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Mesopelagic Fish Biodiversity in the Ligurian Cetacean Sanctuary

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Abstract: Mesopelagic fish belonging to the families Gonostomatidae, Phosichthyidae, Sternoptychidae, Stomiidae, Paralepididae, Myctophidae, Evermannellidae, Microstomatidae, and Nemichthyidae were sampled using an Isaacs Kidd Midwater Trawl (IKMT) in the Ligurian Sea, Northwestern Mediterranean, the core of the Cetacean Sanctuary established in 2001. In September–October 2002 and September 2003, 31 species of mesopelagic fish were captured in oblique hauls from 800 m depth to the surface. The mesopelagic fish fraction represented about 22% of the collected biomass, dominated by two main species of the genus *Cyclothone*, representing 93.8% of total abundance, followed by *Argyropelecus hemigymnus* and *Lampanyctus crocodilus*. The presence of *Valenciennellus tripunctulatus* was a new record for the study area. The family Myctophidae was the most represented in terms of number of species (n = 13). The abundance and biodiversity of mesopelagic fishes were influenced by the sampling period and oceanographic conditions, specifically temperature. While no diel vertical migrations could be observed, day and night samples revealed different compositions of juveniles and adults of *Benthosema glaciale* and *Ceratoscopelus maderensis*. The broad size range of specimens sampled confirmed that adult individuals of *Chauliodus sloani* and *Lampanyctus crocodilus* can inhabit and forage within the water column beyond the slope rather than exclusively near the bottom.

Keywords: mesopelagic zone; fish; biodiversity; Ligurian Sea; NW Mediterranean

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1. Introduction

For descriptive purposes, the mesopelagic ichthyofauna of the Mediterranean Sea can be divided into two groups. The first one is represented by large fish, such as Centrolophidae, Gempylidae, Lampridae, Luvaridae, Trichiuridae, etc., which are caught occasionally or regularly during professional and/or recreational fishing activities [1]. They frequently constitute an appreciated fishery resource, forming a continuum with other fish that live both at the surface and in mesopelagic waters, e.g., swordfish, which are probably the best known mesopelagic hunters [2,3]; tunas, which have similar feeding habits [4,5]; and pelagic sharks, such as *Prionace glauca* (Linnaeus, 1758) [6–8].

The second group is represented by small species (Gonostomatidae, Sternoptychidae, Myctophidae, etc.) of no commercial value, which can be studied only by dedicated sampling.

Studying the qualitative and quantitative aspects of pelagic ichthyofauna is a necessary step towards understanding the trophic web of the Cetacean Sanctuary. Small mesopelagic fish, ranging from the size of *Cyclothone* to larger species, such as *Chauliodus sloani* Bloch & Schneider, 1801 or *Sudis hyalina* Rafinesque, 1810 occupy an important trophic level positioned between mesozooplankton and intermediate or top predators, which include cephalopods, the above-mentioned large fish, and marine mammals. In the study area, the Ligurian Sea, which forms a major part of the established Cetacean Sanctuary, small mesopelagic fish are common prey also for cetaceans such as *Stenella coeruleoalba* (Meyen, 1833), the most abundant species of the Sanctuary, and for *Delphinus delphis* Linnaeus, 1758 [9–12]. It is still a matter of debate whether larger cetacean species such as *Physeter macrocephalus* Linnaeus, 1758; *Ziphius cavirostris* Cuvier, 1823; and *Balaenoptera physalus* Lacépède, 1804 feed, at least occasionally [13], on this kind of prey.

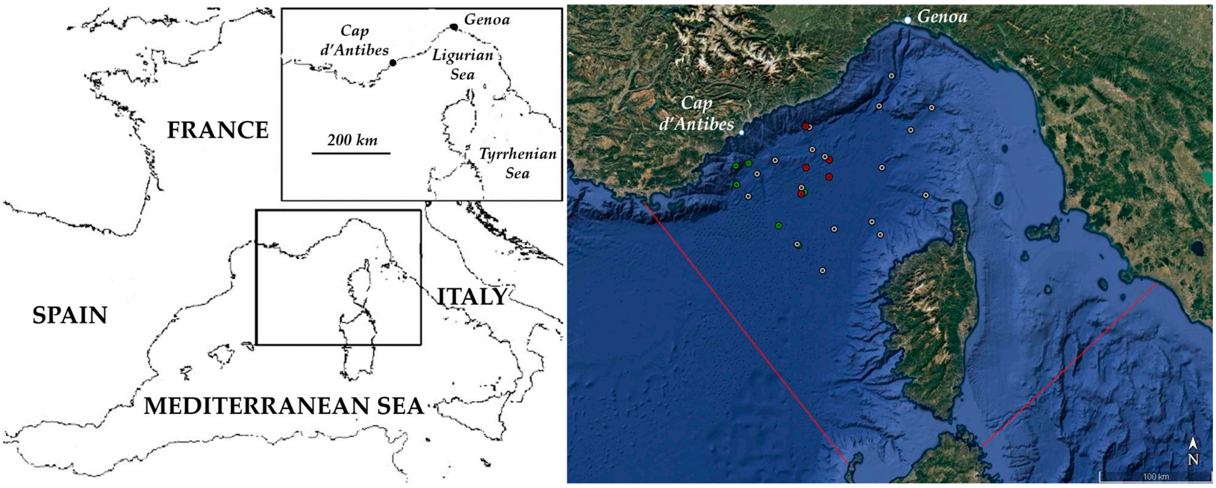
In recent years, other reasons for studying mesopelagic fish are becoming evident, as the Mediterranean Sea ichthyofauna has rapidly been enriched by exotic species coming both from the Atlantic Ocean and the Indo-Pacific Ocean [14]. The Atlantic group of immigrants includes deep sea species such as *Cubiceps capensis* (Smith, 1845) [15,16]; *Hyperoglyphe perciformis* (Mitchill, 1818) [16]; *Halosaurus ovenii* Johnson, 1864 [14]; *Chaunax suttkusi* Caruso, 1989 [14]; *Gephyroberyx darwinii* (Johnson, 1866) [14]; *Beryx splendens* Lowe, 1834 [17]; and *Zenopsis conchifer* (Lowe, 1852) [18]. In the Atlantic, several species that inhabit the slope are known to be moving northward in response to ocean warming [19,20]. In addition, the Indo-Pacific group includes at least one species living in deep waters that has never been recorded in the Red Sea, *Synagrops japonicus* (Doderlein, 1883), whose presence in the Mediterranean Sea could be attributed to transport in ballast water [21]. More recent arrivals of exotic species in the Northwestern Mediterranean area [22–25] are becoming increasingly important due to both global warming and anthropic activities, such as maritime traffic and aquaculture, and it could potentially also affect the small mesopelagic ichthyofauna, but so far, they all belong to shallow water species.

Many studies were performed in the Ligurian Sea by Italian and French teams to investigate the diel migration and vertical distribution of macroplankton and micronekton, which concerned different taxa (euphausiids, copepods, jellyfish, siphonophores, etc.) [26–38]. However, while in other areas of the Western Mediterranean, a number of scientific campaigns were held, in the Ligurian Sea, less attention has been given to the mesopelagic fish community: the Danish Oceanographical expedition with the R/V Thor (1908–1910) [39–41], the Smithsonian Expedition [42], and more recently the R/V Sarmiento de Gamboa expedition in 2009–2010 [43,44] and 2020 [45].

Within the Central and Western Ligurian Sea, our team investigated the community of small mesopelagic fish living in the depth range 0–800 m during several surveys of the R/V Minerva and Urania (1988–1993), which allowed the publication of the first description of the species composition and of their relative abundance in the area [5,46]. Taking into account the above-listed reasons of interest, this paper describes the composition of small mesopelagic ichthyofauna that was obtained in samples collected from the surveys of the R/V Urania and tug Messico (2002 and 2003), comparing it with other studies carried out in the Mediterranean Sea.

