser.* **241**: 41–55, doi:10.3354/meps241041
- CARTES, J. E. 1993. Feeding habits of pasiphaeid shrimps close to the bottom on the Western Mediterranean slope. *Mar. Biol.* **117**: 459–468.
- . 1998. Dynamics of the bathyal Benthic Boundary Layer in the northwestern Mediterranean: Depth and temporal variations in macrofaunal–megafaunal communities and their possible connections within deep-sea trophic webs. *Prog. Oceanogr.* **41**: 111–139, doi:10.1016/S0079-6611(98)00018-4
- , M. ELIZALDE, AND J. C. SORBE. 2001. Contrasting life-histories, secondary production, and trophic structure of Peracarid assemblages of the bathyal suprabenthos from the Bay of Biscay NE Atlantic and the Catalan Sea NW Mediterranean. *Deep-Sea Res. I* **48**: 2209–2232, doi:10.1016/S0967-0637(01)00012-7
- , E. FANELLI, V. PAPIOL, AND L. ZUCCA. 2010. Distribution and diversity of open-ocean, near-bottom macroplankton in the western Mediterranean: Analysis at different spatio-temporal scales. *Deep-Sea Res. I* **57**: 1485–1498, doi:10.1016/j.dsr.2010.08.001
- , A. GRÉMARE, F. MAYNOU, S. VILLORA-MORENO, AND A. DINET. 2002. Bathymetric changes in the distributions of particulate organic matter and associated fauna along a deep-sea transect down the catalan sea slope Northwestern Mediterranean. *Prog. Oceanogr.* **53**: 29–56, doi:10.1016/S0079-6611(02)00023-X
- , F. MAYNOU, D. LLORIS, L. GIL DE SOLA, AND M. GARCÍA. 2009. Influence of trawl type on the composition, abundance and diversity estimated for deep benthopelagic fish and decapod assemblages off the Catalan coasts western Mediterranean. *Sci. Mar.* **73**: 725–737, doi:10.3989/scimar.2009.73n4725
- , V. PAPIOL, AND B. GUIJARRO. 2008. The feeding and diet of the deep-sea shrimp *Aristeus antennatus* off the Balearic Islands Western Mediterranean: Influence of environmental factors and relationship with the biological cycle. *Prog. Oceanogr.* **79**: 37–54, doi:10.1016/j.pocean.2008.07.003
- CLARKE, K. R., AND R. M. WARWICK. 2001. Change in marine communities: An approach to statistical analysis and interpretation, 2nd edition. PRIMER-E.
- COSTA, M. E., K. ERZINI, AND T. C. BORGES. 2005. Reproductive biology of the blackmouth catshark, *Galeus melastomus* Chondrichthyes: Scyliorhinidae off the south coast of Portugal. *J. Mar. Biol. Assoc. UK* **85**: 1173–1183, doi:10.1017/S0025315405012270
- DE PEDRO, N., A. I. GUIJARRO, M. J. DELGADO, M. A. LÓPEZ-PATIÑO, M. L. PINILLOS, AND M. ALONSO-BEDATE. 2001. Influence of dietary composition on growth and energy reserves in tench (*Tinca tinca*). *J. Appl. Ichthyol.* **17**: 25–29, doi:10.1046/j.1439-0426.2001.00274.x
- D'ONGHIA, G., A. TURSI, AND M. BASANISI. 1996. Reproduction of macrourids in the upper slope of the north-western Ionian Sea. *J. Fish Biol.* **49**: 311–317, doi:10.1111/j.1095-8649.1996.tb06084.x
- DRAZEN, J. C. 2002a. A seasonal analysis of the nutritional condition of deep-sea macrourid fishes in the north-east Pacific. *J. Fish Biol.* **60**: 1280–1295, doi:10.1111/j.1095-8649.2002.tb01720.x
- . 2002b. Energy budgets and feeding rates of *Coryphaenoides acrolepis* and *C. armatus*. *Mar. Biol.* **140**: 677–686, doi:10.1007/s00227-001-0747-8
- . 2008. Bypassing the abyssal benthic food web: Macrourid diet in the eastern North Pacific inferred from stomach content and stable isotopes analyses. *Limnol. Oceanogr.* **53**: 2644–2654, doi:10.4319/lo.2008.53.6.2644
- FABIANO, M., AND OTHERS. 2001. Fluxes of phytopigments and labile organic matter to the deep ocean in the NE Atlantic Ocean. *Prog. Oceanogr.* **50**: 89–104, doi:10.1016/S0079-6611(01)00049-0
