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Time-Scale Analysis of Prey Preferences and Ontogenetic Shift in the Diet of European Hake *Merluccius merluccius* (Linnaeus, 1758) in Southern and Central Tyrrhenian Sea

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Abstract: *Merluccius merluccius* is one of the most important Mediterranean benthopelagic predators. It represents a key species for the ecosystem's functioning due to its fundamental role in the energy transferal between different domains and depth strata. The aim of this study was to explore the feeding habits of European hakes in the southern and central Mediterranean Sea, and also to analyze timescale variations and ontogenetic shift in five size length classes. A total of 411 stomachs collected from 2018 to 2020 were analyzed to assess diet and feeding habits. Results confirmed hakes' role as a generalist benthopelagic predator, preying both in the suprabenthic layer and in the entire water column. Concerning the ontogenetic diet shift, juvenile hakes prefer zooplanktonic prey, while larger hakes have a diet mainly based on teleosts and decapods. The variations in diet composition between years, characterized by a fluctuation of cephalopods, bioluminescent teleost species and mesopelagic crustaceans, have highlighted the ability of European hake to model its diet to the geographical and prey availability. These features make analysis of the diet of *M. merluccius* essential to understanding the trophic dynamic existing in bentho-meso-pelagic environments, to improve ecosystem conservation in accordance with ecosystem-based fishery management.

Keywords: apical predators; central Mediterranean; diet; feeding habits; Merluccidae; trophic ecology

1. Introduction

The European hake, *M. merluccius* (Linnaeus 1758), is a benthopelagic predator, widely distributed in the entire Mediterranean Sea and in the northeastern Atlantic Ocean, with a high commercial value and a fundamental role in the trophic ecology of mesopelagic communities. Its bathymetric distribution ranges from 30 to 1000 m, with reported abundance peaks between 50 and 400 m [1,2]. Concerning its commercial value, this species represents an essential resource in the Mediterranean area, caught mainly through trawl and small-scale fisheries [3]. Its economic and social relevance (being one of the most popular and appreciated food resources in western Europe among demersal fishes) has led to an over exploitation of *M. merluccius* stocks, especially in the central and western Mediterranean Sea. According to FAO reports [4,5], European hake showed the highest capture production (20170 t/year) in the Mediterranean and Black Sea, with a worrying

decline in landings since 1980 suggesting there is overfishing of several Mediterranean stocks [3,6]. Indeed, this species suffers from the highest fishing mortality among the demersal species [7], with high risks for stock status, as reported by several authors [8].

In accordance with ecosystem-based fishery management (EBFM), it is essential to focus attention on ecosystem dynamics, to improve management policies for a better regulation of marine environment exploitation, going beyond the single-species models underlying fishery science in the past decades [9]. Understanding the trophic dynamics and the related multispecies interactions is essential to deepen the knowledge concerning ecosystem functioning, focusing on all the processes and relationships which regulate and influence the trophic network and, consequently, the supported fisheries [10–12]. Exploring feeding habits and diet composition, especially of those key species such as apical predators, adding new information about prey–predator interactions and energy transferring in marine communities, is essential to understanding food web structure and trophic dynamics regulating the ecosystem's proper functioning [13].

The heavily exploited Mediterranean basin has in the last decades suffered an exponential decline of piscivorous predators, with a not fully understood impact on the entire ecosystem and communities [14]. For this reason, it is important to analyze the predators' trophic ecology and diet composition, especially of those over exploited such as *M. merluccius*. This species is one of the most important benthopelagic opportunistic predators in the entire Mediterranean area, shifting its diet geographically in relation to prey abundance and composition. It also shows ontogenetic changes in diet, with a planktivorous phase reported in juveniles, mainly preying on Euphausiids, Mysids and small Crustaceans, and an ichthyophagous diet reported for adults, which varies its composition geographically in relation to the ecological features of the area [15–20]. Due to its feeding habits, trophic relationships and daily vertical migrations, European hake plays a fundamental role in the energy flow involving pelagic, demersal and benthic domains [21–25].

The present study aims to investigate the diet and feeding habits of *M. merluccius* in the southern Tyrrhenian Sea, and the ontogenetic variations in prey preferences on a time scale between 2018 and 2020, through the analysis of stomach contents, to deepen the knowledge on the ecological role and the trophic interactions of this species. This is essential both to improve their stock conservation and management, and to monitor the demersal and mesopelagic communities in one of the most heavily impacted Mediterranean geographical areas [26,27]. Indeed, in the southern Tyrrhenian Sea (Geographical sub-area, GSA 10) operates the third trawling fleet for vessel number among the Italians and a well-developed artisanal fishery, characterized by a high variability in target species and fishing types [28–30]. The high fishing pressure acting on the stocks inhabiting this area has led to the creation of trawling ban zones since 1990, with great results on the demersal communities' recovery and restoration [31–35].

2. Materials and Methods

2.1. Study Area

The studied area was the southern and central Tyrrhenian Sea (GSA 10) (Figure 1). According to GFCM-FAO classification, GSA 10 covers an area delimited by the coastline and the joining lines between the two perpendiculars extending off-shore, one at the south (70 miles off Trapani) and one at the north (90 miles off the Circeo promontory). As reported in Figure 1, the coastline extends from the border between Lazio and Campania, near the Garigliano river mouth, and Capo S. Vito, the western border of the Sicilian Tyrrhenian coast.

Figure 1. Maps of the studied area (GSA 10, southern and central Tyrrhenian Sea), Mediterranean Sea in the insert.

The bathymetry of the investigated zones ranges from 10 to 800 m, with an Ocean-like seabed morphology, characterized by the presence of a well-developed continental slope and shelf, abyssal plans, and submarine canyons. The continental shelf is less developed along the north Sicilian coast and along the Calabria and Basilicata coasts, while it is wider along the Lazio and Campania coasts. The northern Sicilian coast is characterized by a steep continental slope with an average depth of 500 m and a distance from the coastline between 4 and 15 km [30,36–38].

The southern and central Tyrrhenian Sea, the deepest in the western Mediterranean area, is considered an oligotrophic basin due to the peculiar seafloor morphology, the scant water and the irregular sediment inflow from the rivers [21,39,40]. Some exceptions are found near the coast with the presence of mesotrophic and eutrophic zones in the Salerno gulf, in front of the Volturno river, in the coast of Napoli and near the Sarno river mouth [30]. In addition to the anthropogenic impact due to the presence of highly populated cities near the coast (such as Napoli), GSA 10 is an area highly exploited by artisanal and trawl fisheries. This, together with the relevant ecological value of many zones, resulted in the institution of the highest number of protected areas among the Italian seas [35,41], in two trawling ban fishing areas in the Castellammare and Patti Gulfs and in one recovery area through artificial reefs in the Castellammare Gulf [31,32,42].

As reported by Vetrano [43], the water column of the southern and central Tyrrhenian Sea is composed of: the Atlantic Water (AW) in the surface layer (depth range between

0 and 200 m), the Levantine Intermediate Water (LIW) in the intermediate layer (depth range between 200 and 700 m) and the Tyrrhenian Deep Water (TDW) in the bottom layer (more than 700 m deep). Concerning the water mass circulation, as large parts of the Mediterranean basin, the entire Tyrrhenian Sea is characterized by a cyclonic circulation, with the peculiar persistence in its southern part of anticyclonic vorticose eddy structures, which move for long distances influencing the chemical and biological properties of the entire western Mediterranean basin. These vortices, generally wind driven [44], are also due to the presence in this area of several isolated seamounts. As reported for the Vanvili [36], these volcanic structures can modify the water properties and the nutrient distribution through vertical exchange. The weak dynamics inside the basin, characterized by the presence of energy only along the coast, and the influence of eddies and vortex structures induce mixing processes resulting in TDW formation [45,46]. This is a peculiar type of water, resulting from the mixing of LIW and the Western Mediterranean Deep Water (WMDW). The southern and central Tyrrhenian Sea is the first part of western Mediterranean Sea crossed by LIW, entering from the Strait of Sicily along the northern Sicily coast, and leaving across the Sardinia–Sicily channel, which in its deepest part is also the entrance area of the WMDW. The LIW flowing along the Sicilian coast causes the formation of a deep mixing area due to LIW's higher density compared to that of the resident Tyrrhenian water [47]. Mixing phenomena, together with the presence of eddies and semi-permanent fronts, are essential in the regulation of reproductive success and recruitments at population scale and feeding activities of juveniles' hake, enhancing the availability of their prey [48–52].

2.2. Samples Collection and Analysis

A total of 411 stomach samples of *M. merluccius* (Table 1) were collected from specimens caught during the MEDITS project (International bottom Trawl Survey in the Mediterranean Sea) and the seasonal biological sampling of catches from commercial fleets (CAMPBIOL project: EU Reg. 1004/2017), carried out between 2018 and 2020. MEDITS trawl surveys were generally performed during the Summer (June/July), except for MEDITS 2020 which, due to operational problems related to COVID-19, was performed in Winter (November/December). Regarding the samples collected during the CAMPBIOL project, they were caught seasonally, using different fishing gears, by the commercial fleets operating in GSA 10.

Table 1. Numbers of sampled and analyzed stomachs (AS), with Vacuity Index (VI%) and numbers of Empty (E), Full < 50% (F < 50), Full > 50% (F > 50) and bursting stomachs (B) by ontogenetic classes in the different years.

Size Classes	2018						2019						2020					
	E	F < 50	F > 50	B	AS	VI %	E	F < 50	F > 50	B	AS	VI %	E	F < 50	F > 50	B	AS	VI %
I	2	5	4	11	22	9.09	5	2	0	0	7	71.42	0	5	2	7	14	0
II	18	4	16	31	69	26.08	6	1	2	5	14	42.85	0	0	0	3	3	0
III	6	11	8	22	47	12.76	11	3	6	8	28	39.28	0	3	2	8	13	0
IV	8	9	12	14	43	18.60	40	25	8	21	94	42.55	8	5	0	6	19	42.10
V	2	2	0	0	4	50	0	1	5	8	14	0	6	4	4	6	20	30
Σ	36	29	40	78	185	19.45	62	32	21	42	157	39.49	14	17	8	30	69	20.28

Once caught, specimens were frozen on board to prevent tissue degradation. After landings, they were transported to the laboratory for stomach sampling, where each individual was measured (TL), weighted (TW) and sexed, and had their degree of sexual maturation determined according to Follesa and Carbonara [53], and their stomach repletion status determined through a macroscopic scale (1: empty; 2: full < 50%; 3: full > 50%; 4: bursting). This was used to calculate the Vacuity Index (VI) as the ratio of empty stomach to the total stomach number (excluding the everted stomachs).

To analyze the diet variations according to the specimen size, five ontogenetic length classes were chosen a priori according to the literature on hakes' biology [20,23,54–56]. All the specimens with a TL less than 10 cm (juveniles' immature specimens) were grouped

into Class I, while the other classes were divided as follows: hakes with a TL between 10.5 and 15 cm (Class II), hakes with a TL between 15.5 and 20 cm (Class III), hakes with a TL between 20.5 and 32.5 cm (Class IV) and hakes with a TL greater than 32.5 cm (Class V).

After sampling, each stomach was stored in ethanol 70% for the content analysis. During the stomach content analysis, each prey was identified to the lowest taxonomic level possible, also measuring its weight, length and digestion degree using a scale from 1 (intact prey) to 3 (highly digested prey). As widely reported in the literature [38,57–64], the hard undigested prey's parts, such as exoskeleton parts (e.g., carapace, telson) for Crustacea, fish otoliths and mouth, and cephalopods beaks, were essential for the identification of the highly digested prey, carefully avoiding their double counting. Only one anatomical part was considered in the numerical evaluation of a specific prey when more than one structure (potentially ascribable to the same prey) occurred in the stomach sample. Moreover, considering that stomach content analysis gives a snapshot of predators' diet, the misidentification and underestimation of prey was carefully avoided including items in advanced digestion state in undetermined categories (e.g., Osteichthyes n.i., Decapoda n.i., Cephalopoda n.i.). Once the content analysis of all the stomachs was performed according to Hyslop, 1980 [65], several indices were calculated for each year and for each prey category: the frequency of occurrence (%F) as the ratio of stomachs containing a prey category to the total not empty stomachs number, the abundance composition (%N) as the ratio of prey item number belonging to a category to the total prey number and the weight composition (W%) as the ratio of wet weight of a category to the total stomach content weight. Finally, all these indices were used to calculate the relative importance index (IRI = %F × (%N + %W)), which was chosen as an indicator of prey preferences [66,67] and used to perform statistical analysis on annual diet composition for each ontogenetic class, also evaluating their annual variation by a comparison between different years.

2.3. Data Analysis

Information on dietary composition was obtained through a combined univariate and multivariate data analysis. One-way ANOVA analysis followed by Tukey's test were performed to assess dietary composition differences between the ontogenetic stage of the *M. merluccius* specimens and between the sampling periods (2018, 2019 and 2020). A square root transformation was applied to the dietary composition matrix; then the Bray–Curtis similarities were calculated. The dendrogram was created by means of the average linkage clustering method. Non-parametric multi-dimensional scaling (nMDS) ordination was applied to the dietary composition matrix to observe temporal and size class effects on dietary composition. A principal component analysis (PCA) was used to visualize diet data. Finally, the similarity percentages procedure (SIMPER) was used to obtain information about the contribution of each prey species to the similarity or dissimilarity in diet composition among trophic clusters within and between the years 2018, 2019 and 2020. Univariate analysis was performed using Sigmaplot V.14 software. All multivariate statistical analyses were performed by using PRIMER6-E and Past (V. 4). p value was set at $p < 0.05$.

2.4. Ethical Statement

Fish specimen collection was authorized by the MEDITS and CAMPBIOL projects (EU Reg. 1004/2017) as part of annual research surveys, all involving lethal sampling. No experiments were conducted, nor were surgical procedures performed. No procedures caused lasting harm to sentient fish, nor were sentient fish subjected to chemical agents. The care and use of collected animals complied with animal welfare guidelines, laws and regulations set by the Italian Government.

3. Results

3.1. European Hake Diet Composition in 2018

In 2018, a total of 185 stomachs were analyzed. As reported in Table 2, the most relevant prey category, according to IRI values, was Osteichthyes n.i. (IRI% = 32.47), followed by *Ceratoscopelus maderensis* (IRI% = 28) and Euphausiacea n.i. (IRI% = 20.16). The other prey categories with an IRI greater than 1 % were: Mysida n.i. (IRI% = 7.37), *Engraulis encrasicolus* (IRI% = 3.94), *Boops boops* (IRI% = 2.18), *Chlorophthalmus agassizi* (IRI% = 2.10) and Dendrobanchiata n.i. (IRI% = 1.02).

Table 2. Diet composition of the whole hake's series from southern and central Tyrrhenian Sea sampled in 2018. In the columns are reported the diet index values (%F, %W, %N, IRI and %IRI) for each prey category.