2. Materials and Methods

In September–October 2002 and September 2003, mesopelagic fish and macroplankton were sampled in the Central and Western Ligurian Sea (Figure 1), by means of a non-closing Isaacs Kidd Midwater Trawl (IKMT; mouth opening 15 feet, 2 mm × 2 mm mesh size in the cod end), with the support of two vessels: the tug Messico and the R/V Urania (SOLMAR project, SIRENA cruises 2002–2003, SACLANTCEN—La Spezia).



(**a**) (**b**)

Figure 1. (**a**) Western Mediterranean Sea, with emphasis on the study area and (**b**) sampling stations (red circles = Messico 02; green circles = Urania 02; white circles = Urania 03) for mesopelagic ichthyofauna with the Cetacean Sanctuary limits (red lines).

Sampling was carried out at 29 stations, divided into three surveys: five in September 2002 (Messico), six in October 2002, and 18 in September 2003 (Urania) (Table 1). The stations were located along transects that were established by the CNR, ISMAR (La Spezia, Santa Teresa), for the study of hydrological characteristics of the area. The sampling grid included two stations located near oceanographic buoys, ODAS (CNR, Italy) and DYFAMED (France), where measures of environmental and biological parameters have been recorded for many years [47–51].

Table 1. List of sampling stations, with the main characteristics by haul.

Survey	Station	Date	Starting Time	Lat. N	Long. E	Minutes Towed	D Flowmeter	Day Night
Messico 2002	ikmt 2	27 September 2002	16:22	43°37′529	7°47′794	127	43,200	D
	ikmt 5 bis	28 September 2002	06:28	43°22′170	8°02′420	130	38,050	D
	ikmt 3	28 September 2002	11:06	43°18′633	7°47′811	131	41,371	D
	ikmt 4	28 September 2002	15:38	43°06′610	7°44′583	125	44,774	D
	ikmt 5	28 September 2002	19:33	43°14′320	8°02′197	117	38,342	N
Urania 2002	929	14 October 2002	10:39	42°42′9650	7°42′7299	131	42,706	D
	931	14 October 2002	18:49	42°52′1360	7°30′4803	133	39,819	N
	934	15 October 2002	09:35	43°10′84	7°04′0300	128	42,217	D
	938	15 October 2002	18:55	43°20′6241	7°11′6296	127	42,319	N
	922	16 October 2002	09:16	43°19′4295	7°03′6332	126	38,881	D
	920	16 October 2002	18:22	43°07′3108	7°46′5213	129	46,035	N
Urania 2003	810	2 September 2003	13:35	43°05′2900	9°03′0600	127	37,561	D
	807	2 September 2003	22:19	43°18′2827	8°35′6630	125	41,192	N
	604	4 September 2003	12:27	44°00′4470	8°42′8538	126	44,547	D
	608	4 September 2003	22:43	43°46′5588	8°34′5315	126	50,064	N
	902	5 September 2003	09:16	43°37′1420	7°50′0810	126	49,604	D
	905	5 September 2003	21:02	43°23′5500	7°59′7170	126	50,863	N
	908	6 September 2003	10:45	42°53′5441	8°28′7835	132	46,518	D
	910	6 September 2003	17:25	42°47′5756	8°33′8997	128	42,262	D
	927	7 September 2003	09:27	42°31′2552	7°57′6526	129	48,032	D
	929 bis	7 September 2003	16:52	42°43′4795	7°41′9068	127	47,040	D
	933	8 September 2003	10:52	43°05′5167	7°11′3967	126	47,040	D
	939	8 September 2003	18:46	43°15′7952	7°16′9228	129	48,400	N
	923	9 September 2003	12:18	43°21′9847	7°28′3443	128	45,467	D
	odas	11 September 2003	20:46	43°45′4478	9°08′1347	128	44,715	N
	dyfamed	12 September 2003	18:30	43°26′8696	7°51′8261	128	46,265	N
	921	13 September 2003	09:54	43°09′4327	7°44′8016	127	45,326	D
	918	13 September 2003	20:00	42°50′4154	8°05′1254	130	47,990	N
	410	16 September 2003	18:02	43°35′3169	8°54′4019	130	47,252	N

The morphology of the Ligurian Sea and its hydrological characteristics are complex; in fact, the slope of the basin presents a segment facing north, from Corsica to Genoa, and another larger segment facing southwest, from Genoa to Cap d'Antibes. The main surface current of Atlantic origin, the Western Corsica Current, flows northward to the Gulf of Genoa and mixes with the Tyrrhenian current, thereby creating the Ligurian Current (or Northern Current), which flows to the southwest. In this way, the central part of the study area is encircled by a large permanent front. At the surface, this cyclonic gyre can probably form a complete ring, which tends to maintain planktonic organisms inside the area. In the vertical plane, the system concerns a water column deeper than 2500 m, where three main water masses can be identified: surface Atlantic Water (AW), Levantine Intermediate Water (LIW), and Deep Water (DW) [50,51]. The essential point is that LIW, where sampled mesopelagic fish mainly live, and in part also DW are influenced by the movements of surface water, with the possibility that temporary structures such as mesoscale vortexes may occur [50,51].

A total of 29 oblique hauls, from 800 m depth to the surface, were carried out at a towing speed of 3 knots, lasting 2 h each. A depth recorder and a flowmeter were positioned inside the net. The filtered volume was calculated by multiplying the flowmeter data by the mouth opening of the net, which was about 17.5 m^2. Table 1 shows the details of each haul.

The whole catch was sorted in the following fractions: fishes (i.e., bony fishes), cephalopods, decapod crustaceans, northern krill, *Meganyctiphanes norvegica* (Sars, 1857), other crustaceans, pteropods, and gelatinous macroplankton. These were measured on board the vessel in terms of displaced volume (milliliters—mL) as a proxy of biomass. Fishes were fixed in a solution of 10% formalin/sea water or frozen immediately at −20 °C. Later on, in the laboratory, they were identified and sorted by species. Individuals of fish belonging to different species were counted (total number) in each station and were standardized into catch-per-unit effort (CPUE), considering 10,000 m^3 of filtered water as unit of effort (ind./10,000 m^3). The main reference textbook for the Mediterranean ichthyofauna is *FNAM* (*Fishes of North-eastern Atlantic and the Mediterranean*) [52], with 12 mesopelagic fish families described for Italian waters [53–56]. Following the Worms Editorial Board [57], we included Astronesthinae, Melanostomiinae, and Chauliodontinae in the family Stomiidae and separated Microstomatidae from the family Argentinidae; we also followed the revision of Paralepididae by Post [58], with changes in nomenclature.

The temperature data of the superficial layer (0–10 m) detected by the DYFAMED 1 buoy (Ligurian Sea, Western Mediterranean) were downloaded and are available on the website donneespubliques.meteofrance.fr (accessed on 1 September 2024).

The fish biodiversity at each station was reported by three indices:

$$\text{Margalef index (species richness): } d = (S - 1)/\log(n)$$

where S is the species number, and n is the number of individuals.

$$\text{Pielou index (species evenness): } J = H'/\log(S)$$

where H′ is the Shannon–Weaver index, and S is the systematic units of the sample.

$$\text{Shannon–Wiener index: } H' = \Sigma\,(n_i/n)\,(\ln(n_i/n))$$

where n_i is the number of individuals of a taxon, and n is the total number of individuals.

