

Article

Deep-Water Cartilaginous Fishes in the Central Mediterranean Sea: Comparison between Geographic Areas with Two Low Impact Tools for Sampling

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Abstract: Baited lander represents a low impact technique, an alternative to the traditional trawl sampling for collecting data on fish diversity and abundance, especially for threatened species such as Chondrichthyes living in sensitive habitats. In this study, distribution and abundance of cartilaginous fish were compared between two geographic areas, the southern Adriatic Sea and the north-western Ionian Sea, with two low impact sampling gears, an experimental bottom longline and a baited lander. Species diversity was evaluated by applying ecological indices and difference in mean abundances were tested using multivariate analysis. A total of 13 species of cartilaginous fish were collected. Significant differences in the assemblage recorded in the same area using different sampling tools were detected and no significant differences were detected among different areas explored with the same method. Using longline, the most abundant species collected in both areas was *Galeus melastomus*, while using lander, the most observed species were *Dalatias licha* in the southern Adriatic Sea and *Hexanchus griseus* in the north-western Ionian Sea. According to IUCN classification, of the 13 species collected, 2 are near threatened and 5 are threatened. A better governance of sensitive habitats coinciding with the essential fish habitat for these species would ensure them a better conservation status.

Keywords: cartilaginous fish; baited lander; longline; Mediterranean Sea



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1. Introduction

More than 1250 species of cartilaginous fish (sharks, rays, and chimaeras) are found throughout the world's oceans and some freshwater systems [1–3]. Sharks are one of the oldest extant radiations of vertebrate animals and they have evolved a wide range of morphological and mechanical features such as facultative parthenogenesis, embryonic diapause, bioluminescence, pockets, saws, hammers, stings, and electricity [2,4]. Sharks are usually near or at the top of the trophic chain, playing an important role in the structure and functioning of the marine ecosystem: they act as a link between food webs and across habitat aggregating, integrating, and transferring energy [2,5–8]. Cartilaginous fish are characterized by a suite of life history traits that lower their ability to tolerate anthropogenic impact [6,9,10]. In fact, chondrichthyans are generally characterized by slow growth, late maturity, low fecundity, long gestation periods, and a long-life span [9–11]. This strategy reduces the capacity to recover and, thereby, increases their vulnerability to human disturbances [5,6,9,12].

A total of 88 chondrichthyes species have been recorded in the Mediterranean Sea, including 48 shark species, 38 batoid species, and 2 chimeras [1]. In this region, the spatial distribution and the abundance of various elasmobranch species have been mainly studied in the trawlable grounds through commercial and experimental trawl surveys [11,13–16].

Relative abundance estimates generated from scientific longline surveys have also been utilized in shark ecology studies, including those investigating declines in shark abundance, habitat use, and demographic population structure and variation in seasonal abundance [17–20]. Moreover, longline surveys allow the collection of samples for studies concerning genetics, diets, age, growth, reproduction, migration, and physiology [21–24]. Nevertheless, this sampling method might be not appropriate for threatened species: during longline surveys, indeed, sharks must be caught and handled out of the water and the captured fishes are exposed to physiological stress and physical trauma that can induce pre- or post-release mortality [21,23–25].

Currently, baited remote underwater video surveys (BRUVS) have become an alternative approach for collecting data on larger-bodied and threatened species including sharks [26–32]. Baited lander represents a low impact non-extractive technique for collecting data on fish diversity, abundance, and behaviour, and can be used as an alternative method to the traditional trawl sampling: the passive nature of this method allows for its use in fragile and protected areas and when dealing with rare and threatened species that can be negatively affected by fishing gears [27–29,33–37].

The use of different sampling system has been validated through the comparison of species composition and abundance determined with different tools. Baited lander efficiency has been compared to those of different fishing methods including trap and longline [21,27,38,39]. These studies suggest that baited lander can provide information on species assemblage and abundance comparable to those generated with traps and longline. Additionally, the observations recorded with BRUVS and remotely operated vehicles (ROVs) have been compared to data collected with bottom beam trawls and prawn trawls, showing the difference in catch composition determined by the different fishing gear [40,41].