TAXON	%F	%W	%N	IRI	%IRI
MOLLUSCA					
Cephalopoda n.i.	3.82	1.07	1.97	11.62	0.64
CRUSTACEA					
Amphipoda					
Amphipoda n.i.	0.64	0.01	0.33	0.21	0.01
Decapoda					
Parapenaeus longirostris	1.27	0.99	0.66	2.10	0.12
Solenocera membranacea	0.64	0.46	0.33	0.50	0.03
Alpheus glaber	1.27	0.11	0.66	0.98	0.05
Chlorotocus crassicornis	1.27	0.28	0.66	1.20	0.07
Processa acutirostris	0.64	0.43	0.99	0.90	0.05
Decapoda n.i.	1.91	0.56	0.99	2.95	0.16
Dendrobranchiata n.i.	5.10	0.98	2.63	18.43	1.02
Euphausiacea					
Meganyctiphanes norvegica	0.64	0.13	4.93	3.22	0.18
Stylocheiron longicorne	0.64	0.01	0.33	0.21	0.01
Euphausiacea n.i.	10.83	1.81	31.91	365.10	20.16
Mysida n.i.	7.01	0.29	18.75	133.42	7.37
OSTEICHTHYES					
Ceratoscopelus maderensis	20.38	13.04	11.84	507.17	28.00
Diaphus holti	1.27	0.00	0.66	0.84	0.05
Boops boops	1.27	30.34	0.66	39.49	2.18
Callionymus sp.	1.27	0.50	0.66	1.47	0.08
Macroramphosus scolopax	1.27	1.94	0.66	3.30	0.18
Maurolicus muelleri	2.55	1.51	1.32	7.21	0.40
Engraulis encrasicolus	3.82	16.70	1.97	71.36	3.94
Peristedion cataphractum	1.27	0.63	0.66	1.64	0.09
Cepola macrophthalma	1.27	0.00	0.66	0.84	0.05
Chlorophthalmus agassizi	5.10	5.49	1.97	38.04	2.10
Notoscopelus elongatus	1.27	0.16	0.66	1.05	0.06
Gobiidae n.i.	1.27	0.50	0.66	1.48	0.08
Sparidae n.i.	1.27	5.92	0.66	8.38	0.46
Osteichthyes n.i.	21.02	16.13	11.84	588.00	32.47

Concerning the different ontogenetic classes, as reported in Table 3 and in Figure 2, the European hakes belonging to Class I showed a clear preference for Euphausiacea n.i. (IRI% = 78.24) and Mysida n.i. (IRI% = 16.72), followed by Osteichthyes n.i. (IRI% = 3.10). In Class II, the Euphausiacea n.i. (IRI% = 4.48) and Mysida n.i. (IRI% = 2.96) were still relevant prey, but the hakes' preferences were significantly oriented toward teleost fishes, such as *C. maderensis* (IRI% = 60.08), Osteichthyes n.i. (IRI% = 25.80) and *Maurolicus muelleri* (IRI% = 3.50). In Class III, the most relevant prey were teleost fishes, with *C. maderensis* as the highest IRI% (36.62), followed by Osteichthyes n.i. (33.33), *E. encrasicolus* (14.68) and *C. agassizi* (7.06). Moreover, Crustaceans showed a high relevance, with Crustacea n.i. (IRI% = 5.02), Euphausiacea n.i. (IRI% = 1.30) and *Parapenaeus longirostris*, (IRI% = 1.13).

Concerning Class IV, the teleost fishes were the most important prey, as confirmed by the highest IRI% values of several categories: Osteichthyes n.i. (29.96), *B. boops* (22.26), *C. maderensis* (18.94), Sparidae n.i (6.01), *E. ancrasicolus* (5.17), *Macroramphosus scolopax* (3.36), *C. agassizi* (2.74) and *Cepola macrophthalma* (2.07). As in Class III, in Class IV Crustaceans were also relevant, mainly Decapoda n.i. (IRI% = 2.28) and *Chlorotocus crassicornis* (IRI% = 2.26), while this was the first class with high IRI% for Cephalopoda n.i. (IRI% = 2.77). For Class V, only empty stomachs were found.

Table 3. Diet composition in the five hake size classes sampled in timeframe 2018/2020. In the column are reported the IRI% values for each prey category.

| TAXON/CLASS	2018					2019					2020				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
MOLLUSCA															
Cephalopoda n.i.	0.88			2.77				1.11			3.92				
CRUSTACEA															
Amphipoda															
Amphipoda n.i.	0.06										0.28				
Decapoda															
Eusergestes arcticus										0.54					0.44
Parapenaeus longirostris		1.13								0.63					
Aristaeomorpha foliacea										0.15					
Solenocera membranacea			0.27										0.98		
Alpheus glaber		0.37								0.55					
Pasiphaea multidentata															2.12
Pasiphaea sivado										0.17					39.50
Chlorotocus crassicornis				2.26						0.27					
Processa acutirostris			0.58										2.36		
Sergestes sp.															0.45
Pasiphaea sp.															1.82
Dardanus sp.														1.07	
Plesionika sp.							2.94			0.15					
Decapoda n.i.		0.39	5.02	2.28					6.41	11.88			0.85		0.88
Dendrobranchiata n.i.															
Stomatopoda															
Parasquilla ferussaci															1.05
Euphausiacea															
Meganyctiphanes norvegica	0.93									0.66		4.31			
Stylocheiron longicorne	0.06											0.28			
Euphausiacea n.i.	78.24	4.48	1.30			100.00	1.13					4.11			
Mysida n.i.	16.72	2.96										71.11			
OSTEICHTHYES															
Ceratoscopelus maderensis		60.08	36.62	18.94						2.94	0.74				3.21
Diaphus holti		0.29													0.56
Lampanyctus crocodilus															0.55
Stomias boa															0.83
Argentina sphyraena										1.62					
Boops boops				22.26							92.51				0.70
Callionymus sp.		0.67													
Macroramphosus scolopax				3.36											0.47
Echiodon dentatus										0.62					
Gaidropsarus biscayensis										0.27					
Maurolicus muelleri		3.50						65.76	0.94						0.49
Engraulis encrasicolus			14.68	5.17					73.71	13.67	0.85			41.17	
Peristedion cataphractum		0.77													0.42
Cepola macrophthalma				2.07											0.91
Physiculus dalwigki										0.12					
Sardina pilchardus										13.25	1.31				
Chlorophthalmus agassizi		0.00	7.06	2.74						0.26					0.83
Nettastoma melanura														1.07	
Trachurus trachurus											2.21			13.55	38.03
Trachurus mediterraneus														48.33	3.30
Trachurus sp.														21.90	
Notoscopelus elongatus				2.18											
Myctophidae n.i.										0.17					
Sparidae n.i.				6.01						0.84					
Carapidae n.i.									0.67	0.12					
Gobiidae n.i.		0.68								0.12					
Macrouridae n.i.										0.13					
Osteichthyes n.i.	3.10	25.80	33.33	29.96			30.17	17.16	52.49	0.77	15.99	100	56.64	14.08	3.45
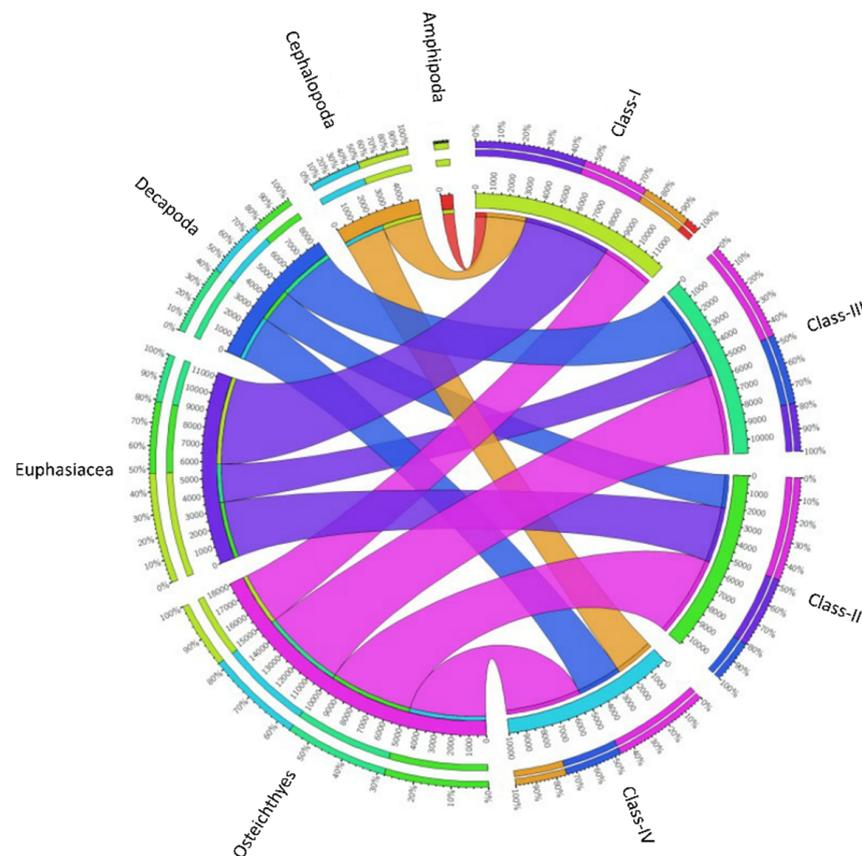
Figure 2. Diet composition of the *Merluccius merluccius* specimens collected from the southern and central Tyrrhenian Sea during 2018. The chord diagram shows the connection among the main taxa found in the stomach contents of different hake size classes investigated. The size classes analyzed are shown on the right of the diagram. Main taxa found are shown on the left. Ribbon size in the chart codifies IRI value associated with hake size classes/prey taxa segment pair.

One-way ANOVA analysis was performed to assess dietary composition differences between the ontogenetic stages of the *M. merluccius* specimens. Indeed, Class I was different from all other classes ($p < 0.05$), while Classes II–IV did not differ significantly in their diets ($p > 0.05$). Cluster analysis and MDS ordination grouped the whole data set obtained from 2018, by size classes, into two main trophic clusters. Cluster A included only the specimens belonging to Class I, while Cluster B included the specimens belonging to Classes II–IV with similar IRI values. In detail, Classes III and IV showed 40% similarity, mainly driven by the large contribution of Osteichthyes (Figure 3 and Table 4).

Table 4. Results of the SIMPER analysis conducted between European hake trophic groups from 2018. The average similarity between cluster groups is reported. The percentage and cumulative contribution of typifying species within-group similarity of the identified hake trophic groups is shown. The table shows results obtained for the trophic cluster B, which includes Classes II–IV. Trophic cluster A = less than 2 size groups.

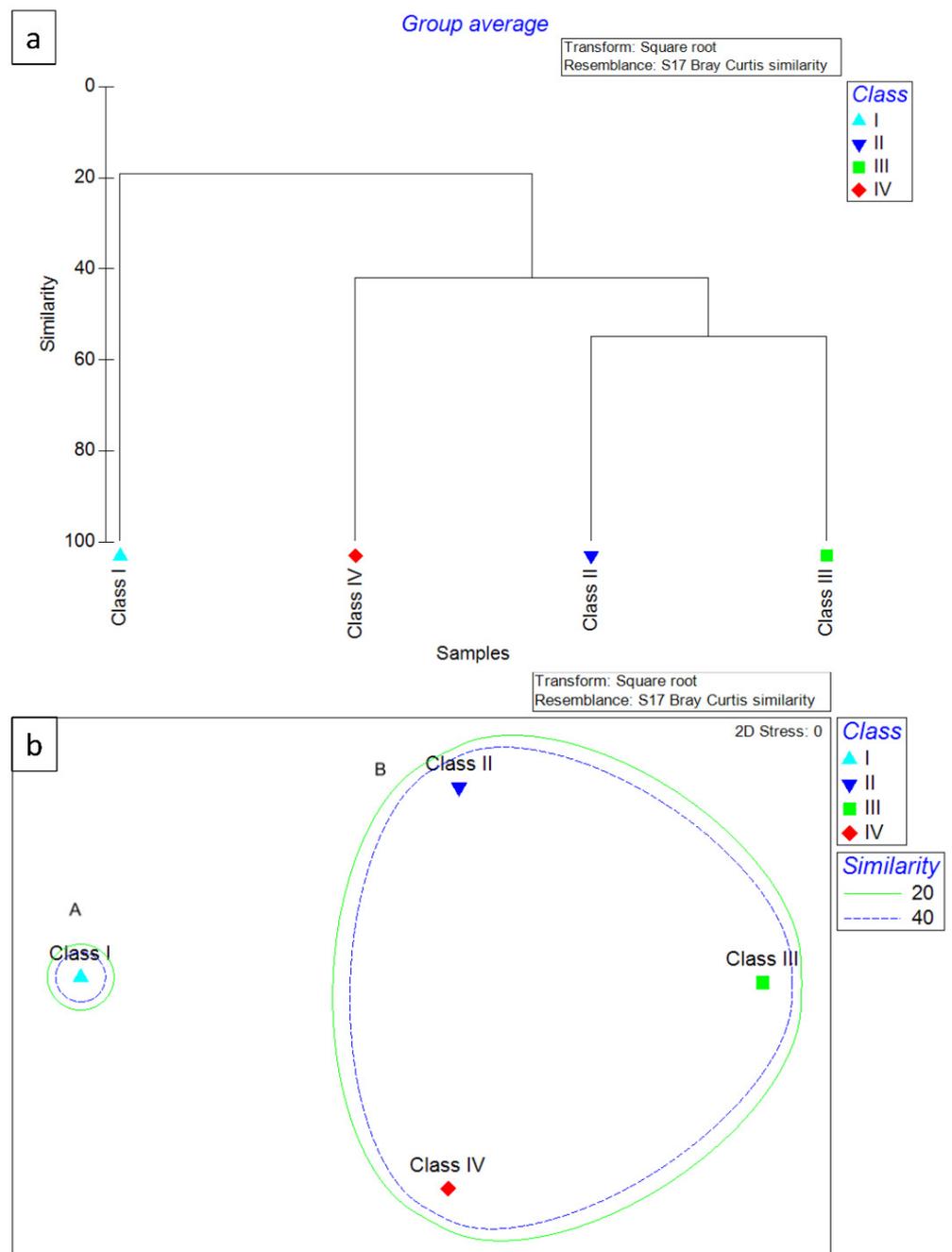| Group B	Species	Av.IRI val	Av.Sim	Contrib%	Cum.%
Average similarity:	Osteichthyes n.i.	28.98	19.2	41.42	41.42
46.36	*C.maderensis*	33.16	17.87	38.55	79.97
	Decapoda n.i.	7.7	3.47	7.48	87.45
	E.encrasicolus	10.6	2.45	5.28	92.74

Figure 3. Dendrogram (**a**) and MDS ordination of Bray–Curtis similarities (**b**) from dietary data (square root transformation) for the 4 hake ontogenetic stages analyzed in 2018. Cluster A included only the specimens belonging to Class I, while Cluster B included the specimens belonging to Classes II–IV.