One-way ANOVA was applied to test differences in diversity among the three surveys; a pairwise Tukey post hoc test was performed. Shapiro–Wilk and Levene tests were performed to verify the normal distribution and homogeneity of variance of tested data.

Multivariate analysis was performed to compare mesopelagic fish assemblages among different stations in terms of abundance (ind./10,000 m^3). In order to reduce bias caused by the most abundant species, values were square root-transformed before applying multivariate methods.

Cluster and Multidimensional Scaling (MDS) analyses were applied by calculating Bray–Curtis similarity matrices, and a one-way analysis of similarity (ANOSIM) was performed in order to test the significant groups. Similarity Percentage analysis (SIMPER) was useful to verify the similarity between groups and identify species responsible for the differences. A two-way PERMANOVA analysis was performed to tests for differences in the composition of the mesopelagic community, considering light conditions (day vs. night samples) and sampling period (September vs. October) as fixed factors. All statistical analysis were performed using the Primer-6 [59,60] and Past 4.01 [61] software.

The most relevant species were measured as total length (TL, mm); length–frequency distributions (LFDs), expressed as percentages of the catch rate (ind./10,000 m^3), were used to investigate modal size classes (cohorts) and differences in behavior among species during the diel vertical migration. To test the significance of the differences between LFDs, a non-parametric Kolmogorov–Smirnov (K-S) two-sample test was applied.

3. Results

3.1. Total Catches and Species List

A total of 29 hauls were made; despite the standardized procedure, the volume of filtered water ranged between 225,366 m^3 and 305,178 m^3 per haul, with an average of 266,452 m^3 (SD = 22,411). Such variability could be explained by differences in the relative directions of the tow and currents that characterize the study area [51,62]. Average volumes of collected organisms were about 925.6 mL per haul, with a catch rate of 35.1 mL/10,000 m^3 of filtered water. The average biomass composition (mL/10,000 m^3) of the different fractions is shown in Figure 2; mesopelagic fishes followed the gelatinous macroplankton, a heterogeneous category that includes salps, ctenophores, and scyphozoans. By volume, the fraction of fish in each haul showed a lower variability (mean = 7.6 mL/10,000 m^3; SD = 2.5) than other fractions.

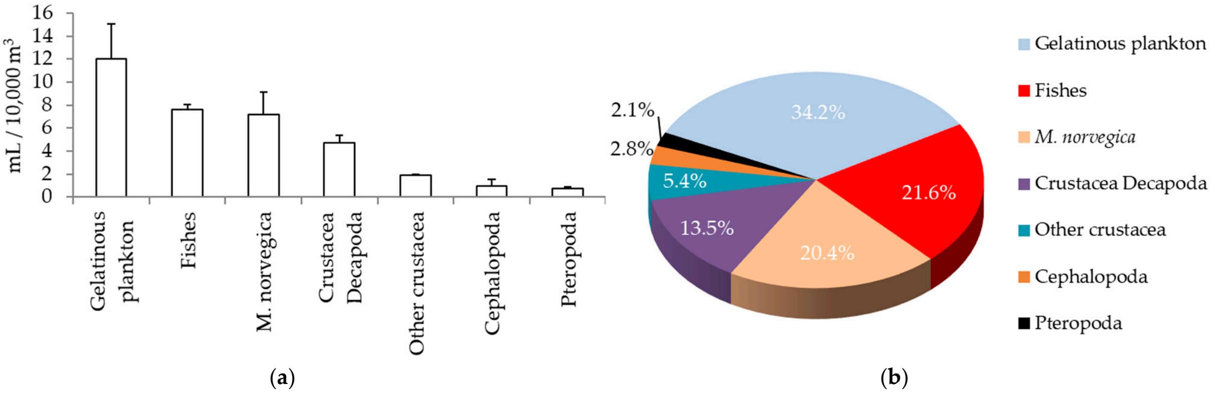


Figure 2. Composition of the total catch in terms of biomass (mL/10,000 m^3): (**a**) average catch rate values for different taxa (bars = SE) and (**b**) average percentage composition.

More than 40,000 mesopelagic fishes, belonging to at least 31 species (9 families), were captured during the three surveys (Table 2). The CPUE of mesopelagic fish was 61.4 ind./10,000 m^3; only two families, Gonostomatidae and Myctophidae, represented 96.9% (59.4 ind./10,000 m^3) of the total catch, followed by *Argyropelecus hemigymnus* Cocco, 1829, with 2.4% (1.45 ind./10,000 m^3). By abundance, the two Cyclothone species alone represented 93.8% of the total (57.6 ind./10,000 m^3).

Table 2. List of mesopelagic fish species caught during the surveys, where n = total number, FO = frequency of occurrence (number of stations in which each species was found) and relative percentage (O%), CPUE = number of individuals of each species caught per 10,000 m^3, and VOL = displaced mL for each species caught per 10,000 m^3.

Species	n	FO	O%	CPUE	VOL
Cyclothone braueri Jespersen & Tåning, 1926 *	31,498	29	100.0	40.76	1.01
Cyclothone pygmaea Jespersen & Tåning, 1926 *	12,995	29	100.0	16.82	0.33
GONOSTOMATIDAE	44,493	-	-	57.58	1.33
Lampanyctus crocodilus (Risso, 1810)	470	29	100.0	0.61	0.89
Lampanyctus pusillus (Johnson, 1890)	53	19	65.5	0.07	0.01
Ceratoscopelus maderensis (Lowe, 1839)	380	28	96.6	0.49	0.04
Hygophum benoiti (Cocco, 1838)	254	24	82.8	0.33	0.01
Hygophum hygomii (Lütken, 1892)	1	1	3.4	0.00	0.00
Benthosema glaciale (Reinhardt, 1837)	196	29	100.0	0.25	0.06
Symbolophorus veranyi (Moreau, 1888)	28	13	44.8	0.04	0.01
Myctophum punctatum Rafinesque, 1810	18	14	48.3	0.02	0.01
Notoscopelus elongatus (Costa, 1844)	17	11	37.9	0.02	0.08
Diaphus rafinesquii (Cocco, 1838)	6	6	20.7	0.01	0.01
Diaphus holti Tåning, 1918	4	4	13.8	0.01	0.00
Lobianchia dofleini (Zugmayer, 1911)	2	2	6.9	0.00	0.00
Electrona risso (Cocco, 1829)	1	1	3.4	0.00	0.00
MYCTOPHIDAE	1430	-	-	1.85	1.11
Argyropelecus hemigymnus Cocco, 1829	1124	29	100.0	1.45	0.24
Maurolicus muelleri (Gmelin, 1789)	3	1	3.4	0.00	0.00
Valenciennellus tripunctulatus (Esmark, 1871)	2	2	6.9	0.00	0.00
STERNOPTYCHIDAE	1129	-	-	1.46	0.24
Vinciguerria attenuata (Cocco, 1838)	104	25	86.2	0.13	0.01
Vinciguerria poweriae (Cocco, 1838)	2	2	6.9	0.00	0.00
Ichthyococcus ovatus (Cocco, 1838)	3	3	10.3	0.00	0.00
PHOSICHTHYDAE	109	-	-	0.14	0.01
Chauliodus sloani Bloch & Schneider, 1801	98	27	93.1	0.13	1.00
Stomias boa (Risso, 1810)	7	6	20.7	0.01	0.01
Borostomias antarcticus (Lönnberg, 1905)	1	1	3.4	0.00	0.01
Bathophilus nigerrimus Giglioli, 1882	1	1	3.4	0.00	0.00
STOMIIDAE	107	-	-	0.14	1.01
Arctozenus risso (Bonaparte, 1840)	38	24	82.8	0.05	0.02
Paralepis coregonoides Risso, 1820	24	14	48.3	0.03	0.00
Lestidiops sphyrenoides (Risso, 1820)	6	6	20.7	0.01	0.00
Paralepididae not identified	37	13	44.8	0.05	0.00
PARALEPIDIDAE	105	-	-	0.14	0.02
Evermannella balbo (Risso, 1820)	9	7	24.1	0.01	0.02
EVERMANNELLIDAE	9	-	-	0.01	0.02
Microstoma microstoma (Risso, 1810)	3	2	6.9	0.00	0.00
MICROSTOMATIDAE	3	-	-	0.00	0.00
Nemichthys scolopaceus Richardson, 1848	2	2	6.9	0.00	0.07
NEMICHTHYDAE	2	-	-	0.00	0.07
Leptocephali	7	4	13.8	0.01	0.00
Not identified fishes	18	10	34.5	0.02	0.00
Total	**47,412**	**-**		**61.36**	**3.81**

* Counted by subsamples.