The Mediterranean Sea is considered a hotspot of extinction risk for chondrichthyan species: in this basin, the chondrichthyes have suffered declines in abundance and biodiversity [16,42–44]. More than half of the assessed species (39 of 73 species recorded in the Mediterranean Sea) are regionally threatened. A total of 31 are most imperiled species and, among these, 20 are classified as critically endangered and 11 as endangered [42]. Moreover, the level of risk may be worse because uncertainty in species status is moderately high: of the 73 species assessed in the Mediterranean Sea, 13 remain data deficient [10,16,42,43]. The main threats that cartilaginous fishes have to endure are overexploitation both as targeted fisheries and incidental catch (by-catch), followed by habitat loss and degradation, pollution, and climate change [5,10,18,43–46].

In the last twenty years, sensitive habitats and marine vulnerable ecosystems (VMEs) have been explored with different low impact non extractive techniques as part of national and international projects carried out in the central Mediterranean ([47] and references therein). The aim of this study is to compare the distribution and abundance of cartilaginous fishes between two geographic areas of the central part of this basin, the southern Adriatic Sea and the north-western Ionian Sea, using two low impact sampling gears, an experimental longline and a baited lander.

2. Materials and Methods

2.1. Area of Study

The study area is located in the Central Mediterranean Sea along the Apulian margin between the southern Adriatic Sea and north-western Ionian Sea. This area is characterized by the presence of several morphological and geological structures that indent the continental shelf [48,49]. Along the Apulian margin, a belt of cold-water coral communities, probably connected by water mass flowing from the southern Adriatic to northern Ionian, has been identified [50–53].

The Bari canyon is a complex morphological structure indenting the southern Adriatic shelf in an west–east direction [48,49,54]. The Bari canyon hosts a diversified community of deep-water cnidarians characterized by the presence of *Madrepora oculata* and *Lophelia pertusa* together with *Desmophyllum dianthus* and *Dendrophyllia cornigera*, *Leiopathes glaberrima* associated with sponges, serpulids, and bryozoans [34,35,53,55]. The benthopelagic fauna distributed in the Bari canyon system is mainly represented by the teleost fishes *Conger conger*, *Helicolenus dactylopterus*, *Merluccius merluccius*, *Phycis blennoides*, and *Pagellus bogaraveo*, whereas *Galeus melastomus* is the most abundant cartilaginous fish [51,56]. In this area, recent explorations have revealed the existence of a new cold-water coral habitat southward to the Bari Canyon system in the area off Monopoli, where living colonies of *M. oculata* have been collected [51].

The north-western Ionian Sea is characterized by the presence of the Santa Maria di Leuca cold-water coral province, the widest and most studied cold-water coral ecosystem known in the Mediterranean [50,53,57]. Dead and living colonies of *L. pertusa* and *M. oculata* are widespread on an area of about 1000 km² at a depth between 300 and 1110 m [50,58,59]. The benthopelagic fauna associated with Santa Maria di Leuca cold-water coral province is characterized by the teleost fishes *C. conger*, *H. dactylopterus*, *M. merluccius*, *P. bogaraveo*, *P. blennoides*, and *Polyprion americanus*, which are targets of fishing activities carried out on the fishing ground surrounding the coral province [60,61]; moreover, the most abundant cartilaginous fish associated with this cold-water coral province is *G. melastomus* [51,60,61].

2.2. Survey Methodology

Data were collected using two different tools, an experimental bottom longline and MEMO baited lander, in two geographic areas of the central Mediterranean, the southern Adriatic Sea and the north-western Ionian Sea (Figure 1, Table 1).

Table 1. Sampling data for each area and tools, with an indication of the depth range explored and number of deployments.

	Longline		MEMO Lander	
	Southern Adriatic Sea	North-Western Ionian Sea	Southern Adriatic Sea	North-Western Ionian Sea
Number of stations	32	39	19	12
Sampling hours (h)	134	152.7	120	103
Depth range (m)	314–612	418–635	427–788	547–792

Using longline, a total of 71 deployments were carried out for a total time of about 287 fishing hours (Table 1). A commercial fishing vessel was hired and equipped with a monofilament longline with 500 hooks and baited with *Sardina pilchardus* as bait (see details in [51]). The soak time lasted about 4 h on average. In order to make a comparison between the used tools, the abundance of the species collected in each deployment was standardized in number of individuals for hour of fishing on the seabed (N/h).