These results were also confirmed with PCA, which highlights the main contribution of Euphausiacea to the differences observed for the size Class I, explaining the 89.36% total variation on the first axis (Figure 4).
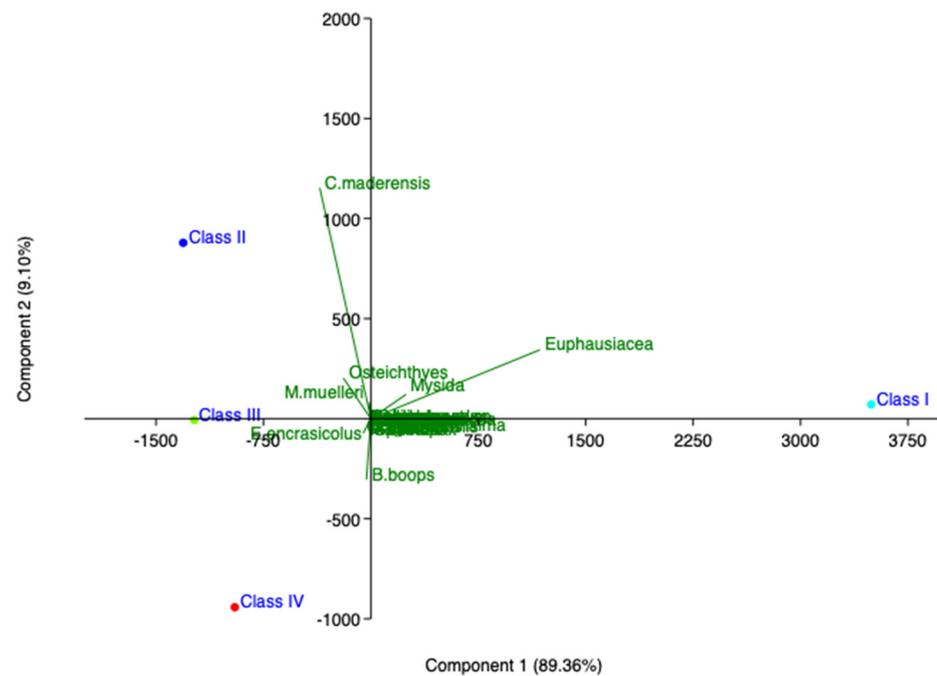
Figure 4. Principal component analysis (PCA) of hake diet in southern and central Tyrrhenian Sea (2018).

3.2. European Hake Diet Composition in 2019

In 2019, a total of 157 stomachs were analyzed. As reported in Table 5, according to IRI% values, the most relevant prey were Osteichthyes n.i. (39.89), followed by *B. boops* (19.92), *E. encrasicolus* (18.43) and *Sardina pilchardus* (7.25). Moreover, mesopelagic fishes, such as *M. muelleri* (IRI% = 2.33) and *C. maderensis* (1.55), and Crustaceans, such as Euphausiacea n.i. (3.93), Decapoda n.i. (1.91) and Dendrobranchiata n.i. (1.78), have confirmed their importance in hakes' diet.

Table 5. Diet composition of the whole hake series from southern and central Tyrrhenian Sea sampled in 2018. In the columns are reported the diet index values (%F, %W, %N, IRI and %IRI) for each prey category.

TAXON	%F	%W	%N	IRI	%IRI
MOLLUSCA					
Cephalopoda n.i.	1.00	0.35	0.77	1.12	0.07
CRUSTACEA					
Decapoda					
Parapenaeus longirostris	1.00	0.57	3.08	3.65	0.22
Aristaeomorpha foliacea	1.00	0.13	0.77	0.90	0.05
Alpheus glaber	2.00	0.13	1.54	3.33	0.20
Chlorotocus crassicornis	1.00	0.10	1.54	1.64	0.10
Eusergestes arcticus	2.00	0.11	1.54	3.29	0.20
Pasiphaea sivado	1.00	0.22	0.77	0.99	0.06
Plesionika sp.	2.00	0.39	1.54	3.86	0.23
Decapoda n.i.	6.00	0.70	4.62	31.87	1.91
Dendrobranchiata n.i.	6.00	0.33	4.62	29.67	1.78
Euphausiacea					
Meganyctiphanes norvegica	1.00	0.21	3.85	4.05	0.24
Euphausiacea n.i.	5.00	0.02	13.08	65.48	3.93
OSTEICHTHYES					
Ceratoscopelus maderensis	5.00	0.54	4.62	25.76	1.55

Table 5. *Cont.*

TAXON	%F	%W	%N	IRI	%IRI
Argentina sphyraena	1.00	0.72	1.54	2.26	0.14
Boops boops	7.00	42.05	5.38	332.06	19.92
Echiodon dentatus	2.00	0.27	1.54	3.61	0.22
Gaidropsarus biscayensis	1.00	0.12	1.54	1.66	0.10
Maurolicus muelleri	5.00	1.60	6.15	38.79	2.33
Engraulis encrasicolus	12.00	14.83	10.77	307.17	18.43
Physiculus dalwigki	1.00	0.00	0.77	0.77	0.05
Sardina pilchardus	6.00	15.51	4.62	120.78	7.25
Chlorophthalmus agassizi	1.00	0.58	0.77	1.35	0.08
Trachurus trachurus	1.00	7.67	0.77	8.44	0.51
Myctophidae n.i.	1.00	0.21	0.77	0.98	0.06
Sparidae n.i.	1.00	2.94	0.77	3.71	0.22
Carapidae n.i.	2.00	0.00	1.54	3.08	0.18
Gobiidae n.i.	1.00	0.00	0.77	0.77	0.05
Macrouridae n.i.	1.00	0.03	0.77	0.80	0.05
Osteichthyes n.i.	23.00	9.68	19.23	664.90	39.89

Concerning the ontogenetic differences, as reported in Table 3 and in Figure 5, Class I showed an absolute preference for Euphausiacea n.i (IRI% = 100). This was not confirmed in Class II (IRI% = 1.13), where in addition there were marked preferences for teleost fishes, mainly *M. muelleri* (IRI% = 65.76), and Decapoda, mainly *Plesionika* sp. (IRI% = 2.94). In Class III, the importance of Decapods in hakes' diet was greater than in smaller specimens, as confirmed by the IRI% value of Decapoda n.i. (6.41). However, the most relevant prey were always teleost fishes, in particular *E. encrasicolus* (IRI% = 73.71) and Osteichthyes n.i. (IRI% = 17.16). In Class IV, the most relevant prey category was Osteichthyes n.i. (IRI% = 52.49), followed by *E. encrasicolus* (IRI% = 13.67), *S. pilchardus* (IRI% = 13.25), Decapoda n.i. (IRI% = 11.88) and *C. maderensis* (IRI% = 2.94). *B. boops* (IRI% = 92.51) was the most relevant prey for the Class V, followed by *Trachurus trachurus* (IRI% = 2.21), *Argentina sphyraena* (IRI% = 1.62) and *S. pilchardus* (IRI% = 1.31).

One-way ANOVA analysis was performed to assess dietary composition differences between the ontogenetic stages of the *M. merluccius* specimens ($p < 0.05$). Indeed, Class IV was significantly different from all other size classes ($p < 0.05$), while the other classes did not differ significantly in their diets ($p > 0.05$).

Cluster analysis and MDS ordination grouped the whole data set obtained from 2019, by size classes, into three main trophic clusters. Cluster A included only the specimens belonging to Class I, cluster B included the specimens belonging to Class V, while cluster C grouped Classes II–IV (Figure 6). In detail, Classes II–IV showed 20% similarity, mainly driven by the large contribution of Osteichthyes (Table 6).

Table 6. Results of the SIMPER analysis conducted between European hake trophic groups from 2019. The average similarity between cluster groups is reported. The percentage and cumulative contribution of typifying species within-group similarity of the identified hake trophic groups is shown. The table shows results obtained for the trophic cluster B, which includes Classes II–IV. Trophic cluster A and C = less than 2 size groups.

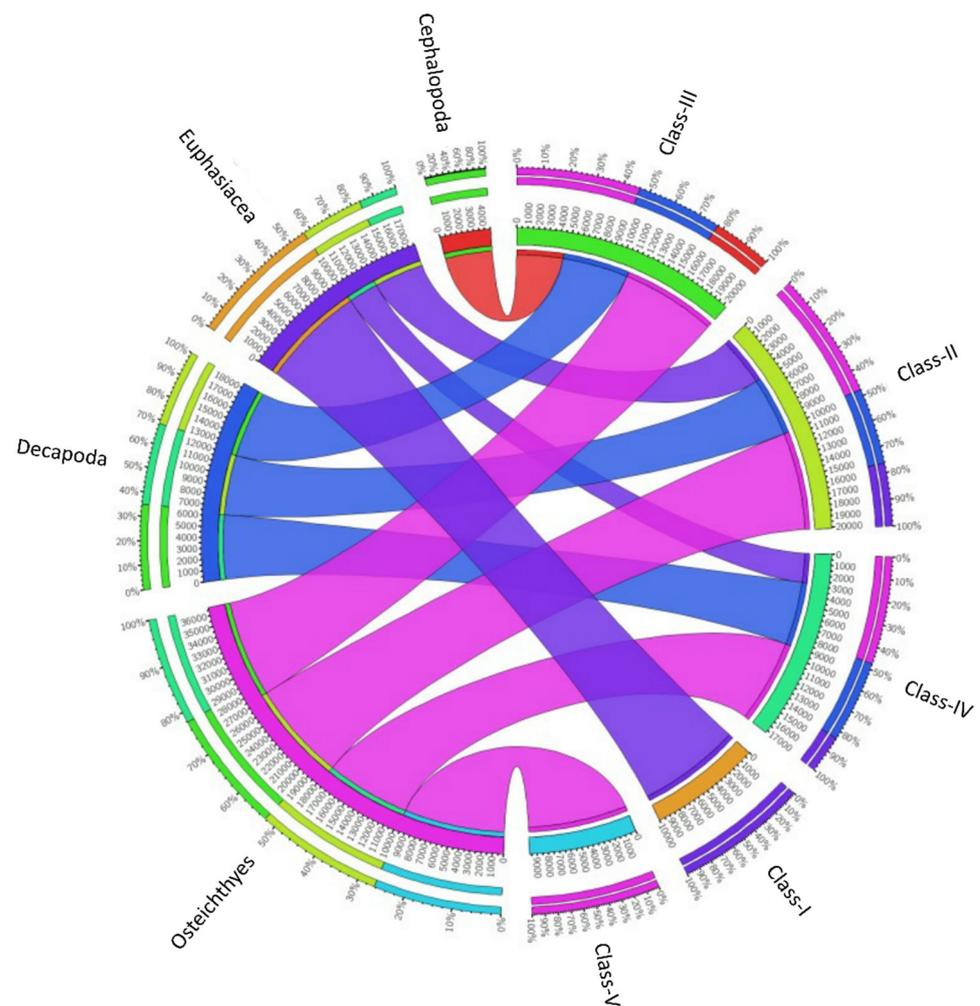| Group	Species	Av.IRI val	Av.Sim	Contrib%	Cum.%
Average similarity:	Osteichthyes n.i.	37.15	23.51	68.76	68.76
34.19	*E. encrasicolus*	27.54	4.16	12.18	80.94
	Decapoda n.i.	11.75	3.88	11.36	92.3

Figure 5. Diet composition of the *Merluccius merluccius* specimens collected from southern and central Tyrrhenian Sea during 2019. The chord diagram shows the connection among the main taxa found in the stomach contents of different hake size classes investigated. The size classes analyzed are shown on the right of the diagram. Main taxa found are shown on the left. Ribbon size in the chart codifies IRI value associated with hake size classes/prey taxa segment pair.

The dominance of Euphausiacea in Class I and Osteichthyes (as *B. boops* and *E. encrasicolus*) in the other ontogenetic classes, as shown by PCA, could explain the 89.36% total variation on the first axis (Figure 7) and the dissimilarity observed between clusters.

3.3. European Hake Diet Composition in 2020

In the 2020 a total of 69 stomachs were analyzed. As reported in Table 7, according to IRI%, the most relevant prey was Osteichthyes n.i. (38.14), followed by Mysida n.i. (17.54), *T. trachurus* (17.14), *Trachurus mediterraneus* (10.21), *Pasiphaea sivado* (5.65), *E. encrasicolus* (3.61) and *Trachurus* sp. (2.52).
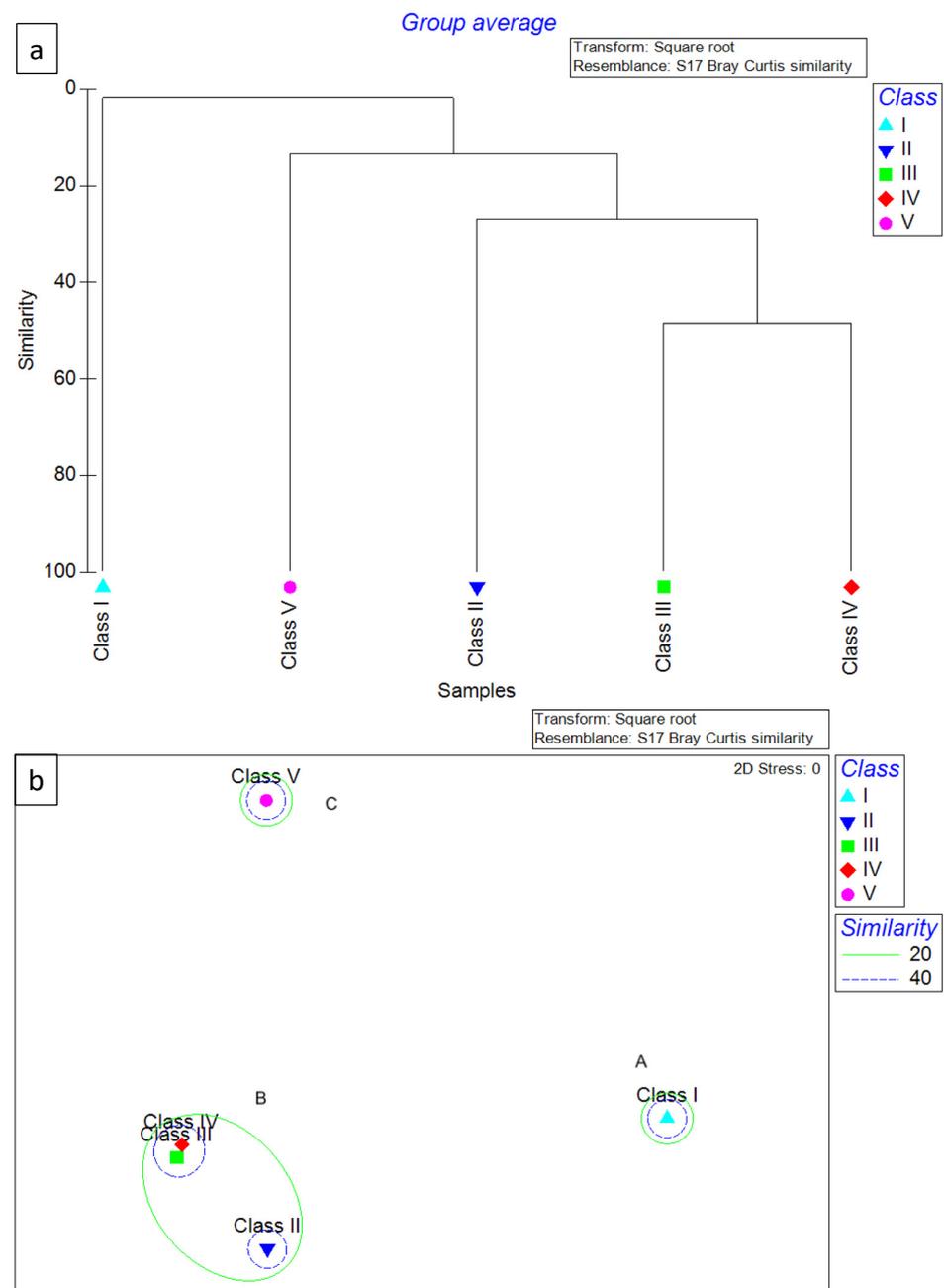
Figure 6. Dendrogram (**a**) and MDS ordination of Bray–Curtis similarities (**b**) from dietary data (square root transformation) for the 5 hake classes analyzed in 2019. Cluster A included only the specimens belonging to Class I, cluster B included the specimens belonging to Class V, while cluster C grouped Classes II–IV.