The group of the rarest species, represented by one to three specimens, included *Valenciennellus tripunctulatus* (Esmark, 1871); this species was recorded for the first time in the study area and, more generally, in the Western Mediterranean. A number of juvenile Paralepididae (n = 37), leptocephali, and larvae remained unidentified, together with some damaged specimens. These unidentified categories amount to 0.13% of the total number (Table 2).

By volume/biomass (mL/10,000 m^3), the top four families were Gonostomatidae, Myctophidae, Stomiidae, and Sternoptychidae (Figure 3), and the top five species were *Cyclothone braueri* Jespersen & Tåning, 1926; *C. sloani*; *Lampanyctus crocodilus* (Risso, 1810); *Cyclothone pygmaea* Jespersen & Tåning, 1926; and *A. hemigymnus*, which represented 90% of the species (Figure 3).

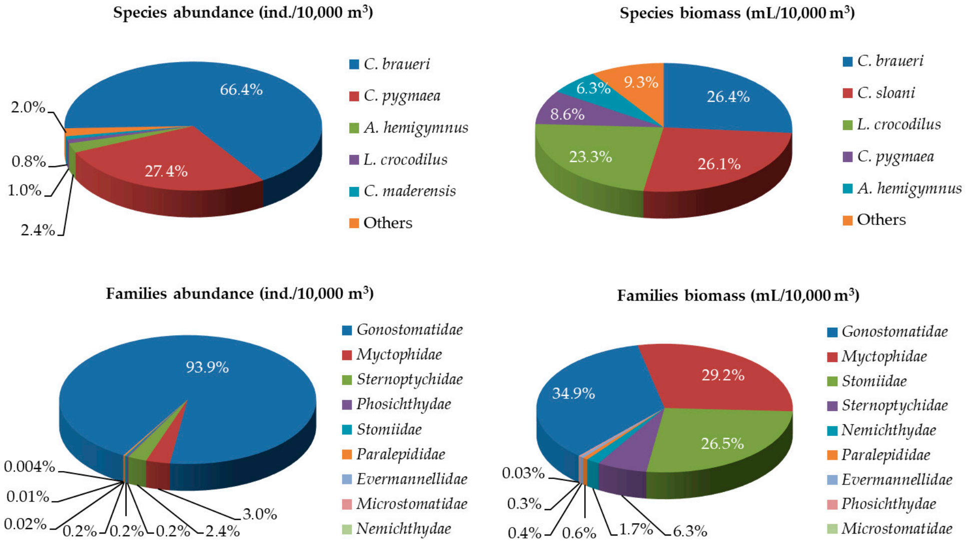


Figure 3. Percentages of the most represented species and families in terms of abundance (ind./10,000 m^3) and biomass (mL/10,000 m^3).

3.2. Biodiversity Indexes

The number of species increased in relation to the number of hauls at different growth rates during the three surveys, reaching a total of 19 species in Messico 2002 (5 hauls carried out in the central–western sector), 24 species in Urania 2002 (6 hauls carried out in the western sector) and 27 species in Urania 2003 (18 hauls covering the whole area) (Figure 4).

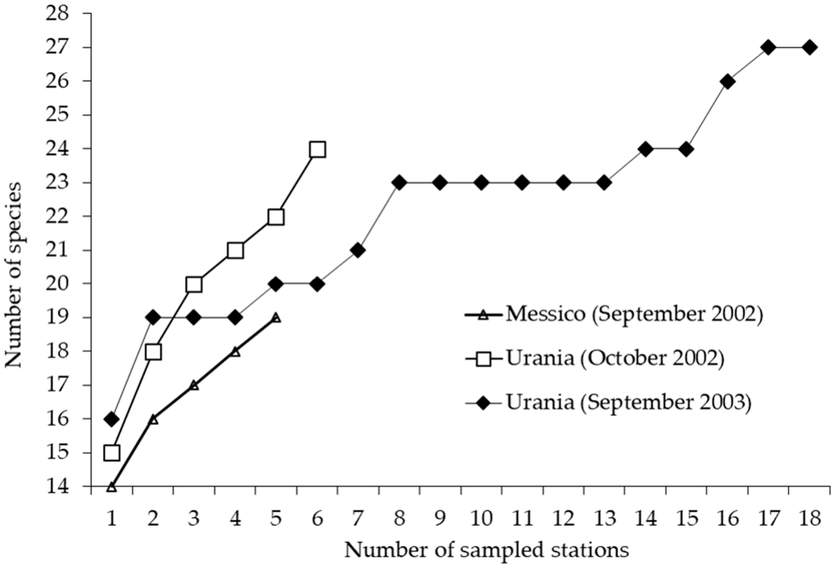


Figure 4. Cumulative number of species in relation to the progress of the sampling in the three surveys.

The number of species per station ranged from 10 to 17 (mean = 13.5; SD = 2.0), but only five were found at all sampling stations: *C. braueri*, *C. pygmaea*, *L. crocodilus*, *Benthosema glaciale* (Reinhardt, 1837), and *A. hemigymnus*.

One-way ANOVA was applied to test the difference between the diversity indices in the three surveys. A significant difference was found among the three indices (Shannon, Pielou, and Margalef; p-value < 0.05). The pairwise post hoc Tukey test (Figure 5) highlighted a difference between Urania 2003 and Urania 2002 in Shannon and Pielou indices but not with the Messico 2002 survey, suggesting that in Urania 2002, a single species dominated the catches more significantly than in Urania 2003. Margalef was significantly higher in Urania 2003 compared to the other surveys ($p < 0.05$), confirming the number of species was significantly higher in Urania 2003 after accounting for the higher number of hauls.

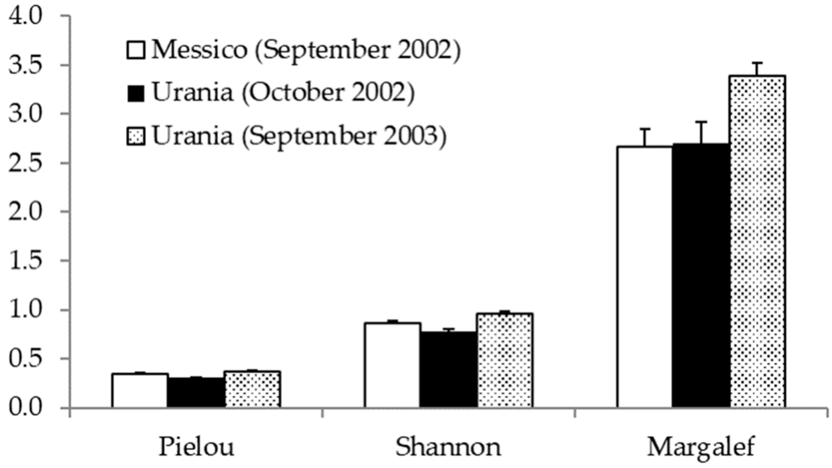


Figure 5. Average values of biodiversity indexes in the three surveys (bars = SE).