- FANELLI, E., AND J. E. CARTES. 2008. Spatio-temporal changes in gut contents and stable isotopes in two deep Mediterranean pandalids: Influence on the reproductive cycle. *Mar. Ecol. Prog. Ser.* **355**: 219–233, doi:10.3354/meps07260
- , AND ———. 2010. Temporal variations in the feeding habits and trophic levels of three deep-sea demersal fishes from the western Mediterranean Sea, based on stomach contents and stable isotope analyses. *Mar. Ecol. Prog. Ser.* **402**: 213–232, doi:10.3354/meps08421
- , ———, AND V. PAPIOL. 2011. Food web structure of deep-sea macrozooplankton and micronekton off the Catalan slope: Insight from stable isotopes. *J. Mar. Syst.* **87**: 79–89, doi:10.1016/j.jmarsys.2011.03.003
- , ———, ———, AND C. LÓPEZ-PÉREZ. 2013. Environmental drivers of megafaunal assemblage composition and biomass distribution over mainland and insular slopes of the Balearic Basin (Western Mediterranean). *Deep-Sea Res. I* **78**: 79–94, doi:10.1016/j.dsr.2013.04.009
- , V. PAPIOL, J. E. CARTES, AND O. RODRIGUEZ-ROMEU. 2014. Trophic ecology of *Lampanyctus crocodilus* on north-west Mediterranean Sea slopes in relation to reproductive cycle and environmental variables. *J. Fish Biol.* **84**: 1654–1688, doi:10.1111/jfb.12378

- , J. REY, P. TORRES, AND L. GIL DE SOLA. 2007. Feeding habits of the blackmouth catshark *Galeus melastomus* Rafinesque, 1810 and the velvet belly lantern shark *Etmopterus spinax* (Linnaeus, 1758) in the western Mediterranean. *J. Appl. Ichthyol.* **25**: 83–93, doi:10.1111/j.1439-0426.2008.01112.x
- FERNÁNDEZ-ARCAJA, U., E. RAMÍREZ-LLODRA, L. RECASENS, G. ROTLLANT, H. MURUA, AND J. B. COMPANY. 2012. Population structure and reproductive patterns of the NW Mediterranean deep-sea macrourid *Trachyrincus scabrus* Rafinesque, 1810. *Mar. Biol.* **159**: 1885–1896, doi:10.1007/s00227-012-1976-8
- GAGE, J. D., AND P. A. TYLER. 1991. Deep-sea biology: A natural history of organism at the deep-sea floor. Cambridge University Press.
- GINGER, M. L., AND OTHERS. 2001. Organic matter assimilation and selective feeding by holothurians in the deep-sea: Some observations and comments. *Prog. Oceanogr.* **50**: 407–423, doi:10.1016/S0079-6611(01)00063-5
- GREEN, B. S. 2008. Maternal effects in fish populations. *Adv. Mar. Biol.* **54**: 1–105, doi:10.1016/S0065-2881(08)00001-1
- HUDSON, I. R., D. W. POND, D. S. M. BILLETT, P. A. TYLER, R. S. LAMPITT, AND G. A. WOLFF. 2004. Temporal variations in fatty acid composition of deep-sea holothurians: Evidence of benthic-pelagic coupling. *Mar. Ecol. Prog. Ser.* **281**: 109–120, doi:10.3354/meps281109
- IKEN, K., T. BREY, U. WAND, J. VOIGT, AND P. JUNGHANS. 2001. Food web structure of the benthic community at the Porcupine Abyssal Plain NE Atlantic: A stable isotope analysis. *Prog. Oceanogr.* **50**: 383–405, doi:10.1016/S0079-6611(01)00062-3
- KEMP, K. M., K. P. P. FRASER, M. A. COLLINS, AND I. G. PRIEDE. 2008. Seasonal variation in the white muscle biochemical composition of deep-sea macrourids in the North-east Atlantic. *Mar. Biol.* **155**: 37–49, doi:10.1007/s00227-008-1004-1
- KOSLOW, J. A. 1996. Energetic and life-history patterns of deep-sea benthic, benthopelagic and seamount-associated fish. *J. Fish Biol.* **49**: 54–74, doi:10.1111/j.1095-8649.1996.tb06067.x
- LABORDE, P., J. URRUTIA, AND V. VALENCIA. 1999. Seasonal variability of primary production in the Cap-Ferret Canyon area Bay of Biscay during the ECOFER cruises. *Deep-Sea Res. II* **46**: 2057–2079, doi:10.1016/S0967-0645(99)00055-7