Concerning ontogenetic classes, as reported in Table 3 and Figure 8, in Class I Mysida n.i. (IRI% = 71.11) was the most relevant prey category, followed by Osteichthyes n.i. (IRI% = 15.99), *Meganyctiphanes norvegica* (IRI% = 4.31), Euphausiacea n.i. (IRI% = 4.11) and Cephalopoda n.i. (IRI% = 3.92). The diet of hakes belonging to Class II was totally dominated by Osteichthyes n.i., which was also the most relevant prey category in Class III (IRI% = 54.64), followed by *E. encrasicolus* (IRI% = 41.17) and *Processa acutirostris* (IRI% = 2.36). The diet of Class IV was dominated by *T. mediterraneus* (IRI% = 48.33), followed by *Trachurus* sp. (IRI% = 21.90), Osteichthyes n.i. (IRI% = 14.08) and *T. trachurus* (IRI% = 13.55).
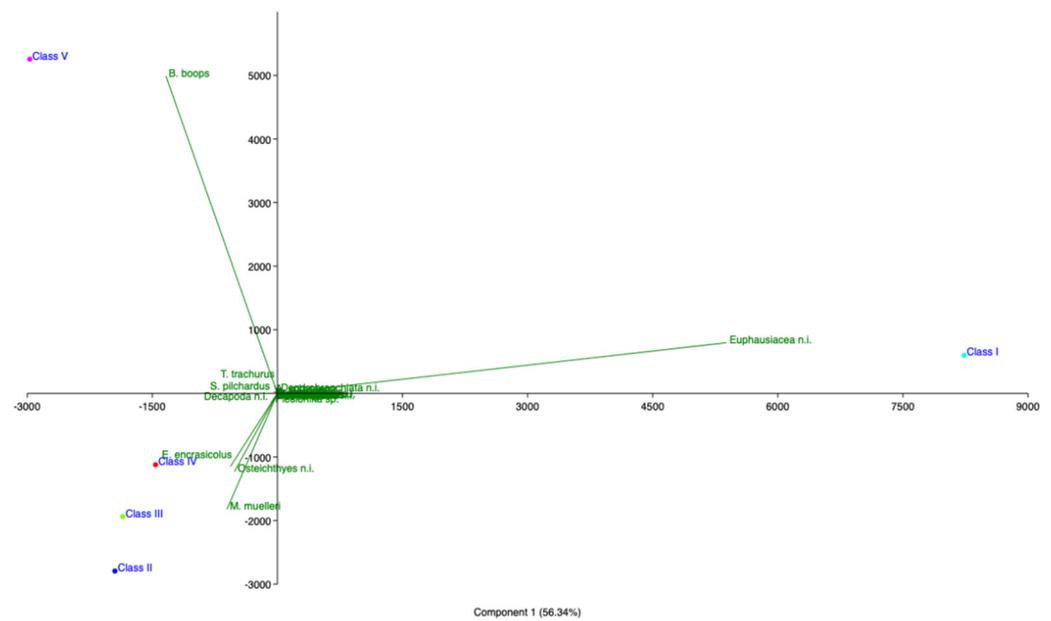
Figure 7. Principal component analysis (PCA) of hake diet in southern and central Tyrrhenian Sea (2019).

Table 7. Diet composition of the whole hake series from southern and central Tyrrhenian Sea sampled in 2020. In the columns are reported the diet index values (%F, %W, %N, IRI and %IRI) for each prey category.

TAXON	%F	%W	%N	IRI	%IRI
MOLLUSCA					
Cephalopoda n.i.	4.60	0.02	2.68	12.43	0.84
CRUSTACEA					
Amphipoda					
Amphipoda n.i.	1.15	0.01	0.67	0.78	0.05
Decapoda					
Eusergestes arcticus	1.15	0.09	0.67	0.87	0.06
Solenocera membranacea	1.15	0.33	0.67	1.16	0.08
Pasiphaea multidentata	2.30	0.66	1.34	4.61	0.31
Pasiphaea sivado	5.75	3.73	10.74	83.15	5.65
Processa acutirostris	1.15	0.32	2.01	2.68	0.18
Sergestes sp.	1.15	0.11	0.67	0.90	0.06
Pasiphaea sp.	1.15	0.51	2.68	3.67	0.25
Dardanus sp.	1.15	0.01	0.67	0.79	0.05
Decapoda n.i.	3.45	0.27	1.34	5.57	0.38
Stomatopoda					
Parasquilla ferussaci	1.15	0.64	1.34	2.28	0.15
Euphausiacea					
Meganyctiphanes norvegica	1.15	0.09	10.07	11.68	0.79
Stylocheiron longicorne	1.15	0.01	0.67	0.78	0.05
Euphausiacea n.i.	3.45	0.04	2.01	7.08	0.48
Mysida n.i.	10.34	0.12	24.83	258.12	17.54
OSTEICHTHYES					
Ceratoscopelus maderensis	2.30	1.03	2.01	7.00	0.48
Diaphus holti	1.15	0.40	0.67	1.23	0.08
Boops boops	1.15	0.78	0.67	1.67	0.11
Engraulis encrasicolus	4.60	8.86	2.68	53.07	3.61
Macroramphosus scolopax	1.15	0.17	0.67	0.97	0.07
Maurolicus muelleri	1.15	0.23	0.67	1.04	0.07
Lampanyctus crocodilus	1.15	0.39	0.67	1.23	0.08

Table 7. *Cont.*

TAXON	%F	%W	%N	IRI	%IRI
Peristedion cataphractum	1.15	0.04	0.67	0.82	0.06
Nettastoma melanura	1.15	0.01	0.67	0.79	0.05
Chlorophthalmus agassizi	1.15	1.14	0.67	2.08	0.14
Cepola macrophthalma	1.15	1.35	0.67	2.32	0.16
Stomias boa	1.15	1.14	0.67	2.08	0.14
Trachurus mediterraneus	5.75	22.12	4.03	150.29	10.21
Trachurus trachurus	5.75	40.55	3.36	252.32	17.14
Trachurus sp.	3.45	8.74	2.01	37.08	2.52
Osteichthyes n.i.	25.29	6.10	16.11	561.45	38.14
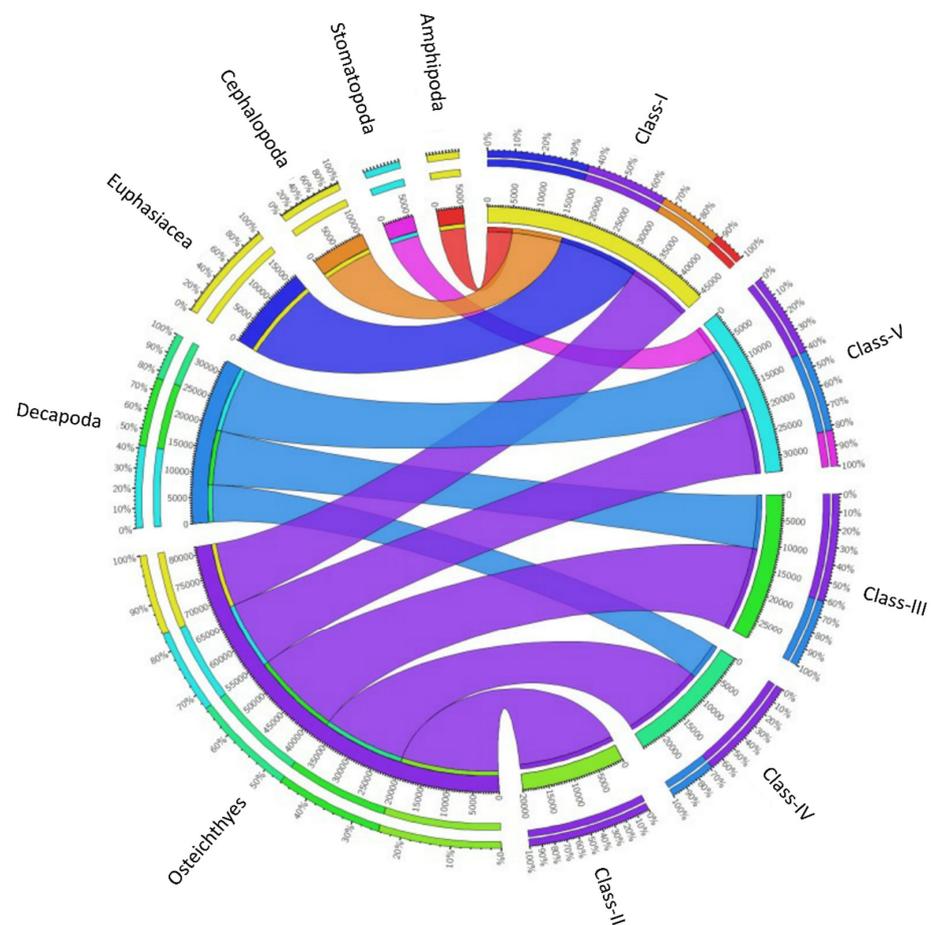
Figure 8. Diet composition of the *Merluccius merluccius* specimens collected from southern and central Tyrrhenian Sea during 2020. The chord diagram shows the connection among the main taxa found in the stomach contents of different hake size classes investigated. The size classes analyzed are shown on the right of the diagram. Main taxa found are shown on the left. Ribbon size in the chart codifies IRI value associated with hake size classes/prey taxa segment pair.

One-way ANOVA analysis was performed to assess dietary composition differences between the size classes of the *M. merluccius* specimens ($p < 0.05$). Indeed, only Classes II and V differ significantly in their diets ($p < 0.05$).

Cluster analysis and MDS ordination grouped the whole data set obtained from 2020, by size classes, into three trophic clusters (Figure 9). Cluster A included only the specimens belonging to Class I, cluster B included the specimens belonging to Classes II and III while the third one (cluster C) included Classes IV and V. In detail, the trophic clusters A and C showed 20% similarity, while cluster B showed 40% similarity.
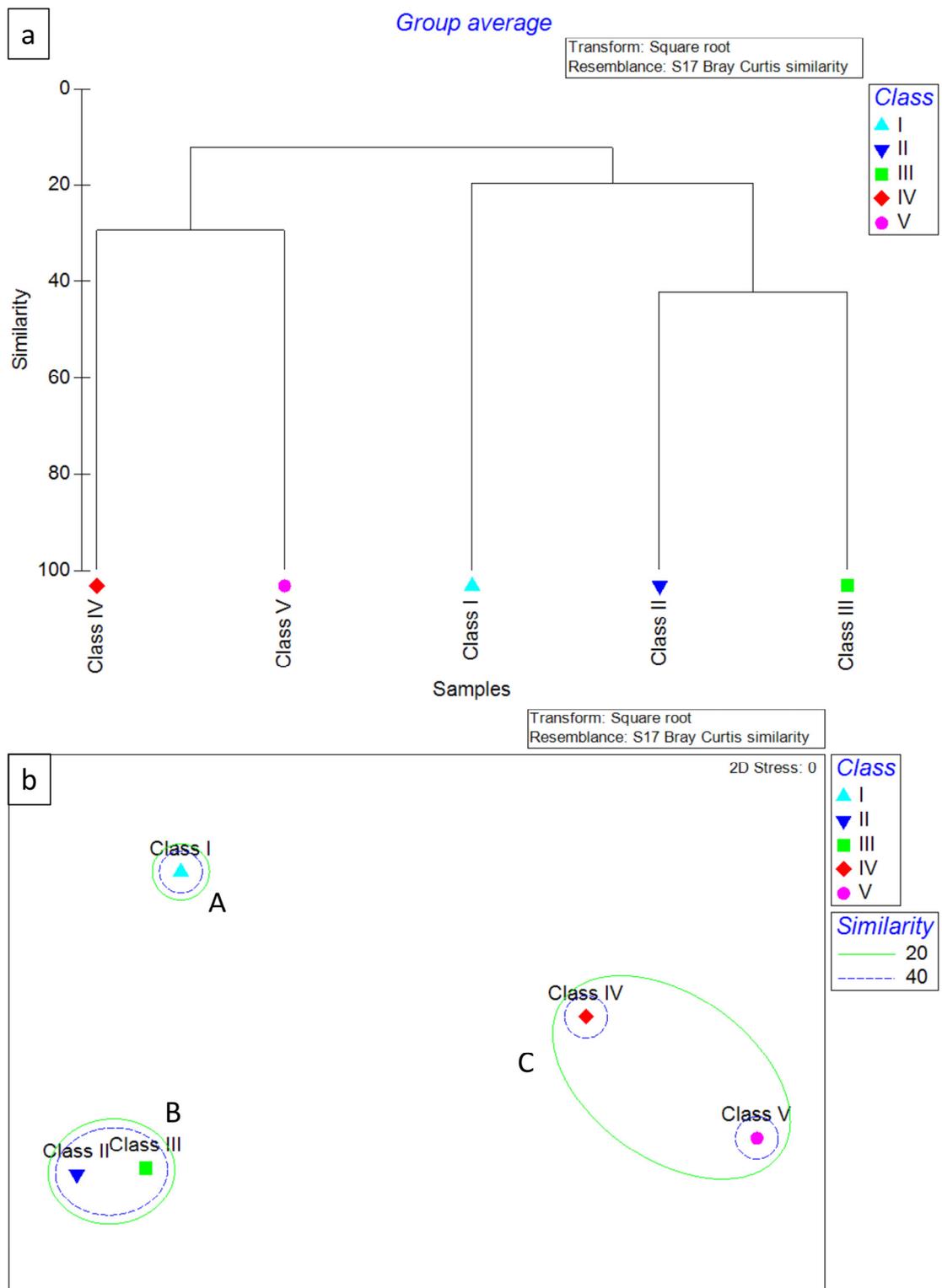
Figure 9. Dendrogram (**a**) and MDS ordination of Bray–Curtis similarities (**b**) from dietary data (square root transformation) for the 5 hake classes analyzed in 2020. Cluster A included only the specimens belonging to Class I, cluster B included the specimens belonging to Classes II and III while the third one (cluster C) included Classes IV and V.