3.3. Mesopelagic Fish Assemblages/Communitys

The cluster analysis showed a high similarity (>75%) among the stations. A first separation occurred at a level of 80% of similarity, where four groups of stations were identified (Figure 6a).

Similar results were obtained by the MDS analysis, which confirmed the presence of two main groups characterized by the samples from the Urania 2002 survey (group c) and by a group comprising the stations of Messico 2002 and Urania 2003 (group b) (Figure 6b). Three hauls, namely 807, 810, and 918 (Urania 2003), had anomalous results.

The analysis of similarity (one-way ANOSIM) was performed considering the a priori hypothesis (H_0) that there were no differences in species composition between the groups identified. The pairwise test, at 80% similarity level, detected a significant difference only between two groups (b and c) (global R = 0.83; $p < 0.01$) and not between the others.

The species contribution to the overall mesopelagic similarity (80% level) is reported in Table 3. SIMPER analysis showed that five species contributed to about 90% of the similarity: *C. braueri*, *C. pygmaea*, *A. hemigymnus*, *L. crocodilus*, and *Ceratoscopelus maderensis* (Lowe, 1839).

The two species of genus *Cyclothone* and *A. hemigymnus*, in terms of average catch rate, gave a different contribution between the two main groups (b, c): 78.4 ind./10,000 m^3 (group c) against 26.2 ind./10,000 m^3 (group b) in *C. braueri*, 29.6 ind./10,000 m^3 (group c) against 13.8 ind./10,000 m^3 (group b) in *C. pygmea*, and 2.6 ind./10,000 m^3 (group c) against 0.8 ind./10,000 m^3 (group b) in *A. hemigymnus*.

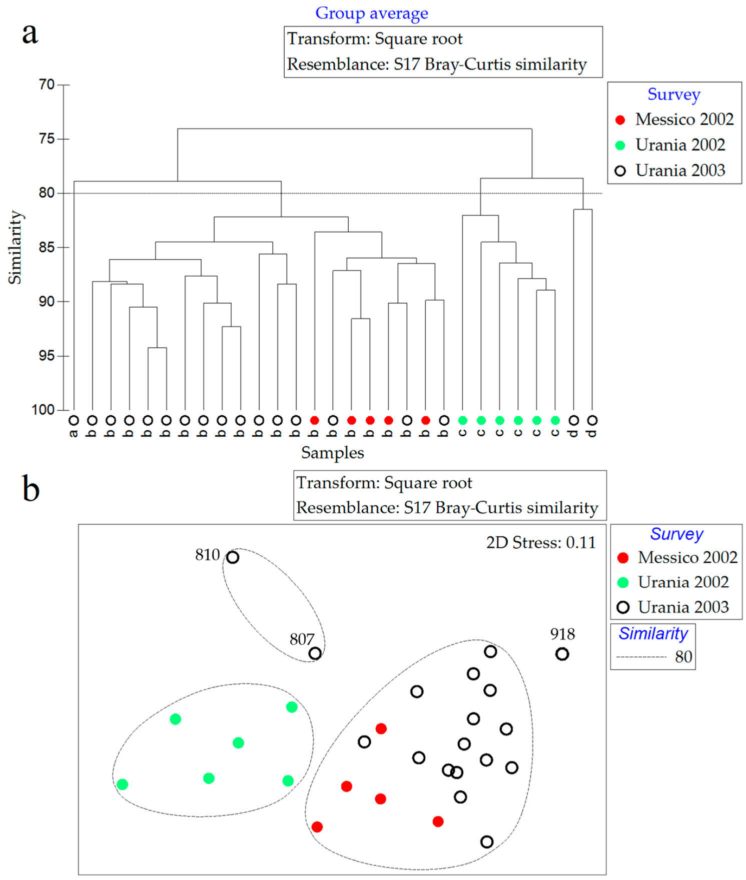


Figure 6. Cluster (**a**) and MDS (**b**) analysis at 80% of similarity among the stations.

Table 3. Similarity percentage (SIMPER) analysis within groups (80% similarity) identified by cluster and MDS and percentage contribution per species (cut-off level 90%).

	Group b (Average Similarity: 84.13)			
Species	**Av.Abund**	**Av.Sim**	**Contrib%**	**Cum.%**
Cyclothone braueri	5.34	34.85	41.42	41.42
Cyclothone pygmaea	3.81	25.32	30.1	71.52
Argyropelecus hemigymnus	0.97	5.86	6.96	78.48
Lampanyctus crocodilus	0.69	4.29	5.1	83.58
Ceratoscopelus maderensis	0.58	3.07	3.65	87.24
Benthosema glaciale	0.49	2.77	3.29	90.53
	Group c (Average Similarity: 84.78)			
Species	**Av.Abund**	**Av.Sim**	**Contrib%**	**Cum.%**
Cyclothone braueri	8.79	41.56	49.02	49.02
Cyclothone pygmaea	5.36	24.2	28.54	77.56
Argyropelecus hemigymnus	1.58	7.02	8.28	85.84
Lampanyctus crocodilus	0.8	3.47	4.09	89.93
Ceratoscopelus maderensis	0.46	1.89	2.22	92.15
	Group d (Average Similarity: 81.47)			
Species	**Av.Abund**	**Av.Sim**	**Contrib%**	**Cum.%**
Cyclothone braueri	8.09	36.61	44.94	44.94
Cyclothone pygmaea	3.15	15.26	18.73	63.66
Argyropelecus hemigymnus	1.89	8.81	10.81	74.48
Ceratoscopelus maderensis	1.49	4.23	5.19	79.67
Lampanyctus crocodilus	1.29	4.11	5.05	84.72
Chauliodus sloani	0.68	3.13	3.85	88.57
Benthosema glaciale	0.66	2.64	3.24	91.81

The PERMANOVA analysis, used to test the mesopelagic fish assemblage, revealed a significant difference among sampling periods ($p < 0.001$) (Table 4), while light presence (day/night samples) was not significant ($p > 0.05$). The difference due to the sampling period (September vs. October) was affected by the catch rate that, in October (Urania 2002), was more than twice (mean = 112.5 ind./10,000 m^3, SD = 31.8) that of the samples from September (Messico 02 and Urania 03) (mean = 50.2 ind./10,000 m^3, SD = 16.1).

Table 4. PERMANOVA analysis used to test the effect of the sampling period (September vs. October) and light (day vs. night samples) on mesopelagic fish assemblage.

Source	Sum of Sqrs	df	Mean Square	F	*p*
Sampling period (S/O)	0.6614	1	0.6614	14.5830	0.0001
Light (D/N)	0.0189	1	0.0189	0.4175	0.5046
Residual	1.1338	25	0.0454		
Total	1.3986	28			

This effect could be related to the variation in the water temperature of the superficial layer (0–10 m), as detected by the DYFAMED buoy that, in September (Messico 2002 and Urania 2003), showed higher results (~20.9 °C (SD = 0.1) and ~21.3 °C (SD = 0.2)) than in October (Urania 2002) (~18.1 °C (SD = 0.1)) (Figure 7).