- MACPHERSON, E. 1979. Ecological overlap between Macrourids in the Western Mediterranean Sea. *Mar. Biol.* **53**: 149–159, doi:10.1007/BF00389186
- . 1981. Resource partitioning in a Mediterranean demersal fish community. *Mar. Ecol. Prog. Ser.* **4**: 183–193, doi:10.3354/meps004183
- MADURELL, T., AND J. E. CARTES. 2005. Trophodynamics of a deep-sea demersal fish assemblage from the bathyal eastern Ionian Sea Mediterranean. *Deep-Sea Res. I* **52**: 2049–2064, doi:10.1016/j.dsr.2005.06.013
- , AND ———. 2006. Trophic relationships and food consumption of slope dwelling macrourids from the bathyal Ionian Sea eastern Mediterranean. *Mar. Biol.* **148**: 1325–1338, doi:10.1007/s00227-005-0158-3
- , ———, AND M. LABROPOULOU. 2004. Changes in the structure of fish assemblages in a bathyal site of the Ionian Sea eastern Mediterranean. *Fish. Res.* **66**: 245–260, doi:10.1016/S0165-7836(03)00205-4
- MAMOURIDIS, V., J. E. CARTES, S. PARRA, E. FANELLI, AND J. I. SAIZ-SALINAS. 2011. A temporal analysis on the dynamics of deep-sea macrofauna: Influence of environmental variability off Catalonia coasts western Mediterranean. *Deep-Sea Res. I* **58**: 323–337, doi:10.1016/j.dsr.2011.01.005
- MARSHALL, C. T., N. A. YARAGINA, Y. LAMBERT, AND O. S. KJESBU. 1999. Total lipid energy as a proxy for total egg production by fish stocks. *Nature* **402**: 288–290, doi:10.1038/46272
- MASSUTÍ, E., B. MORALES-NIN, AND C. STEFANESCU. 1995. Distribution and biology of five grenadier fish Pisces: Macrouridae from the upper and middle slope of the northwestern Mediterranean. *Deep-Sea Res. I* **423**: 307–330, doi:10.1016/0967-0637(95)00003-0
- MERRETT, N. R., AND N. B. MARSHALL. 1981. Observations on the ecology of deep-sea bottom-living fishes collected off north-west Africa (08°–27°N). *Prog. Oceanogr.* **9**: 185–244, doi:10.1016/0079-6611(80)90002-6
- MIQUEL, J. C., S. W. FOWLER, J. LA ROSA, AND P. BUAT-MENARD. 1994. Dynamics of the downward flux of particles and carbon in the open North Western Mediterranean Sea. *Deep-Sea Res. I* **41**: 243–261, doi:10.1016/0967-0637(94)90002-7
- MORALES-NIN, B., AND OTHERS. 2003. Size influence in zonation patterns in fishes and crustaceans from deep-water communities of the Western Mediterranean. *J. Northwest Atl. Fish. Soc.* **31**: 413–430.
- MURUA, H., AND F. SABORIDO-REY. 2003. Female reproductive strategies of marine fish species of the North Atlantic. *J. Northwest Atl. Fish. Soc.* **33**: 23–31, doi:10.2960/J.v33.a2
- PAPIOL, V., J. E. CARTES, E. FANELLI, AND F. MAYNOU. 2012. Influence of environmental variables on the spatio-temporal dynamics of benthic-pelagic assemblages in the middle slope of the Balearic Basin NW Mediterranean. *Deep-Sea Res. I* **61**: 84–99, doi:10.1016/j.dsr.2011.11.008
- , ———, ———, AND P. RUMOLO. 2013. Trophic web structure and seasonality of slope megafauna in the NW Mediterranean elucidated by stable isotopes: Relationship with available food sources. *J. Sea Res.* **77**: 53–69, doi:10.1016/j.seares.2012.10.002
- PUIG, P., J. B. COMPANY, F. SARDÀ, AND A. PALANQUES. 2001. Responses of deep-water shrimp populations to intermediate nepheloid layer detachments on the Northwestern Mediterranean continental margin. *Deep-Sea Res. I* **48**: 2195–2207, doi:10.1016/S0967-0637(01)00016-4
- REY, J., L. GIL DE SOLA, AND E. MASSUTÍ. 2005. Distribution and biology of the blackmouth catshark *Galeus melastomus* in the Alboran Sea Southwestern Mediterranean. *J. Northwest Atl. Fish. Soc.* **35**: 215–223.