Results obtained by PCA, with 94% total variation in the first axis (Figure 10), confirm the results obtained by SIMPER and ANOVA, resulting in a greater dissimilarity between Classes II and V due to the difference in the percentage of Osteichthyes (Table 8) and crustaceans found in the diet of the two classes.
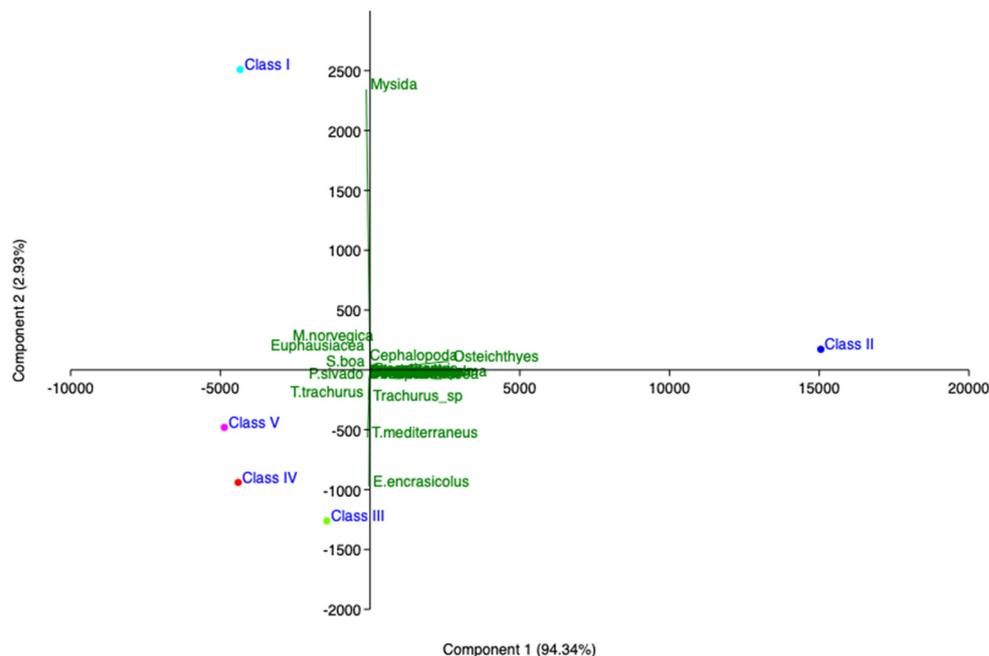
Figure 10. Principal component analysis (PCA) of hake diet in southern and central Tyrrhenian Sea (2020).

Table 8. Results of the SIMPER analysis conducted between European hake trophic groups from 2020. The average similarity between cluster groups is reported. The percentage and cumulative contribution of typifying species within-group similarity of the identified hake trophic groups is shown. The table shows results obtained for the trophic clusters B (including size Classes II and III) and C (including size Classes IV and V). Trophic cluster A = less than 2 size groups.

Group B	Species	Av.IRI val	Av.Sim	Contrib%	Cum.%
Average similarity: 42.33	Osteichthyes n.i.	100.3	42.33	100	100
Group C					
Average similarity: 29.36	*T. trachurus*	24.79	17.86	60.81	60.81
	Osteichthyes n.i.	16.02	5.8	19.76	80.57
	T. mediterraneus	26.31	5.71	19.43	100

3.4. Temporal-Scale Variations in European Hake Prey Preference

Concerning diet composition in the entire analyzed timeframe (2018/2020), the most relevant prey were Osteichthyes n.i. (IRI% = 38.17), followed by Euphausiacea n.i. (IRI% = 17.78), *B. boops* (IRI% = 8.78) and Mysida n.i. (IRI% = 5.07).

To detect significant temporal variation in European hake diet, all data obtained during 2018–2020 were analyzed using univariate and multivariate analyses. The One-way Analysis of Variance showed that the specimens belonging to Classes I and III did not differ in their diet among the sampling periods analyzed in this study ($p > 0.05$), while significant differences were detected within Classes II ($p = 0.07$), IV ($p = 0.014$) and V ($p < 0.05$, only for 2019 and 2020) in specimens collected during the timeframe investigated. As evident from Table 3, the most relevant differences among specimens belonging to Class II were detected for *C. maderensis*, Osteichthyes n.i., *M. muelleri* and *Plesionika* sp., while, concerning Class IV

for *T. mediterraneus*, they were Osteichthyes n.i., *E. encrasicolus* and *C. maderensis.* Specimens belonging to Class V showed differences in diet composition among years mainly for *B. boops*, *T. trachurus* and *P. sivado.*

Cluster analysis and MDS ordination grouped the whole data set into four trophic clusters. Cluster A included only the specimens belonging to Class I collected during 2018 and 2019. This trophic cluster showed the highest average similarity (58.22%), mainly attributable to the dominance of Euphausiacea n.i. (Table 9). Cluster B grouped the specimens belonging to Class I collected during 2020, and Classes II–IV of all three years, except for Class IV samples collected during 2020. Osteichtyes n.i. mainly contributed to the similarity observed for this trophic group with an average similarity of 33.46 %. Cluster C included Classes IV and V from 2020, showing the lowest average similarity (29.36%) resulting from the large contribution given by the species *T. trachurus* (contr. 60.81%). Each trophic cluster showed 20% similarity.

Table 9. Results of the SIMPER analysis conducted between European hake trophic groups collected between 2018 and 2020. The average similarity between cluster groups is reported. The percentage and cumulative contribution of typifying species within-group similarity of the identified hake trophic groups is shown. The table show results obtained for the trophic clusters A–C. Trophic cluster D = less than 2 size groups.

Group A	Species	Av.IRI val	Av.Sim	Contrib%	Cum.%
Average similarity: 58.22	Euphausiacea	83.2	58.22	100	100
Group B					
Average similarity: 33.46	Osteichthyes n.i.	47.31	22.39	66.92	66.92
	E. encrasicolus	18.42	3.77	11.28	78.2
	Decapoda	7.31	2.22	6.62	84.83
	C. maderensis	11.93	1.98	5.92	90.75
Group C					
Average similarity: 29.36	*T. trachurus*	24.79	17.86	60.81	60.81
	Osteichthyes n.i.	16.02	5.8	19.76	80.57
	T. mediterraneus	26.31	5.71	19.43	100

Finally, Cluster D only included specimens belonging to Class V from 2019 (Figure 11).

Results obtained by PCA, with 63.41% total variation on the first axis (Figure 12), confirm the results obtained by SIMPER and ANOVA, resulting in a greater dissimilarity between Classes II (2020) and V (2019) due to the difference in the percentage of Osteichthyes (Table 9) found in the diet of the two classes.
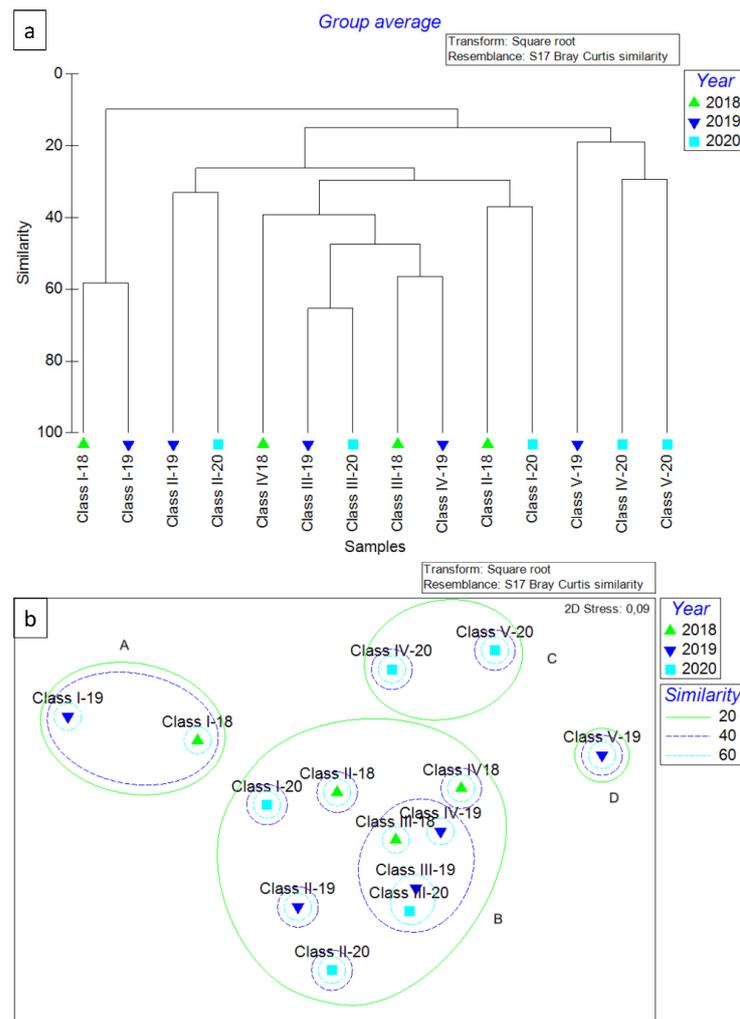
Figure 11. Dendrogram (**a**) and MDS ordination of Bray–Curtis similarities (**b**) from dietary data (square root transformation) for the all hake classes analyzed in 2018–2020. Cluster A included the specimens belonging to Class I collected during 2018 and 2019. Cluster B grouped the specimens belonging to Class I collected during 2020, and Classes II–IV of all three years, except for Class IV samples collected during 2020. Cluster C included Classes IV and V from 2020. Finally, Cluster D only included specimens belonging to Class V from 2019.
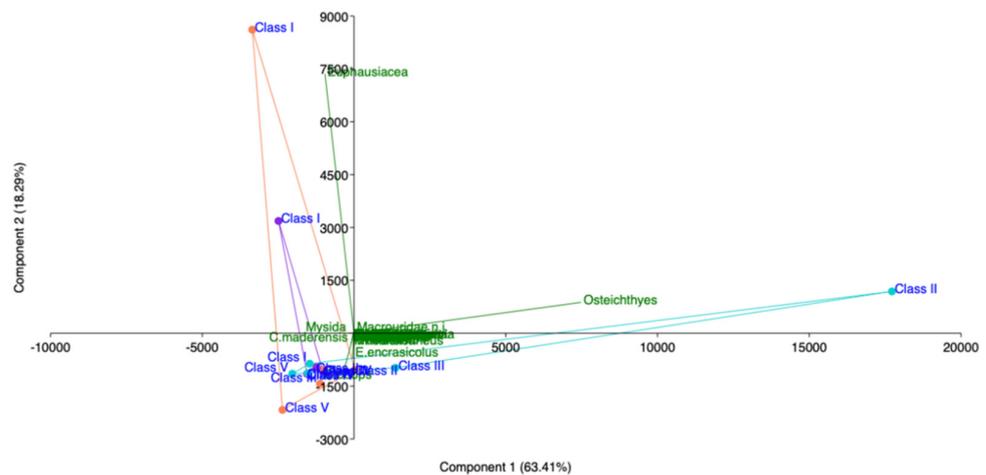
Figure 12. Principal component analysis (PCA) of hake diet in southern and central Tyrrhenian Sea (2018–2020). Violet: 2018; Orange: 2019; Turquoise: 2020.

4. Discussion

4.1. Diet Composition and Feeding Habits

The data provided by the present study on *M. merluccius* diet in the time frame between 2018 and 2020 confirmed its ecological role as a generalist benthopelagic predator in the southern and central Tyrrhenian Sea. Results showed a diet composition and feeding habits in line with literature from other geographical areas [17,19–21,23,51,68–75], highlighting its ability to prey on creatures both in the suprabenthic layer and in the entire water column. This ability was confirmed in all the analyzed years by the presence among prey of species profoundly connected with the benthic environment (e.g., *Cepola macrophthalma*, *Callionymus* sp., *Echiodon dentatus*, *P. longirostris*, *Alpheus glaber*), mesopelagic bioluminescent species (e.g., *C. maderensis*, *M. muelleri*, *Diaphus* sp.) and nektobenthic species performing large horizontal and vertical migrations (e.g., *Trachurus* sp., *M. scolopax*, *C. agassizi*, *P. sivado*, *C. crassicornis*). This large spectrum of prey exploited by *M. merluccius*, also composed by those inhabiting deeper habitats, makes this species an essential predator, confirming its key role in the energy exchanges between different depth layers and trophic levels [18,21,23], with the large hakes also being a prey of bigger predators (such as large pelagic fishes) [76], while the juveniles are often cannibalized by larger hakes [20,22]. However, our results show the absence of cannibalism in the analyzed area. This could be strictly related to the recruit's availability, the presence/absence of a nursery area, variations in population size structures and main prey availability, as reported by previous studies from other geographical areas [20,22,55,69]. The high occurrence of recruits (conspecific alternate prey) together with the depletion of the main prey, can trigger an increase in cannibalism [77,78]. The absence of cannibalism among the analyzed hake specimens may indicate a high availability of prey, with a large spectrum of species, which together with the relative low incidence of recruits [2,79,80], could reduce the need for intraspecific predation in *M. merluccius*. Further analyses are required to deepen the knowledge on hake cannibalism in GSA 10, investigating mainly larger hakes' diet and recruit distribution, with the studies [81] on nursery area and population size structures from this geographical area being less relative.

Another interesting aspect shown by the results was the low occurrence of cephalopod prey in hake diet composition if compared with literature from other geographical areas, such as the Adriatic Sea and the Strait of Sicily [18,20,82]. This could be mainly related to the ecological features of GSA 10, characterized by a high occurrence of mesopelagic bioluminescent fishes, benthopelagic and mesopelagic crustaceans, and small pelagic species, which may be preferred as prey compared to cephalopods. Moreover, these last seem to be highly sensitive to environmental conditions, such as temperature variations due to climate changes and nutrient concentration variations, influencing their distribution and population dynamics [83–86]. All these factors, together with an unfavorable oceanographic feature of the studied area, could induce a low occurrence of those cephalopod species mainly preyed on in the other Mediterranean geographical areas (such as *Rondeletiola minor*, *Alloteuthis* sp., *Illex coindetii*, *Sepia officinalis*, *Sepietta* sp.) in zones and depths exploited by hakes.

4.2. Ontogenetic Variations in Diet Composition

Concerning the diet composition in the different ontogenetic stages, results confirmed the planktivorous feeding habits of juvenile hakes belonging to Class I in the three analyzed years. The main prey in this ontogenetic class were Euphausiids (mainly *M. norvegica* and *Stylocheiron longicorne*) and not identified Mysida, confirming the essential contribution of planktonic crustaceans for the livelihood of this predator in its early life. As reported in other Mediterranean geographical area [18,20,69,71,87], juvenile hakes' aggregations near the shelf-break favor their foraging on school-forming zooplanktonic and micronektonic species. These are preyed on during their daily vertical migrations along the water column [17,51,88]. The presence among prey in this ontogenetic class and in Class II, mainly in 2018 and 2020, of small Osteichthyes, as lanternfish, could be confirm the importance

of the planktonic trophic chain in the energy support of the entire ecosystem. Indeed, zooplanktonic proliferation, related to phytoplanktonic blooms, may attract other planktivorous mesopelagic micronekton, such as lanternfishes, which become in turn prey of hakes together with Euphausiids.