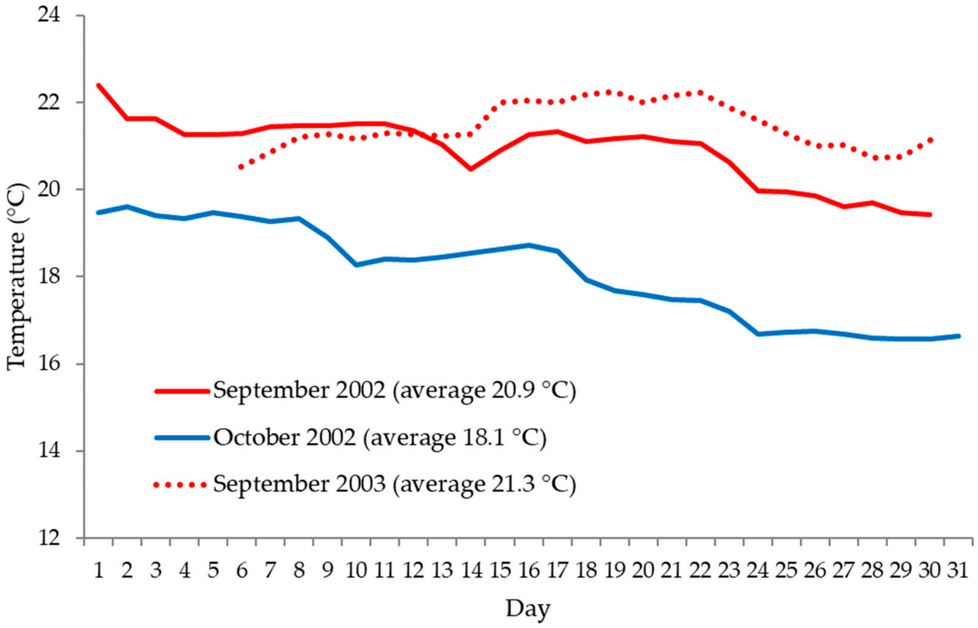


Figure 7. Mean sea surface temperature registerd by the Boa Dyfamed between August and October 2002/2003. Measurements are not available for the first 6 days of September 2003.

3.4. Length Groups and Daily Variations in Species

The LFDs of five mesopelagic fish species revealed that catches were mainly represented by juveniles and, for some species, by the entire population, including subadult and adult specimens (Figure 8).

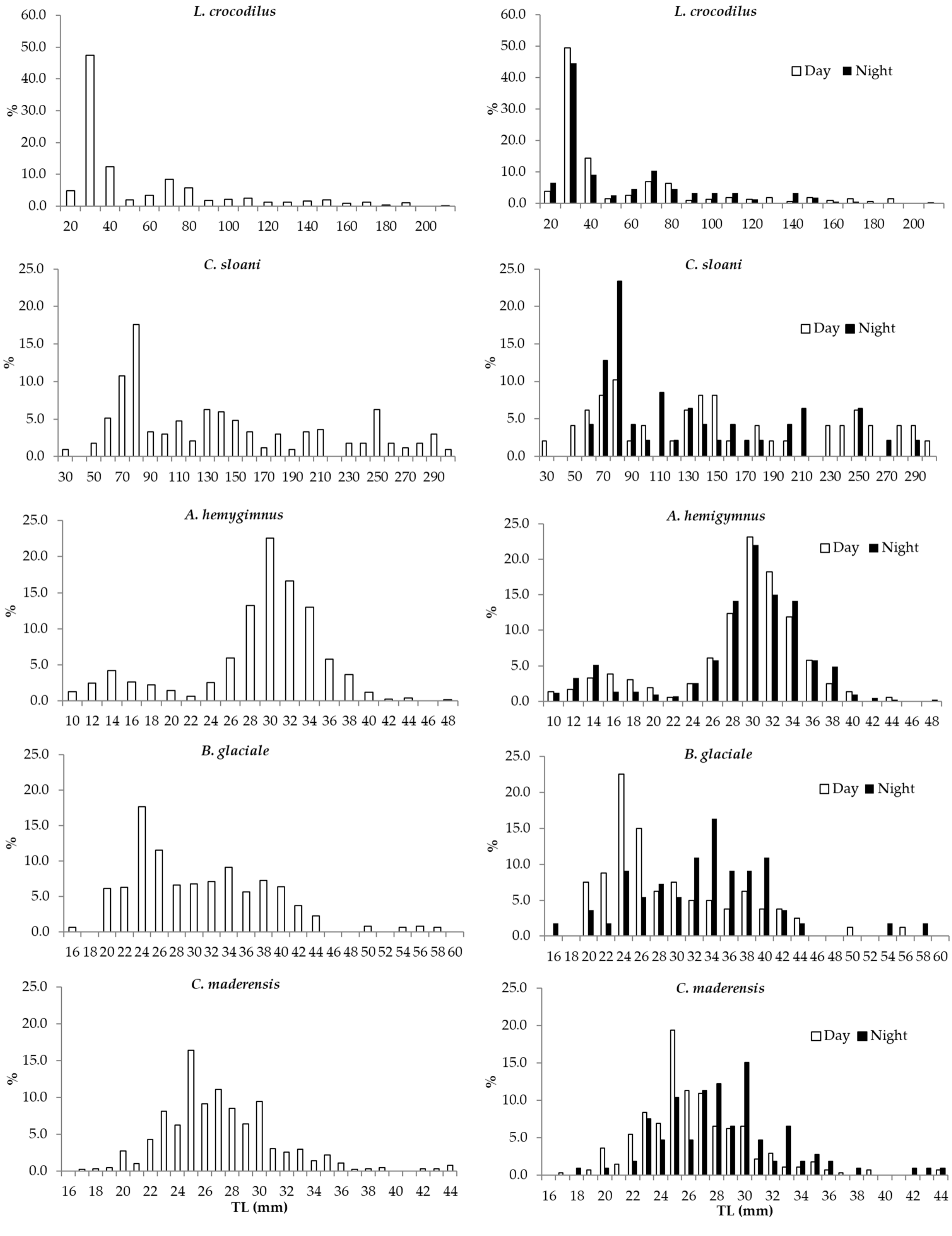


Figure 8. Length–frequency distributions of five mesopelagic species: on the left is the total sample, and on the right, the day/night samples are separated.

The jewel lanternfish *L. crocodilus* showed a polymodal distribution, with the main one comprising juveniles (modal size at 30 mm TL), representing more than 60% of the entire sample, and a second modal distribution at 70 mm TL followed by two less distinctive distributions comprising sub-adult and adult specimens. No significant differences (K-S test: p-value > 0.05) were detected between LFDs observed during day/night samples.

The Sloane's viperfish *C. sloani* showed a polymodal distribution as well, with the main one comprising juveniles (modal size at 80 mm TL), representing 40% of the total sample, and at least 3–4 mixed distributions comprising sub-adult and adult specimens. No significant differences (K-S test: p-value > 0.05) were detected between LFDs observed during day/night samples.

The half-naked hatchetfish *A. hemigymnus* showed a clear division of the population into two size groups, namely the juveniles (modal size at 14 mm) and the adults and sub-adults (modal size at 30 mm TL), which represented more than 80% of the sample. The LFDs did not show significant differences (K-S test: p-value > 0.05) between specimens caught in day/night samples.

The glacier lantern fish *B. glaciale* showed a bimodal distribution comprising juveniles (24 mm) and subadult/adult individuals (34 mm TL). A significant statistical difference (K-S test: p-value < 0.05) was detected between day/night samples, with a predominance of the juveniles fraction (<28 mm TL) during the daily sample.

The Madeira lantern fish *C. maderensis* showed a distribution mainly comprising juveniles with a predominant modal size of 25 mm and one less visible at 30 mm TL. A significant statistical difference (K-S test: p-value < 0.05) was detected between day/night distributions, with a predominance of smaller individuals (<28 mm TL) in the daily samples.

4. Discussion

This study allowed the description of the mesopelagic fish community of the Ligurian Sea in offshore waters, integrating the available knowledge on the mesopelagic community of the Mediterranean Sea.