Using the MEMO baited lander, a total of 31 deployments were carried out between 427 and 792 m, for a total time of 223 h of video record (Table 1).

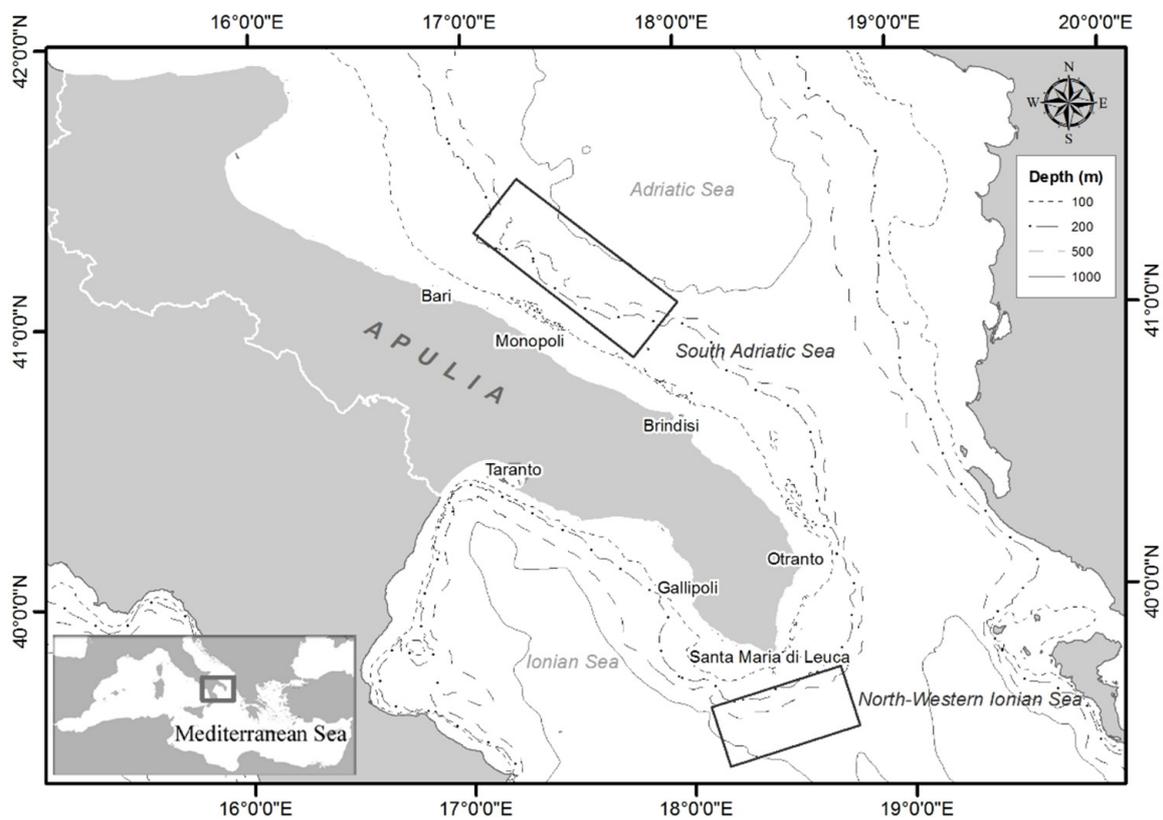


Figure 1. Map of the study area with an indication of the areas in which baited lander and longline were used.

The MEMO lander consists of a stainless steel frame (\varnothing 2.15 m; h 1.65 m) equipped with two video cameras (HD Multi SeaCam), two white LED lights, and a multiparametric probe (see details in [62]). A continuous connection is maintained via the acoustic modem with an onboard PC software platform, making images and sensor data available on the vessel. During each deployment, the lander was baited with a fresh specimen of *Scomber scombrus*. The bait produces an odour plume that attracts the animals in the field of video cameras. The videos recorded by both video cameras during each deployment were analysed using the Adobe Premier Pro software and the animals recorded by MEMO were identified to the lower taxonomical level using morphological characteristics. For each species, MaxN was recorded as the maximum number of individuals of the same species recorded at the same time in the field of the camera and MaxN per hour was used to standardize the abundance as N/h for each deployment.