According to previous literature for this and other geographical areas [15–18,20,21,51,69,72,82], the prey composition in the other ontogenetic classes have confirmed the transition of European hakes during their growth to a diet mainly based on teleost and decapod consumption (mainly after the first year of age, TL > 15 cm). This tendency is reported by results for all the years, with a similar diet composition among hakes belonging to Class II, mainly based on small teleost, as mesopelagic bioluminescent prey (e.g., *C. maderensis*, *M. muelleri*), Euphasiids, and larger nektobentic species (such as *B. boops*, *E. encrasicolus*, *Trachurus* sp., *C. agassizi*, *A. sphyrena*) in the other size classes, with an increase in pelagic and benthopelagic decapod consumption (*P. longirostris*, *S. membranacea*, *Pasiphae* sp., *C. crassicornis*) in Classes III and IV. These diet shifts may be related to morphological changes associated with hake growth, as increase in mouth opening, visual acuity and hearing [89,90]. Indeed, all these changes can improve the hunting ability of this species, making it able to feed on larger mobile fish and benthopelagic species, as reported also for other predators [64,91,92]. Moreover, according to several authors [17,69], this increase in larger prey consumption could also be related to the large energy demand required for sexual maturation and gonadal development in post recruits and adults specimens.

4.3. Temporal Scale Variations in Diet Composition

To our best knowledge, the present paper is the first study exploring the temporal scale variability in hakes' diet from the southern and central Tyrrhenian Sea. Previous studies from this area were focused on the seasonal ontogenetic changes in diet, assessing the importance as prey of mesopelagic bioluminescent prey in hakes from northern Sicily, with only one paper investigating the entirety of GSA 10 [16,21,23]. Concerning this last paper, it showed clear differences in prey composition and hakes' size distribution in 2018 if compared to results of the present paper for the same year. This could be strictly related to the differences between sampling methods. Indeed, in the present paper, all the samples for 2018 were caught by trawling, having been provided through the MEDITS project. Concerning the other paper of D'Iglio et al. [23], most samples were provided by biological sampling of catches from commercial fleets (CAMPBIOL project), with hakes caught using different sampling methods. For this reason, despite involving the same sampling area and year, the results showed by each paper for 2018 are different, with the absence of hakes belonging to Class V in the present paper (due to the rapid ascent of trawling nets, which cause stomach eversion in larger hakes).

Herein, data on *M. merluccius* diet composition showed a temporal variability reflecting an opportunistic feeding habit, shaped on the temporal and spatial availability and prey distribution. European hakes confirmed their ability to adapt the diet to the temporal (annual or seasonal) and spatial distribution of the different prey categories, as widely reported in literature from other geographical areas [15,17,18,20,51,69]. This is an essential skill to survive in over-exploited oligotrophic ecosystems, such as the southern and central Tyrrhenian Sea, being able to feed on all the available prey belonging to the main categories, Osteichthyes and Crustaceans.

Prey belonging to the cephalopod category (Cephalopoda n.i.), even if with a lesser occurrence than other categories, showed variations between years, with the highest IRI% values in stomachs collected in 2020 belonging to hakes of Class I. This annual fluctuation may reflect changes in composition of nektobenthic species related to several ecological factors. These may depend on changes in predator population and distribution [14], and on environmental conditions, such as water temperature and nutrients. Indeed, as reported in the Atlantic Ocean, these factors can influence the population size and the distribution of cephalopods [83–86]. Moreover, feeding on cephalopods was not a prerogative of juvenile hakes. As shown by results, post recruits and adults belonging to size Classs IV (mainly in

2019) and III (mainly in 2018) also feed on this prey category, even if less than juveniles in 2020. Further analysis of cephalopod populations and their distribution in the southern and central Tyrrhenian Sea, also analyzing the annual variations in composition and abundance through the study of time series on demersal assemblages, are required to understand these dynamics and the ecological/environmental causes which may allow variations on population dynamics and species composition of this taxa.

Concerning the annual variations in decapod prey composition, an interesting aspect was the large amount of Pasiphaeidae species (mainly *P. sivado*) detected in hake gut contents from specimens collected in 2020. The large amounts of adult individuals (belonging to size Class V) which showed a preference for this prey category once again underline the essential relevance of prey inhabiting deeper environments than hakes for their livelihood, representing a clear case of inverse energy flow between different bathymetric layers [15,21,23,51,69,93]. The glass shrimp *P. sivado*, as the other Pasiphaeids present in the Mediterranean Sea, are a worldwide-distributed caridean decapod inhabiting the epi-, meso- and bathypelagic environments, with a size-dependent distribution and a key ecological role. It is one of the favorite prey of many supra benthic predators, thanks to its daily vertical migrations [94–97]. According to [98], this species feeds on benthic prey (gammarids, isopods and macruran decapods) during the nighttime, while in the daytime it performs vertical migrations to feed on plankton (mainly Euphausiids, chaetognaths) and small fishes, becoming in turn prey of benthopelagic predators, such as *M. merluccius*, with energy transferal between benthic and pelagic environments. The higher occurrence of Pasiphaeidae species in gut content of hakes from 2020 than the other years could be related to variations in environmental factors, resulting in an increase in population size, with Pasiphaeids reproductive processes and population dynamics being highly sensitive to environmental variations and fluctuation in prey availability and distribution [97].

Osteichthyes showed the most marked annual variations in species composition and occurrence. As reported by statistical analysis, the larger fluctuations in occurrence were detected for lanternfishes (*M. muelleri*, *C. maderensis*) and nektobenthic fishes (*B. boops*, *Trachurus* sp., *E. encrasicolus*, *C. agassizi*, *S. pilchardus*), with the benthic species occurring always as occasional prey, mainly in adult hakes belonging to Classes IV and V. This demonstrates the main hunting strategy of post recruits and adult European hakes in the studied area, ambushing its favorite pelagic prey, represented by fast swimming teleost and lanternfish, in the water column. Concerning the annual fluctuation of lanternfishes (*M. muelleri* and *C. maderensis*), these showed an evident decreasing in occurrence between 2018 and 2020. This is a strange trend, since mesopelagic bioluminescent fishes are widely distributed in the entire Tyrrhenian Sea, and as reported in literature, they represent essential prey for juvenile, recruit and post-recruit hakes, especially in shelf-break environments [21,23,51,69]. Focusing attention on the annual variations in prey preferences among the different ontogenetic stages, as shown by statistical analysis, the significant differences detected between years for Classes II, IV and V were also mainly related to *C. maderensis* and other nektobenthic species (mainly *B. boops*, *E. encrasicolus*, *Trachurus* sp.) confirming the importance of broadening the knowledge base concerning all these prey categories and their trophic relations with benthopelagic predators.

All these annual fluctuations in prey occurrence could be related to several factors, such as: (i) the seasonal variability in distribution and abundance of the different species, which in turn depends on the seasonal variations of nutrients, on the oceanographic condition of the area and on the availability of planktonic/micronektonic prey; (ii) daily temporal variations of hake feeding activities and intensity, influencing the rate of stomach fullness and undigested prey availability, easier to be identified at a species level than heavily digested prey found in hakes sampled long after their feeding period; (iii) small local scale variability in primary production and consequently in prey distribution, influencing the diet composition of sampled hakes. Several studies are required to deepen the knowledge on the population dynamics of prey species, adding new information on their temporal and spatial distribution. Further analysis supported by innovative molecular and stable

isotopes techniques are required to deepen the knowledge on the importance of different teleost species in hakes and other benthopelagic predators' diets [18,72,82]. These are essential to confirming diet composition, and avoiding the problems related to a high degree of digestion of prey parts, which reduces identification accuracy, causing an inevitable underestimation of the different species. Moreover, several studies with different sampling designs are required to evaluate seasonal and small-scale spatial variations in hake feeding habits and diet composition. These should be such as to analyze the differences in feeding activities and habits related to different seasons, periods of the day and different zones of the studied area with several oceanographic, ecological and environmental features. This is essential especially in the southern and central Tyrrhenian Sea, characterized by high heterogeneity in both environmental and fishing activity features between the northern (Campania and Lazio coasts) and southern zones (Sicilian and Calabrian coasts), as highlighted by several studies on other demersal species [28].

4.4. On an EBFM (Ecosystem-Based Fishery Management) Point of View

The trophic relationships between benthic and pelagic domains are essential for the effective functioning of the entire marine ecosystem. Deepening the knowledge of feeding activities and prey preferences of voracious benthopelagic predators could add precious information about interspecific ecological dynamics occurring in the different geographical areas. These predators, especially those such as hakes with marked ontogenetic diet shifts, moving among habitats according to different day periods, seasons, life cycles and prey availability, allow energy transferal between domains (benthic, pelagic and demersal) with a fundamental coupling between them [25,98–101]. The ability of European hakes to feed on planktonic, pelagic and benthic species shown by the results, according to season and ontogenetic stage, makes them a fundamental link among marine domains and a key species to understand and analyze marine trophic interactions in a holistic ecosystemic view [16,99–101]. This is an essential point of view to better understand and analyze the impacts, both direct and indirect, of fisheries on marine ecosystems, especially regarding those of mixed and heterogenous fisheries on oligotrophic environments, such as the southern and central Mediterranean Sea. Indeed, fisheries, through the expansion of fishery grounds to the deepest environments, the impact on ecologically relevant taxa, the depletion of benthic communities due to trawl fishing and the cascade effect related to removal of large predators, can cause severe stressors to ecosystems by altering the trophic relationships and dynamics in relative short temporal scales [24,31,32,42,102–110]. For this reason, it is essential to improve proper ecosystem-based fishery management, especially in those geographical areas with less and fragmentary information, enhancing and increasing the knowledge base concerning feeding habits of commercially and ecologically relevant predators, with the analysis of data from a large temporal scale and growing attention on seasonality in prey availability and ontogenetic diet shifts.

5. Conclusions

The data on diet composition and feeding habits of *M. merluccius* between 2018 and 2020 provided by the present paper represent a useful tool to analyze the interspecific trophic relationships occurring in the southern and central Tyrrhenian Sea. This area, characterized by an accentuated anthropogenetic impact and a lack on trophic ecology data concerning the main relevant and exploited species, represents a perfect study ground to better understand the trophic interactions and energy transferal occurring between species inhabiting different marine domains and habitats, also evaluating how fishery activities can alter them, through the alteration of the interspecific dynamics and the food web of an entire area. In accordance with previous literature from the studied area, results have confirmed the importance of deep species (as mesopelagic teleost and decapods) and species with high commercial value (as *B. boops*, *T. trachurus*, *T. mediterraneus*) in hakes' diet, highlighting the sensitivity of this species to anthropogenic pressure. The ontogenetic diet shifts confirmed the planktivorous diet of juvenile hakes belonging to

Class I, the mesopelagic bioluminescent teleost predation behavior in Class II and a diet mainly composed by decapods and teleosts in Classes III–V. Moreover, the opportunistic feeding habits confirmed by temporal scale analysis on diet highlight the ability of this species in adapting its diet to available prey. The high variability in occurrence and species composition of teleost, decapod and cephalopod prey confirm the key role of *M. merluccius* in monitoring the ecological dynamics in demersal marine environments. Further analysis applying stable isotopes and metabarcoding techniques are required to understand the entire trophic web structure, elucidating feeding habits and temporal variations in prey composition of *M. merluccius* and other benthopelagic predators. Deepening the knowledge of this key species for the ecosystem's effective functioning is fundamental to improve their conservation, enhancing fishery management and consequently the preservation of the entire marine ecosystem. Moreover, it will be necessary to couple an improved knowledge base concerning population dynamics and distribution of ecologically relevant species with data on trophic interspecific relationships to look at the functioning of all the ecosystems of the southern and central Tyrrhenian Sea, developing the best management policies for the most sustainable and least dangerous fisheries. 1D461

Institutional Review Board Statement: Ethical review and approval were waived for this study, due to fish specimens collection was authorized by the MEDITS and CAMPBIOL projects as part of annual research surveys, all involving lethal sampling. No experiments were conducted, nor were surgical procedures performed. No procedures caused lasting harm to sentient fish, nor were sentient fish subjected to chemical agents. The care and use of collected animals complied with animal welfare guidelines, laws and regulations set by the Italian Government.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

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References

1. Orsi Relini, L.; Papaconstantinou, C.; Jukic-Peladic, S.; Souplet, A.; De Sola, L.G.; Piccinetti, C.; Kavadas, S.; Rossi, M. Distribution of the Mediterranean hake populations (*Merluccius merluccius* smiridus Rafinesque, 1810) (Osteichthyes: Gadiformes) based on six years monitoring by trawl-surveys: Some implications for management. *Sci. Mar.* **2002**, *66*, 21–38. [CrossRef]
2. Sion, L.; Zupa, W.; Calculli, C.; Garofalo, G.; Hidalgo, M.; Jadaud, A.; Lefkaditou, E.; Ligas, A.; Peristeraki, P.; Bitetto, I.; et al. Spatial distribution pattern of european hake, *Merluccius merluccius* (Pisces: Merlucciidae), in the mediterranean sea. *Sci. Mar.* **2019**, *83*, 21–32. [CrossRef]
3. STECF. *Mediterranean Assessments Part 1 (STECF-15–18)*; JRC 98676, EUR 27638; Publications Office of the European Union: Luxembourg, 2015; Volume 1, ISBN 978-92-79-54141-4.
4. FAO. *Fishery and Aquaculture Statistics (Capture Productoin)*; FAO: Rome, Italy, 2018.
5. FAO. *The State of World Fisheries and Aquaculture 2020*; Sustainability in action; FAO: Rome, Italy, 2020.
6. GFCM. *Working Group on Stock Assessment of Demersal 655 Species (WGSAD)*; FAO: Rome, Italy, 2016.
7. FAO. *Report of the Fortieth Session of the General Fisheries Commission for the Mediterranean (GFCM)*; FAO: Rome, Italy; St. Julian's, Malta, 2016.
8. Russo, T.; Bitetto, I.; Carbonara, P.; Carlucci, R.; D'Andrea, L.; Facchini, M.T.; Lembo, G.; Maiorano, P.; Sion, L.; Spedicato, M.T.; et al. A holistic approach to fishery management: Evidence and insights from a central mediterranean case study (Western Ionian Sea). *Front. Mar. Sci.* **2017**, *4*, 193. [CrossRef]
9. Schaefer, M.B. Some considerations of population dynamics and economics in relation to the management of the commercial marine fisheries. *Fish. Econ.* **1957**, *1*, 33–45. [CrossRef]

10. Pikitch, E.K.; Santora, C.; Babcock, E.A.; Bakun, A.; Bonfil, R.; Conover, D.O.; Dayton, P.; Doukakis, P.; Fluharty, D.; Heneman, B.; et al. Ecosystem-based fishery management. *Science* **2004**, *305*, 346–347. [CrossRef]

11. Longo, C.; Hornborg, S.; Bartolino, V.; Tomczak, M.T.; Ciannelli, L.; Libralato, S.; Belgrano, A. Role of trophic models and indicators in current marine fisheries management. *Mar. Ecol. Prog. Ser.* **2015**, *538*, 257–272. [CrossRef]

12. Duffy, J.E.; Cardinale, B.J.; France, K.E.; McIntyre, P.B.; Thébault, E.; Loreau, M. The functional role of biodiversity in ecosystems: Incorporating trophic complexity. *Ecol. Lett.* **2007**, *10*, 522–538. [CrossRef]

13. Leroux, S.J.; Loreau, M. Theoretical perspectives on bottom-up and top-down interactions across ecosystems. In *Trophic Ecology: Bottom-Up and Top-Down Interactions Across Aquatic and Terrestrial Systems*; Cambridge University Press: Cambridge, UK, 2015; pp. 3–28. ISBN 9781139924856.