The sample of small mesopelagic fish obtained by the 2002–2003 surveys in the Ligurian Sea was represented by 31 species belonging to nine families, plus some unidentified juveniles. During previous surveys carried out in the same area (1988–1993) using the same IKMT net [5,46], nine additional species belonging to four families were recorded: *Zu cristatus* (Bonelli, 1819), *Trachipterus trachypterus* (Gmelin, 1789), *Regalecus glesne* Ascanius, 1772 (three large mesopelagic species found in larval stages), *Nansenia oblita* (Facciolà, 1887), *Gonostoma denudatum* Rafinesque, 1810, *Diaphus metopoclampus* (Cocco, 1829), *S. hyalina, Lestidiops jayakari* (Boulenger, 1889), and a species of *Notoscopelus* different from *N. elongatus* [5]. The last one was first indicated as *Notoscopelus kroyeri* (Malm, 1861), as in the Smithsonian list [42], but following [63], it was later identified as *Notoscopelus bolini* Nafpaktitis, 1975 [64]. The absence of these species in the present surveys could be due to chance, given that they were already listed in the group of "rare" fish (less than 0.01%). Another possibility is that some species are "rarely caught" in mesopelagic surveys, as they are associated with the shelf/slope and can be found only when sampling is carried out above this zone [43]. For example, *Maurolicus muelleri* (Gmelin, 1789) is surely not rare but probably lives in more coastal waters on the slope [43,65]. In fact, it is a common prey of *Micromesistius poutassou* (Risso, 1827) at epibathyal levels of the slope [66,67], and mass strandings can occur in winter on the shore of the Western Ligurian Riviera [68–70]. On the other hand, in the present sample, *V. tripunctulatus* was found, a species new to the Western Mediterranean Sea at time of the 2003 survey. Commonly present on the western side of Gibraltar, is rare in the Mediterranean, and it was recorded only in the Strait of Messina, the Southern Tyrrhenian Sea, and the Adriatic Sea [71]. In the Urania survey (2003), this fish was found twice, at stations 410 and 810, positioned along the transect Gulf of Genoa, north of Corsica: it was absent in repeated sampling previously carried out at the same stations during the 1990s. From these observations, considering the geographical distribution of all records and the water circulation in the Mediterranean [72], two alternative hypotheses may be formulated: (a) *V. tripunctulatus* is an Atlantic stray, and (b) there is a Mediterranean population, which is rarely sampled and more widely distributed than it was previously thought.

It is well known that the Mediterranean mesopelagic fish community is less diverse than the adjacent Atlantic community [45,73]. It includes four endemic species, *C. pygmaea*, *N. elongatus*, *L. crocodilus* [74], and *Nansenia iberica* Matallanas, 1985. This latter species is reported as being present in the Catalan Sea [75] and around the Balearic Islands [76], taking into account the difficulties in distinguishing it from *N. oblita* and the other species of the genus. In this context, the Ligurian Sea can be considered the area with the highest mesopelagic fish community richness of the Mediterranean Basin [43,45,65]. This was already shown by the Smithsonian Expedition [42]; from five stations (southwest of the Balearic Islands, west of Corsica, the Central Tyrrhenian Sea, the Central Ionian Sea, and south of Crete), a total of 21,574 specimens were collected, belonging to 35 species. The highest numbers of species were found at the stations positioned in the Central Mediterranean, with 29 species west of Corsica and 30 species in the Tyrrhenian Sea, including an Atlantic stray, *Diogenichthys atlanticus* (Tåning, 1928) (only one postlarva found in the westernmost station) and *Paravocettinops trilinearis* Kanazawa and Maul, 1967 (later on determined as a juvenile phase of *N. scolopaceus*). At the station west of Corsica, *N. bolini* (under the name *N. kroyeri*) and *G. denudatum* were found but not *N. oblita*, *D. metopoclampus*, *S. hyalina*, and *Borostomias antarcticus* (Lönnberg, 1905), which were found instead in the Ligurian Sea in the present and/or past surveys. Of these, *B. antarcticus* was discovered in the 1960s offshore Monaco and was later recorded in the Gulf of Genoa [77]. Some species, which were recorded in the Western Mediterranean and have sometimes been mentioned in the literature [78], were later on deleted as junior synonyms (i.e., *Parabathophilus gloriae* Matallanas, 1984, present accepted name *Bathophilus nigerrimus* Giglioli, 1882, or misidentification of other species as *Lampanyctus intricarius* Tåning, 1928) [79]. More recently, scientific cruises conducted in the Western Mediterranean (Balearic Islands and Alboran Sea) in 2009–2010 and 2020 recorded a total of 25 and 22 species, respectively [43,45]. In the Eastern Mediterranean, during the Smithsonian Expedition, only 17 species were found at the station south of Crete, a number comparable to a recent cruise conducted in the Gulf of Corinth in 2018-2019, with 15 species recorded [65], despite the fact that Golani reported 27 species of small mesopelagic fishes in the Eastern Mediterranean [80].

Thus, we can assume that small mesopelagic fish that are currently known to inhabit the Mediterranean also occur in the Sanctuary. They are represented by 40 species belonging to 13 families; only two species were missing: *N. iberica* found off Blanes [75] and the Balearic Islands [76] and *Sygmops elongatus* (Günther, 1878) found off the Balearic Islands [81] and in the Southern Tyrrhenian Sea [82] (under the name of *Gonostoma elongatum*), suggesting a possible immigration from the Atlantic.

At the time of the Smithsonian Mediterranean study, the station closest to our study area (Ligurian Sea—west of Corsica), showed a remarkable abundance of many of the listed species (17 of 29). In fact, the catch (in terms of N per hour) reached the maximum that was registered during the expedition: the top species were *C. braueri* (564/h vs. 511/h in the present study), *C. pygmaea* (500/h vs. 211/h), and, in order of decreasing abundance, *C. maderensis*, *B. glaciale*, *Hygophum benoiti* (Cocco, 1838), *Vinciguerria attenuata* (Cocco, 1838), *Arctozenus risso* (Bonaparte, 1840), *A. hemigymnus*, *Paralepis coregonoides* (Risso, 1820), and *Lampanyctus pusillus* (Johnson, 1890) [46]. Our study confirms the high abundance of the species *C. braueri*, which plays a key role in the food web of the Ligurian Sea [38] as much as the northern krill, *M. norvegica* [83]. Differently from other studies, the third and fourth most abundant species were, respectively, *A. hemigymnus* and *L. crocodilus*, while a lower presence of *B. glaciale* and *C. maderensis*, the two myctophids frequently reported in other areas of the Western Mediterranean [42,43,45], was observed. Although the characteristics of the net (mouth opened) did not allow to establish in detail the vertical distribution of the different species, it is clear how the presence of *Cyclothone* spp. strongly influenced the abundance distribution of all our samples. The dominant presence of this group is probably linked to the presence of a permanent layer, mainly around 200–400 m, that does not migrate towards the surface, which was identified in the Ligurian Sea and in the Balearic area [38,43]. Comparison of the Smithsonian data with the present series suggests that the basic structure of the fish community in the Ligurian Sea has remained the same over the years in terms of the dominant species, in both its qualitative and quantitative aspects.