- ROTLLANT, G., J. MORANTA, E. MASSUTÍ, B. MORALES-NIN, AND F. SARDÀ. 2002. Reproductive biology of three gadiform fish species through the Mediterranean deep-sea range 147–1850 m. *Sci. Mar.* **662**: 157–166.
- SABATÉS, A., M. P. OLIVAR, J. SALAT, I. PALOMERA, AND F. ALEMANY. 2007. Physical and biological processes controlling the distribution of fish larvae in the NW Mediterranean. *Prog. Oceanogr.* **74**: 355–376, doi:10.1016/j.pocean.2007.04.017
- SBRANA, M., V. CHERICONI, AND F. BIAGI. 1998. Biologia riproduttiva e fecondità di *Micromesistius poutassou* Risso, 1826 del Mar Tirreno settentrionale. *Biol. Mar. Mediterr.* **52**: 107–116. [Reproductive biology and fecundity of *Micromesistius poutassou* Risso, 1826 in the northern Tyrrhenian Sea.]
- SHERMAN, K., W. SMITH, W. MORSE, M. BERMAN, J. GREEN, AND L. EJSYMONT. 1984. Spawning strategies of fishes in relation to circulation, phytoplankton production, and pulses in zooplankton off the northeastern United States. *Mar. Ecol. Prog. Ser.* **18**: 1–19. , doi:10.3354/meps018001
- SNELGROVE, P. V. R., AND C. R. SMITH. 2002. A riot of species in an environmental calm; the paradox of the species-rich deep sea. *Oceanogr. Mar. Biol.* **40**: 311–342.

- STEFANESCU, C., B. MORALES-NIN, AND E. MASSUTÍ. 1994. Fish assemblages on the slope in the Catalan Sea western Mediterranean: Influence of a submarine canyon. *J. Mar. Biol. Assoc. UK* **74**: 499–512, doi:[10.1017/S0025315400047627](https://doi.org/10.1017/S0025315400047627)
- STOWASSER, G., R. McALLEN, G. J. PIERCE, M. A. COLLINS, C. F. MOFFAT, I. G. PRIEDE, AND D. W. POND. 2009. Trophic position of deep-sea fish—assessment through fatty acid and stable isotope analyses. *Deep-Sea Res. I* **56**: 812–826, doi:[10.1016/j.dsr.2008.12.016](https://doi.org/10.1016/j.dsr.2008.12.016)
- SWYNNERTON, G. H., AND E. B. WORTHINGTON. 1940. Note on the food of fish in Haweswater Westmoreland. *J. Anim. Ecol.* **9**: 183–187, doi:[10.2307/1454](https://doi.org/10.2307/1454)
- TIESZEN, L. L., T. W. BOUTTON, K. G. TESDAHL, AND N. H. SLADE. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for ^{13}C analysis of diet. *Oecologia* **57**: 32–37, doi:[10.1007/BF00379558](https://doi.org/10.1007/BF00379558)
- TSIKLIRAS, A. C., K. I. STERGIU, AND E. ANTONOPOULOU. 2010. Spawning period of Mediterranean marine fishes. *Rev. Fish Biol. Fish.* **20**: 499–538, doi:[10.1007/s11160-010-9158-6](https://doi.org/10.1007/s11160-010-9158-6)
- VIDUSSI, F., J. C. MARTY, AND J. CHIAVÉRINI. 2000. Phytoplankton pigment variations during the transition from spring bloom to oligotrophy in the northwestern Mediterranean Sea. *Deep-Sea Res. I* **47**: 423–445, doi:[10.1016/S0967-0637\(99\)00097-7](https://doi.org/10.1016/S0967-0637(99)00097-7)

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