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

15. Bozzano, A.; Recasens, L.; Sartor, P. Diet of the european hake *Merluccius merluccius* (Pisces: Merluciidae) in the Western Mediterranean (Gulf of Lions). *Sci. Mar.* **1997**, *61*, 1–8.

16. Sinopoli, M.; Fanelli, E.; Anna, G.D.; Badalamenti, F.; Pipitone, C. Assessing the effects of a trawling ban on diet and trophic level of hake, *Merluccius merluccius*, in the southern Tyrrhenian Sea. *Sci. Mar.* **2012**, *76*, 677–690. [CrossRef]

17. Carpentieri, P.; Colloca, F.; Cardinale, M.; Belluscio, A.; Ardizzone, G.D. Feeding habits of European hake (*Merluccius merluccius*) in the central Mediterranean Sea. *Fish. Bull.* **2005**, *103*, 411–416.

18. Fanelli, E.; Rumolo, P.; Barra, M.; Basilone, G.; Genovese, S.; Bonanno, A. Mesoscale variability in the trophic ecology of the European hake *Merluccius merluccius* in the Strait of Sicily. *Hydrobiologia* **2018**, *821*, 57–72. [CrossRef]

19. Mellon-Duval, C.; Harmelin-Vivien, M.; Métral, L.; Loizeau, V.; Mortreux, S.; Roos, D.; Fromentin, J.M. Ecología trófica de la merluza europea en el golfo de león, mediterráneo noroccidental. *Sci. Mar.* **2017**, *81*, 7–18. [CrossRef]

20. Carrozzi, V.; Di Lorenzo, M.; Massi, D.; Titone, A.; Ardizzone, G.; Colloca, F. Prey preferences and ontogenetic diet shift of European hake *Merluccius merluccius* (Linnaeus, 1758) in the central Mediterranean Sea. *Reg. Stud. Mar. Sci.* **2019**, *25*, 100440. [CrossRef]

21. Modica, L.; Cartes, J.E.; Velasco, F.; Bozzano, A. Juvenile hake predation on Myctophidae and Sternoptychidae: Quantifying an energy transfer between mesopelagic and neritic communities. *J. Sea Res.* **2015**, *95*, 217–225. [CrossRef]

22. Preciado, I.; Punzón, A.; Velasco, F. Spatio-temporal variability in the cannibalistic behaviour of European hake *Merluccius merluccius*: The influence of recruit abundance and prey availability. *J. Fish. Biol.* **2015**, *86*, 1319–1334. [CrossRef] [PubMed]

23. D'Iglio, C.; Porcino, N.; Savoca, S.; Profeta, A.; Perdichizzi, A.; Armeli, E.; Davide, M.; Francesco, S.; Rinelli, P.; Giordano, D. Ontogenetic shift and feeding habits of the European hake (*Merluccius merluccius* L., 1758) in Central and Southern Tyrrhenian Sea (Western Mediterranean Sea): A comparison between past and present data. *Ecol. Evol.* **2022**, *12*, e8634. [CrossRef] [PubMed]

24. Griffiths, J.R.; Kadin, M.; Nascimento, F.J.A.; Tamelander, T.; Törnroos, A.; Bonaglia, S.; Bonsdorff, E.; Brüchert, V.; Gårdmark, A.; Järnström, M.; et al. The importance of benthic–pelagic coupling for marine ecosystem functioning in a changing world. *Glob. Chang. Biol.* **2017**, *23*, 2179–2196. [CrossRef] [PubMed]

25. Agnetta, D.; Badalamenti, F.; Colloca, F.; D'Anna, G.; Di Lorenzo, M.; Fiorentino, F.; Garofalo, G.; Gristina, M.; Labanchi, L.; Patti, B.; et al. Benthic-pelagic coupling mediates interactions in Mediterranean mixed fisheries: An ecosystem modeling approach. *PLoS ONE* **2019**, *14*, e0210659. [CrossRef]

26. FAO. *The State of Mediterranean and Black Sea Fisheries 2020*; General Fisheries Commission for the Mediterranean: Rome, Italy, 2020; ISBN 9789251337240.

27. Albano, M.; Panarello, G.; Di Paola, D.; D'Angelo, G.; Granata, A.; Savoca, S.; Capillo, G. The mauve stinger *Pelagia noctiluca* (Cnidaria, Scyphozoa) plastics contamination, the Strait of Messina case. *Int. J. Environ. Stud.* **2021**, *78*, 977–982. [CrossRef]

28. Perdichizzi, A.; D'Iglio, C.; Giordano, D.; Profeta, A.; Ragonese, S.; Rinelli, P. Comparing life-history traits in two contiguous stocks of the deep-water rose shrimp *Parapenaeus longirostris* (H. Lucas, 1846) (Crustacea: Decapoda) in the Southern Tyrrhenian Sea (Central Mediterranean Sea). *Fish. Res.* **2022**, *248*, 106206. [CrossRef]

29. Edizioni Scientifiche Italiane (Ed.) IREPA Ecological aspect. In *Fisheries and Biodiversity*; MiPAAF: Rome, Italy, 2011.

30. Cataudella, S.; Spagnolo, M. *The State of Italian Marine Fisheries and Aquaculture*; MiPAAF: Rome, Italy, 2011.

31. Mangano, M.C.; Kaiser, M.J.; Porporato, E.M.D.; Lambert, G.I.; Spanò, N. Trawling disturbance effects on the trophic ecology of two co-generic Astropectinid species. *Mediterr. Mar. Sci.* **2015**, *16*, 538–549. [CrossRef]

32. Mangano, M.C.; Bottari, T.; Caridi, F.; Porporato, E.M.D.; Rinelli, P.; Spanò, N.; Johnson, M.; Sarà, G. The effectiveness of fish feeding behaviour in mirroring trawling-induced patterns. *Mar. Environ. Res.* **2017**, *131*, 195–204. [CrossRef] [PubMed]

33. Rinelli, P.; Ragonese, S.; Consoli, P.; Florio, G.; Romeo, T.; Perdichizzi, F.; Greco, S. Monitoring no-taken areas: The cases of the Gulfs of Castellammare and Patti (northern coasts of Sicily). *Biol. Mar. Mediterr.* **2004**, *11*, 82–87.

34. Potoschi, A.; Battaglia, P.; Rinelli, P.; Perdichizzi, F.; Manganaro, A.; Greco, S. Fluctuations in bottom trawl yelds in the area with partial protection in the Gulf of Patti (Northern Sicily) along 20 years of monitoring. *Biol. Mar. Mediterr.* **2006**, *13*, 149–157.

35. Sanfilippo, M.; Capillo, G.; Spanò, N.; Manganaro, A. Evaluation of water variables in no-take zone of ustica marine protected area (Southern Tyrrhenian Sea). *Braz. Arch. Biol. Technol.* **2016**, *59*, 1–10. [CrossRef]

36. Budillon, G.; Gasparini, G.P.; Schroeder, K. Persistence of an eddy signature in the central Tyrrhenian basin. *Deep Res. Part II Top. Stud. Oceanogr.* **2009**, *56*, 713–724. [CrossRef]

37. Budillon, G.; Cotroneo, Y.; Fusco, G.; Rivaro, P. Variability of the Mediterranean deep and bottom waters: Some recent evidences in the western basin. In *CIESM Workshop Monographs*; Briand, F., Ed.; CIESM Publisher: Monaco, 2009; Volume 38, pp. 57–63.

38. D'Iglio, C.; Albano, M.; Famulari, S.; Savoca, S.; Panarello, G.; Di Paola, D.; Perdichizzi, A.; Rinelli, P.; Lanteri, G.; Spanò, N.; et al. Intra- and interspecific variability among congeneric Pagellus otoliths. *Sci. Rep.* **2021**, *11*, 16315. [CrossRef]

39. Ribera d'Alcalà, M.; Civitarese, G.; Conversano, F.; Lavezza, R. Nutrient ratios and fluxes hint at overlooked processes in the Mediterranean Sea. *J. Geophys. Res. Oceans* **2003**, *108*, 1–16. [CrossRef]

40. Ribera d'Alcalà, M.; Brunet, C.; Conversano, F.; Corato, F.; Lavezza, R. Nutrient and pigment distributions in the southern Tyrrhenian Sea during mid-summer and late fall 2005. *Deep Res. Part II Top. Stud. Oceanogr.* **2009**, *56*, 676–686. [CrossRef]

41. La Mesa, G.; Guidetti, P.; Bussotti, S.; Cattaneo-Vietti, R.; Manganaro, A.; Molinari, A.; Russo, G.F.; Spanò, N.; Vetrano, G.; Tunesi, L. Rocky reef fish assemblages at six Mediterranean marine protected areas: Broad-scale patterns in assemblage structure, species richness and composition. *Ital. J. Zool.* **2013**, *80*, 90–103. [CrossRef]

42. Mangano, M.C.; Kaiser, M.J.; Porporato, E.M.D.; Spanò, N. Evidence of trawl disturbance on mega-epibenthic communities in the Southern Tyrrhenian Sea. *Mar. Ecol. Prog. Ser.* **2013**, *475*, 101–117. [CrossRef]

43. Vetrano, A.; Napolitano, E.; Iacono, R.; Schroeder, K.; Gasparini, G.P. Tyrrhenian Sea circulation and water mass fluxes in spring 2004: Observations and model results. *J. Geophys. Res. Oceans* **2010**, *115*, 1–18. [CrossRef]

44. Artale, V.; Astraldi, M.; Buffoni, G.; Gasparini, G.P. Seasonal variability of gyre-scale circulation in the northern Tyrrhenian Sea. *J. Geophys. Res.* **1994**, *99*, 14127–14137. [CrossRef]

45. Astraldi, M.; Gasparini, G.P. The Seasonal Characteristics of the Circulation in the Tyrrhenian Sea. In *Seasonal and Interannual Variability of the Western Mediterranean Sea*; La Violette, P.E., Ed.; American Geophysical Union: Washington, DC, USA, 1994; Volume 46.

46. Zodiatis, G.; Gasparini, G. Pietro Thermohaline staircase formations in the Tyrrhenian Sea. *Deep Res. Part I Oceanogr. Res. Pap.* **1996**, *43*, 655–678. [CrossRef]

47. Sparnocchia, S.; Gasparini, G.P.; Astraldi, M.; Borghini, M.; Pistek, P. Dynamics and mixing of the Eastern Mediterranean outflow in the Tyrrhenian basin. *J. Mar. Syst.* **1999**, *20*, 301–317. [CrossRef]

48. Bakun, A. Fronts and eddies as key structures in the habitat of marine fish larvae: Opportunity, adaptive response and competitive advantage. *Sci. Mar.* **2006**, *70*, 105–122. [CrossRef]

49. Fortibuoni, T.; Bahri, T.; Camilleri, M.; Garofalo, G.; Gristina, M.; Fiorentino, F. Nursery and spawning areas of deep-water rose shrimp, *Parapenaeus longirostris* (Decapoda: Penaeidae), in the Strait of Sicily (Central Mediterranean Sea). *J. CrustaC. Biol.* **2010**, *30*, 167–174. [CrossRef]

50. Garofalo, G.; Fortibuoni, T.; Gristina, M.; Sinopoli, M.; Fiorentino, F. Persistence and co-occurrence of demersal nurseries in the Strait of Sicily (central Mediterranean): Implications for fishery management. *J. Sea Res.* **2011**, *66*, 29–38. [CrossRef]

51. Cartes, J.E.; Rey, J.; Lloris, D.; Gil De Sola, L. Influence of environmental variables on the feeding and diet of European hake (*Merluccius merluccius*) on the Mediterranean Iberian coasts. *J. Mar. Biol. AssoC. U. K.* **2004**, *84*, 831–835. [CrossRef]

52. Hidalgo, M.; Massutí, E.; Moranta, J.; Cartes, J.; Lloret, J.; Oliver, P.; Morales-Nin, B. Seasonal and short spatial patterns in European hake (*Merluccius merluccius* L.) recruitment process at the Balearic Islands (western Mediterranean): The role of environment on distribution and condition. *J. Mar. Syst.* **2008**, *71*, 367–384. [CrossRef]

53. Follesa, M.C.; Carbonara, P. *Atlas of the Maturity Stages of Mediterranean Fishery Resources*; Studies and Reviews n. 99; FAO: Rome, Italy, 2019; ISBN 9789251319758.

54. Kacher, M.; Amara, R. Distribution and growth of 0-group European hake in the Bay of Biscay and Celtic Sea: A spatial and inter-annual analyses. *Fish. Res.* **2005**, *71*, 373–378. [CrossRef]

55. Mahe, K.; Amara, R.; Bryckaert, T.; Kacher, M.; Brylinski, J.M. Ontogenetic and spatial variation in the diet of hake (*Merluccius merluccius*) in the Bay of Biscay and the Celtic Sea. *ICES J. Mar. Sci.* **2007**, *64*, 1210–1219. [CrossRef]

56. Morote, E.; Olivar, M.P.; Bozzano, A.; Villate, F.; Uriarte, I. Feeding selectivity in larvae of the European hake (*Merluccius merluccius*) in relation to ontogeny and visual capabilities. *Mar. Biol.* **2011**, *158*, 1349–1361. [CrossRef]

57. D'iglio, C.; Natale, S.; Albano, M.; Savoca, S.; Famulari, S.; Gervasi, C.; Lanteri, G.; Panarello, G.; Spanò, N.; Capillo, G. Otolith Analyses Highlight Morpho-Functional Differences of Three Species of Mullet (Mugilidae) from Transitional Water. *Sustainability* **2022**, *14*, 398. [CrossRef]

58. Lombarte, A.; Tuset, V.M. Chapter3- Morfometría de otolitos. In *Métodos de Estudios Con Otolitos: Princípios y Aplicaciones/Métodos de Estudos Com Otólitos: Princípios e Aplicações*; Volpedo, A.V., Vaz-dos-Santos, A.M., Eds.; PIESCE-SPU: Buenos Aires, Argentina, 2015; p. 31.