The peculiarity of this research was that sampling stations were positioned in offshore waters generally deeper than 2000 m on the pelagic domain. This, in some way, may influence the catches, which showed a high similarity at all stations (>75%). Moreover, the distribution of mesopelagic fish fauna was uniform over the whole investigated area, probably influenced by its oceanographic specifically in the autumn (October). In fact, diversity indices (Shannon and Pielou) were significantly lower for the Urania 2002 survey held in October, confirming the presence of a few dominant species (*Cyclothone* spp. and *A. hemigymnus*) considered as non-migrant or weakly migrant species in other studies [38,43,45,65]. This result might be attributable to the sea surface temperatures registered in September, which were still high compared to temperatures in October (Figure 7). This increase in temperature together with the salinity observed in the superficial layer (0–200 m) and even in deep waters (200–600 m) between 2002 and 2006 [84] was attributed to a propagation of the Eastern Mediterranean Transient in the Western Mediterranean Sea [85–87]. This kind of scenario might have affected the composition of the mesopelagic community in the water column and created a vertical stratification delaying the vertical mixing of different strata [49,88]. The pelagic domain in the study area is deeply conditioned by events of dense water formation caused by the decrease in temperature due to cold wind activity in the autumn/winter season, which increases evaporation, increasing the salinity (density) and generating the vertical mixing of the water column [51,84]. Moreover, the Ligurian Sea is characterized by a counterclockwise gyre [50,51]; an important permanent geostrophic front exists between the peripheral zone Corsica Current and the Northern Current, where massive surface currents flow (i.e., the Ligurian Current), and a central zone of divergence that is characterized by slow currents and complex physical dynamics, where upwelling occurs [27,51,89–91].

In the literature, it was demonstrated that vertical distribution of mesopelagic fauna (zooplankton and micronekton) was variable and conditioned by many different factors: temperature, salinity, productivity, food availability, nutrient, season, bathymetry, and light [26–38,43,45,75,86,92]. Among these, light is considered one of the primary factors of diel migration in mesopelagic fish and their prey [26–38,43,45,75,86,92]. However, in our study, mesopelagic fish assemblages did not appear to be influenced by light conditions (day vs. night samples). This outcome could be attributed to our sampling approach, which employed oblique hauls spanning the entire water column (0–800 m) rather than focusing on a fixed layer, as in other studies [43,45,65]. Despite this limitation, our fishing gear (IKMT) proved effective in terms of both catchability (relative abundance) and selectivity (size range sampling) for various species. This effectiveness was achieved through a combination of a wide mouth opening (17.5 m^2) and a fine internal mesh size (2 mm × 2 mm). Smaller nets were shown to be particularly suitable for collecting juveniles, while larger nets were better suited for capturing greater quantities of species and broader size ranges of specimens [43,63]. The efficiency of our gear enabled us to sample a wider range of sizes and life stages of *C. sloani* and *L. crocodilus* compared to other regions in the Western Mediterranean Sea [43,45]. The polymodal distributions observed in both species due to the presence of large specimens confirm a longer life span of these species [93–95]; furthermore, it might indicate that adult individuals, over the length of first maturity (L_{50}) [96,97], can also live and prey in the water column outside the slope and not only near the bottom [43], where they are frequently caught by trawls [98–100]. *A. hemigymnus* has a short life span, as confirmed by the bimodal distribution and a study carried out in the same area [101]; its population is composed by juveniles and in large part by adults specimens (L_{50}: 25 mm SL [102]), without a clear difference between day/night length distributions. This evidence confirms the habits of *A. hemigymnus*, which is considered a weakly migrant species without a clear vertical stratification of sizes [43,65]; furthermore, in the Balearic area, *A. hemigymnus* exhibited a daily mode that was larger (19–25 mm SL) than at night (14–16 mm SL) [43]. *B. glaciale* has a long life span of up to 7–8 years old [103] but reaches sexual maturity early (L_{50}: 30 mm SL). In other Mediterranean areas, it is one of the more abundant species, together with *C. maderensis* [43,45]. Our samples were composed by two cohorts, a smaller one composed by juveniles detected during daily sampling and one of sub-adult/adults observed during the night samples. The absence of the largest individuals might be linked to the fact that mature specimens (>30 mm SL) were found deeper near the bottom, while juveniles (<30 mm) were frequently found between 0–80 m and 400–600 m depth at night [43]. *C. maderensis* has a brief life span (about 1 year old) and quickly reaches maximum size [104,105]. Our sample almost totally comprised juveniles (L_{50}: 38 mm SL; [106]). The significant difference between day/night samples could be related to a bias due to the different growth rates of the two cohorts detected in our sample; this does not allow considerations regarding the vertical distribution in different life stages (juveniles/adults) but simply observation of the presence of juveniles during day and night sampling in equal measure. In other studies, *C. maderensis* was detected mainly near the surface at night [43], while during the daytime, adults remained in mesopelagic layers (200–1000 m) [105]. On the basis of sizes and sporadic biological data, our sample of small mesopelagic fishes could tentatively be assigned to three trophic levels: (1) at the base, the very abundant *Cyclothone* spp.; (2) at the intermediate position, *A. hemygimnus* and myctophids, which feed on both mesozooplanktonic and nektonic crustaceans and small fish, including *Cyclothone* spp. [98,101,107]; and (3) at the highest level, *C. sloani*, which feeds on myctophids and gonostomids [106,107]. These trophic levels are mirrored by the decreasing biomass of the corresponding families: (1) Gonostomatidae; (2) Sternoptychidae plus Myctophidae; and (3) Stomiidae (Chauliodontinae). Mesopelagic fishes represent important prey to a diversity of higher-trophic-level predators of the Cetacean Sanctuary (tuna, swordfish, and cetaceans). In past studies carried out in the Ligurian Sea, we observed that the preferred fish prey of the swordfish were Paralepididae and large mesopelagic species of Lampriformes [108,109]. *C. sloani* was found in the stomach contents of the common dolphin *D. delphis*, which is at present relatively rare in the Sanctuary [9]. On the other hand, the very common *S. coeruleoalba* feeds on members of the genera *Ceratoscopelus*, *Chauliodus*, *Diaphus*, *Electrona*, *Hygophum*, *Lampanyctus*, *Lobianchia*, *Myctophum*, *Notoscopelus*, *Stomias*, and *Symbolophorus* [5,10–12]. This is clearly due to the fact that wild animals feed on not only the most abundant species but also those that, on the basis of scientific sampling, seem to be rare. These patterns probably have a great influence on the distributions of large pelagic predators, and swordfish present the best yield-per-unit effort of the Ligurian professional fishery in the Northwestern sector [110,111], and cetaceans were always observed in large numbers in this area [112].

In conclusion, we acknowledge that our sample represents only a portion of the pelagic ichthyofauna; this is an obvious effect regarding all pelagic animals with good swimming capabilities when sampling is carried out using “scientific” nets. In fact, considering studies conducted on the diet of large pelagic predators or even monitoring professional fishing gear [1,3,5,7–13,109–111], the situation appears slightly different. Often, there may be very abundant populations of some fish species considered “rare”, which remain “hidden” in an environment that is very difficult to study and sample, and it would be more accurate to define them as "rarely sampled" [113]. Some examples include Evermannellidae (i.e., *Evermannella balbo* (Risso, 1820)) and Paralepididae (i.e *S. hyalina*) but also large fish such as Trachypteridae (i.e., *Z. cristatus*), Luvaridae (i.e., *Luvarus imperialis* Rafinesque, 1810), and Centrolophidae (i.e., *Centrolophus niger* (Gmelin, 1789)) that represent a by-catch species of the mesopelagic longlines fishery targeting swordfish in the Ligurian Sea [1]. For these reason, a multidisciplinary combination of different studies appears to be a necessary step for the future, even to identify potential good environmental status (GES) in the Marine Strategy Framework Directive (MSFD) for pelagic environmental/ecosystems of the Mediterranean area and, for the Pelagos Sanctuary area, to better understand the dynamics of the pelagic domain for improving the management strategies and tools for the conservation of cetaceans.

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