2.3. Data Analysis

The abundances for each species, for both longline and baited lander deployments, were standardized over the sampling time as number of individuals per hour (N/h). Species diversity was evaluated applying the Margalef species richness, Shannon–Wiener diversity, and Pielou’s evenness ecological indices [63]. Cumulative curves were calculated through EstimateS 8.2 [64] in order to assess whether the number of sampling stations was sufficient to describe the species richness identified by the different tools. The slope (b) of the linear regression through the last five sub-samples was used, where $b \leq 0.05$ signified acceptable leveling off of the curve [65].

Non-metric multidimensional scaling (nMDS) ordination, based on a Bray–Curtis dissimilarity matrix, was applied to reveal multivariate patterns in the species assemblages [66]. Differences in mean abundances of species between the two explored areas and between longline and baited lander were tested using one-way analysis of similarities (ANOSIM, [66]) and one-way permutational multivariate analysis of variance (PERMANOVA, [67]); in both tests, a Bray–Curtis dissimilarity index was applied. Similarity percentage analysis (SIMPER) and indicator species analysis (IndVal) were used to determine which species are responsible for the dissimilarity among areas and sampling tools [66,68]. Statistical analyses were performed within PRIMER v6 with PERMANOVA add-on software and using the software PAST ver. 4.05 [69–71].

3. Results

A total of 13 species of cartilaginous fish were collected. In both areas, a total of 10 species were recorded (Table 2). In the southern Adriatic Sea, seven species were recorded using both MEMO lander and longline, whereas in the north-western Ionian Sea, five species were recorded using the lander and eight were collected by means of longline. *Centrophorus granulosus* and *Etmopterus spinax* were the only species collected in the two areas with both sampling tools. In both areas, *G. melastomus* was the most abundant species collected using longline. The greatest mean values observed using the lander were obtained for *Dalatias licha* and *Hexanchus griseus* in the southern Adriatic and north-western Ionian, respectively.

Table 2. Mean abundance (N/h) of cartilaginous fishes collected in the southern Adriatic and north-western Ionian Sea using longline and MEMO baited lander.

Species	Longline		MEMO Lander	
	Southern Adriatic Sea	North-Western Ionian Sea	Southern Adriatic Sea	North-Western Ionian Sea
<i>Centrophorus granulosus</i> (Bloch & Schneider, 1801)	0.01	0.04	0.08	0.01
<i>Chimaera monstrosa</i> Linnaeus, 1758	0.05			
<i>Dalatias licha</i> (Bonnaterre, 1788)	0.01		0.11	0.01
<i>Dipturus oxyrinchus</i> (Linnaeus, 1758)		0.02	0.01	0.03
<i>Etmopterus spinax</i> (Linnaeus, 1758)	0.14	0.32	0.06	0.02
<i>Galeus melastomus</i> Rafinesque, 1810	3.78	4.03	0.10	
<i>Hexanchus griseus</i> (Bonnaterre, 1788)			0.03	0.09
<i>Leucoraja circularis</i> (Couch, 1838)		0.02		
<i>Leucoraja fullonica</i> (Linnaeus, 1758)		0.04		
<i>Prionace glauca</i> (Linnaeus, 1758)		0.01		
<i>Pteroplatytrygon violacea</i> (Bonaparte, 1832)	0.03	0.07		
<i>Scyliorhinus canicula</i> (Linnaeus, 1758)	0.02			
<i>Somniosus rostratus</i> (Risso, 1827)			0.01	

The number of samples was sufficient to describe the species distribution in both areas and collected with different tools as the resulting cumulative curves reached the asymptote ($b \leq 0.05$), although with different slope values (Figure 2).