59. Tuset, V.M.; Lombarte, A.; Assis, C.A. Otolith atlas for the western Mediterranean, north and central eastern AtlantiC. *Sci. Mar.* **2008**, *72*, 7–198. [CrossRef]

60. Clarke, M.R. Significance of cephalopod beaks. *Nature* **1962**, *193*, 560–561. [CrossRef]

61. Clarke, M.R. The identification of cephalopod 'beaks' and the relationship between break size and total body weight. *Deep Sea Res. Oceanogr. Abstr.* **1964**, *11*, 312. [CrossRef]

62. Fischer, W. Méditerranée et mer Noire. *Fiches FAO D'identification des Especes Pour les Besoins la Peche* **1987**, *1*, 334.

63. Jawad, L.A.; Sabatino, G.; Ibáñez, A.L.; Andaloro, F.; Battaglia, P. Morphology and ontogenetic changes in otoliths of the mesopelagic fishes *Ceratoscopelus maderensis* (Myctophidae), *Vinciguerria attenuata* and *V. poweriae* (Phosichthyidae) from the Strait of Messina (Mediterranean Sea). *Acta Zool.* **2018**, *99*, 126–142. [CrossRef]

64. D'Iglio, C.; Savoca, S.; Rinelli, P.; Spanò, N. Diet of the Deep-Sea Shark *Galeus melastomus* Rafinesque, 1810, in the Mediterranean Sea: What We Know and What We Should Know. *Sustainability* **2021**, *13*, 3962. [CrossRef]
65. Hyslop, E.J. Stomach contents analysis—A review of methods and their application. *J. Fish. Biol.* **1980**, *17*, 411–429. [CrossRef]
66. Cortés, E. A critical review of methods of studying fish feeding based on analysis of stomach contents: Application to elasmobranch fishes. *ProC. Can. J. Fish. Aquat. Sci.* **1997**, *17*, 411–429. [CrossRef]
67. Hacunda, J.S. Trophic relationships among demersal fishes in a coastal area of the Gulf of Maine. *Fish. Bull.* **1981**, *79*, 775–788.
68. Papaconstantinou, C.; Caragitsou, E. The food of hake (merluccius merluccius) in greek seas. *Vie Et Milieu* **1987**, *37*, 21–29.
69. Cartes, J.E.; Hidalgo, M.; Papiol, V.; Massutí, E.; Moranta, J. Changes in the diet and feeding of the hake *Merluccius merluccius* at the shelf-break of the Balearic Islands: Influence of the mesopelagic-boundary community. *Deep Res. Part I Oceanogr. Res. Pap.* **2009**, *56*, 344–365. [CrossRef]
70. Guichet, R. The diet of european hake (*Merluccius merluccius*) in the northern part of the bay of biscay. *ICES J. Mar. Sci.* **1995**, *52*, 21–31. [CrossRef]
71. Bozzano, A.; Sardà, F.; Ríos, J. Vertical distribution and feeding patterns of the juvenile European hake, *Merluccius merluccius* in the NW Mediterranean. *Fish. Res.* **2005**, *73*, 29–36. [CrossRef]
72. Riccioni, G.; Stagioni, M.; Piccinetti, C.; Libralato, S. A metabarcoding approach for the feeding habits of European hake in the Adriatic Sea. *Ecol. Evol.* **2018**, *8*, 10435–10447. [CrossRef]
73. Ferraton, F.; Harmelin-Vivien, M.; Mellon-Duval, C.; Souplet, A. Spatio-temporal variation in diet may affect condition and abundance of juvenile European hake in the Gulf of Lions (NW Mediterranean). *Mar. Ecol. Prog. Ser.* **2007**, *337*, 197–208. [CrossRef]
74. Payne, A.I.L.; Rose, B.; Leslie, R.W. Feeding of hake and a first attempt at determining their trophic role in the South African west coast marine environment. *S. Afr. J. Mar. Sci.* **1987**, *5*, 471–501. [CrossRef]
75. Stagioni, M.; Montanini, S.; Vallisneri, M. Feeding habits of European hake, *Merluccius merluccius* (Actinopterygii: Gadiformes: Merlucciidae), from the northeastern Mediterranean sea. *Acta Ichthyol. Piscat.* **2011**, *41*, 277–284. [CrossRef]
76. Battaglia, P.; Andaloro, F.; Consoli, P.; Esposito, V.; Malara, D.; Musolino, S.; Pedà, C.; Romeo, T. Feeding habits of the Atlantic bluefin tuna, *Thunnus thynnus* (L. 1758), in the central Mediterranean Sea (Strait of Messina). *Helgol. Mar. Res.* **2013**, *67*, 97–107. [CrossRef]
77. Persson, L.; Byström, P.; Wahlström, E. Cannibalism and competition in Eurasian perch: Population dynamics of an ontogenetic omnivore. *Ecology* **2000**, *81*, 1058–1071. [CrossRef]
78. Post, D.M.; Kitchell, J.F.; Hodgson, J.R. Interactions among adult demography, spawning date, growth rate, predation, overwinter mortality, and the recruitment of largemouth bass in a northern lake. *Can. J. Fish. Aquat. Sci.* **1998**, *55*, 2588–2600. [CrossRef]
79. Druon, J.N.; Fiorentino, F.; Murenu, M.; Knittweis, L.; Colloca, F.; Osio, C.; Mérigot, B.H.; Garofalo, G.; Mannini, A.; Jadaud, A.H.; et al. Modelling of European hake nurseries in the Mediterranean Sea: An ecological niche approach. *Prog. Oceanogr.* **2015**, *130*, 188–204. [CrossRef]
80. Bartolino, V.; Colloca, F.; Sartor, P.; Ardizzone, G. Modelling recruitment dynamics of hake, *Merluccius merluccius*, in the central Mediterranean in relation to key environmental variables. *Fish. Res.* **2008**, *92*, 277–288. [CrossRef]
81. Profeta, A.; Giordano, D.; Perdichizzi, F.; Perdichizzi, A.; Busalacchi, B.; Potoschi, A.; Rinelli, P. Spatio-Temporal Distribution of Recruits of Hake (*Merluccius merluccius* L.) in the Calabrian Tyrrhenian Sea (Central Mediterranean). *Rapp. Comm. Int. Mer Medit* **2007**, *2004*, 98122.
82. Riccioni, G.; Stagioni, M.; Manfredi, C.; Tinti, F.; Piccinetti, C.; Libralato, S. DNA metabarcoding suggests dietary niche partitioning in the Adriatic European hake. *Sci. Rep.* **2022**, *12*, 1–13. [CrossRef]
83. Sims, D.W.; Genner, M.J.; Southward, A.J.; Hawkins, S.J. Timing of squid migration reflects North Atlantic climate variability. *ProC. R. SoC. B Biol. Sci.* **2001**, *268*, 2607–2611. [CrossRef]
84. Hastie, L.C.; Pierce, G.J.; Wang, J.; Bruno, I.; Moreno, A.; Piatkowski, U.; Robin, J.P. Cephalopods in the North-Eastern Atlantic: Species, Biogeography, Ecology, Exploitation and Conservation. *Oceanogr. Mar. Biol. An. Annu. Rev.* **2016**, *47*, 111–190. [CrossRef]
85. Oesterwind, D.; ter Hofstede, R.; Harley, B.; Brendelberger, H.; Piatkowski, U. Biology and meso-scale distribution patterns of North Sea cephalopods. *Fish. Res.* **2010**, *106*, 141–150. [CrossRef]
86. Oesterwind, D.; Barrett, C.J.; Sell, A.F.; Núñez-Riboni, I.; Kloppmann, M.; Piatkowski, U.; Wieland, K.; Laptikhovsky, V. Climate change-related changes in cephalopod biodiversity on the North East Atlantic Shelf. *Biodivers. Conserv.* **2022**, *31*, 1491–1518. [CrossRef]
87. Arneri, E.; Morales-Nin, B. Aspects of the early life history of European hake from the central AdriatiC. *J. Fish. Biol.* **2000**, *56*, 1368–1380. [CrossRef]
88. Orsi Relini, L.; Zamboni, A.; Fiorentino, F.; Relini, G. Vulnerabilità luce-dipendente del nasello (*Merluccius merluccius*) giovanile. *Biol. Mar. Medit.* **1997**, *4*, 262–268.
89. Lombarte, A.; Popper, A.N. Quantitative analyses of postembryonic hair cell addition in the otolithic endorgans of the inner ear of the european hake, *Merluccius merluccius* (gadiformes, teleostei). *J. Comp. Neurol.* **1994**, *345*, 419–428. [CrossRef]
90. Mas-Riera, J. Changes during growth in the retinal structure of three hake species, *Merluccius* spp. (Teleostei: Gadiformes), in relation to their depth distribution and feeding. *J. Exp. Mar. Biol. Ecol.* **1991**, *152*, 91–104. [CrossRef]

91. Bozzano, A.; Murgia, R.; Vallerga, S.; Hirano, J.; Archer, S. The photoreceptor system in the retinae of two dogfishes, *Scyliorhinus canicula* and *Galeus melastomus*: Possible relationship with depth distribution and predatory lifestyle. *J. Fish. Biol.* **2001**, *59*, 1258–1278. [CrossRef]

92. D'Iglio, C.; Albano, M.; Tiralongo, F.; Famulari, S.; Rinelli, P.; Savoca, S.; Spanò, N.; Capillo, G. Biological and Ecological Aspects of the Blackmouth Catshark (*Galeus melastomus* Rafinesque, 1810) in the Southern Tyrrhenian Sea. *J. Mar. Sci. Eng.* **2021**, *9*, 967. [CrossRef]

93. Modica, L.; Bozzano, A.; Velasco, F. The influence of body size on the foraging behaviour of European hake after settlement to the bottom. *J. Exp. Mar. Biol. Ecol.* **2013**, *444*, 46–54. [CrossRef]

94. Orsi-Relini, L.; Relini, G. The glass shrimp Pasiphaea sivado in the food chains of the Ligurian Sea. In *Trophic Relationships in the Marine Environment, Proceedings 24th European Marine Biology Symposium, Oban, Scotland, 4–10 October 1989*; Aberdeen University Press: Aberdeen, Scotland, 1990; pp. 334–346.

95. Golani, D.; Galil, B. Trophic relationships of colonizing and indigenous goatfishes (Mullidae) in the eastern Mediterranean with special emphasis on decapod crustaceans. *Hydrobiologia* **1991**, *218*, 27–33. [CrossRef]

96. Cartes, J.E.; Sardá, F.; Company, J.B.; Lleonart, J. Day-night migrations by deep-sea decapod crustaceans in experimental samplings in the Western Mediterranean sea. *J. Exp. Mar. Biol. Ecol.* **1993**, *171*, 63–73. [CrossRef]

97. Company, J.B.; Cartes, J.E.; Sardà, F. Biological patterns and near-bottom population characteristics of two pasiphaeid decapod crustacean species, *Pasiphaea sivado* and *P. multidentata*, in the north-western Mediterranean Sea. *Mar. Biol.* **2001**, *139*, 61–73. [CrossRef]

98. Cartes, J.E. Feeding habits of pasiphaeid shrimps close to the bottom on the Western Mediterranean slope. *Mar. Biol.* **1993**, *117*, 459–468. [CrossRef]

99. Marcus, N.H.; Boero, F. Minireview: The importance of benthic-pelagic coupling and the forgotten role of life cycles in coastal aquatic systems. *Limnol. Oceanogr.* **1998**, *43*, 763–768. [CrossRef]

100. Baustian, M.M.; Hansen, G.J.A.; de Kluijver, A.; Robinson, K.; Henry, E.N.; Knoll, L.B.; Rose, K.C.; Carey, C.C. Linking the bottom to the top in aquatic ecosystems: Mechanisms and stressors of benthic-pelagic coupling. In *Proceedings of the Eco-DAS X Symposium Proceedings*; The Association for the Sciences of Limnology and Oceanography: Waco, TX, USA, 2014; Volume 3, pp. 25–47.

101. Savoca, S.; Grifó, G.; Panarello, G.; Albano, M.; Giacobbe, S.; Capillo, G.; Spanó, N.; Consolo, G. Modelling prey-predator interactions in Messina beachrock pools. *Ecol. Modell.* **2020**, *434*, 109206. [CrossRef]

102. Puig, P.; Canals, M.; Company, J.B.; Martín, J.; Amblas, D.; Lastras, G.; Palanques, A.; Calafat, A.M. Ploughing the deep sea floor. *Nature* **2012**, *489*, 286–289. [CrossRef]

103. Tecchio, S.; Coll, M.; Christensen, V.; Company, J.B.; Ramírez-Llodra, E.; Sardà, F. Food web structure and vulnerability of a deep-sea ecosystem in the NW Mediterranean Sea. *Deep Res. Part I Oceanogr. Res. Pap.* **2013**, *75*, 1–15. [CrossRef]

104. Shannon, L.; Coll, M.; Bundy, A.; Gascuel, D.; Heymans, J.J.; Kleisner, K.; Lynam, C.P.; Piroddi, C.; Tam, J.; Travers-Trolet, M.; et al. Trophic level-based indicators to track fishing impacts across marine ecosystems. *Mar. Ecol. Prog. Ser.* **2014**, *512*, 115–140. [CrossRef]

105. Sciberras, M.; Hiddink, J.G.; Jennings, S.; Szostek, C.L.; Hughes, K.M.; Kneafsey, B.; Clarke, L.J.; Ellis, N.; Rijnsdorp, A.D.; McConnaughey, R.A.; et al. Response of benthic fauna to experimental bottom fishing: A global meta-analysis. *Fish Fish.* **2018**, *19*, 698–715. [CrossRef]

106. Howarth, L.M.; Waggitt, J.J.; Bolam, S.G.; Eggleton, J.; Somerfield, P.J.; Hiddink, J.G. Effects of bottom trawling and primary production on the composition of biological traits in benthic assemblages. *Mar. Ecol. Prog. Ser.* **2018**, *602*, 31–48. [CrossRef]

107. Eddy, T.D.; Lotze, H.K.; Fulton, E.A.; Coll, M.; Ainsworth, C.H.; de Araújo, J.N.; Bulman, C.M.; Bundy, A.; Christensen, V.; Field, J.C.; et al. Ecosystem effects of invertebrate fisheries. *Fish Fish.* **2017**, *18*, 40–53. [CrossRef]

108. Pusceddu, A.; Bianchelli, S.; Martín, J.; Puig, P.; Palanques, A.; Masqué, P.; Danovaro, R. Chronic and intensive bottom trawling impairs deep-sea biodiversity and ecosystem functioning. *ProC. Natl. Acad. Sci. USA* **2014**, *111*, 8861–8866. [CrossRef] [PubMed]

109. Prugh, L.R.; Stoner, C.J.; Epps, C.W.; Bean, W.T.; Ripple, W.J.; Laliberte, A.S.; Brashares, J.S. The rise of the mesopredator. *Bioscience* **2009**, *59*, 779–791. [CrossRef]

110. Tecchio, S.; Coll, M.; Sardà, F. Structure, functioning, and cumulative stressors of Mediterranean deep-sea ecosystems. *Prog. Oceanogr.* **2015**, *135*, 156–167. [CrossRef]