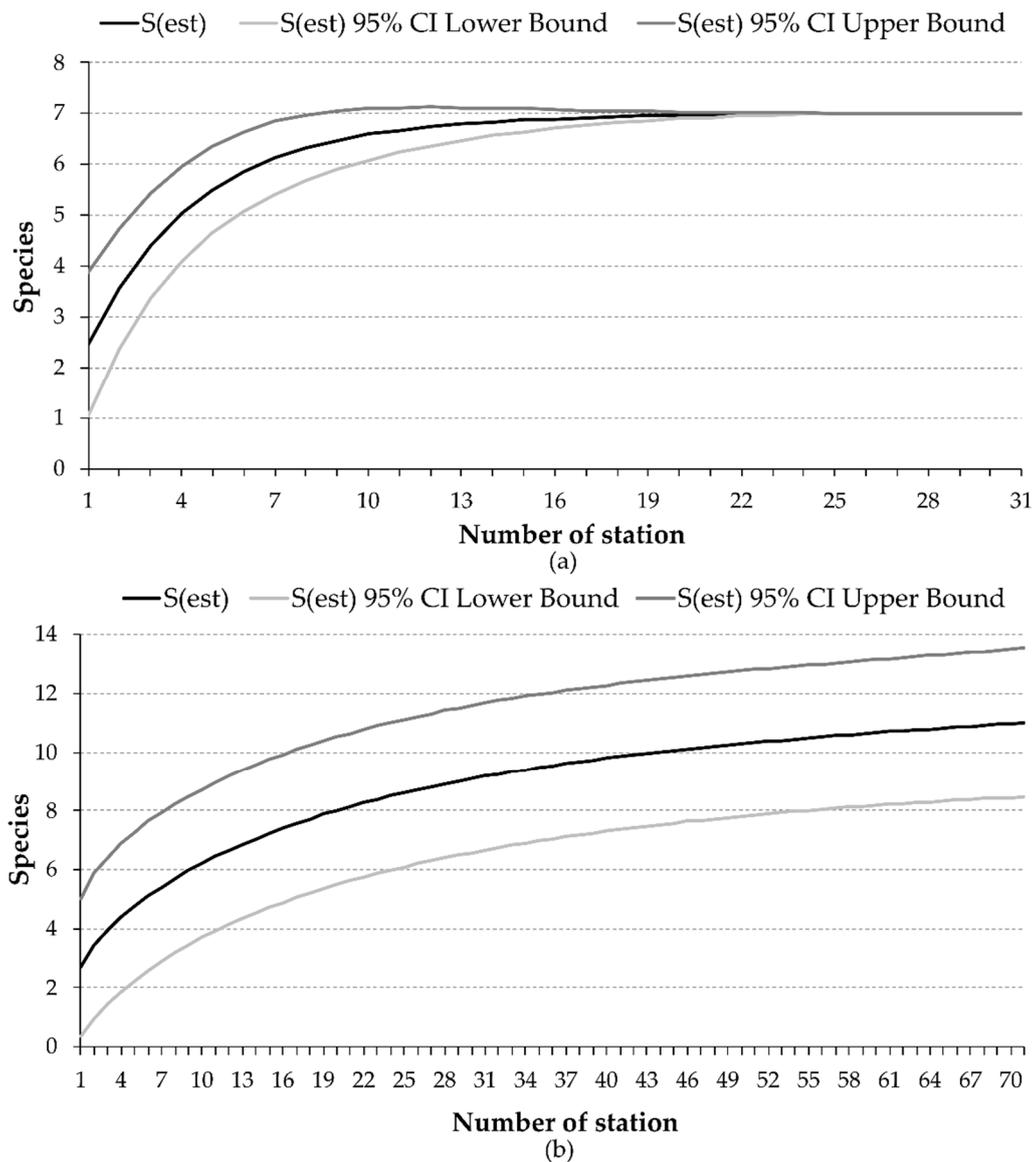


Figure 2. Cumulative curves for total deployments in the southern Adriatic Sea and north-western Ionian Sea using baited lander (a) and longline (b).

In each area, the values of ecological indices provided using the lander were greater than those obtained from longline. For the lander, the values were slightly greater in the southern Adriatic than in the north-western Ionian, while for the longline, it was the opposite (Table 3).

Table 3. Ecological indices computed in the southern Adriatic Sea and north-western Ionian Sea using longline and baited lander.

	Longline		MEMO Lander	
	Southern Adriatic Sea	North-Western Ionian Sea	Southern Adriatic Sea	North-Western Ionian Sea
Shannon–Wiener	0.30	0.48	1.72	1.33
Margalef	0.96	1.08	1.61	1.52
Pielou	0.16	0.23	0.89	0.83

A separation between the species assemblages recorded with longline and MEMO lander, with overlapping areas, was observed using nMDS (Figure 3).

This reflects the pattern showed by both ANOSIM and PERMANOVA, which detected significant differences in the assemblage recorded in the same area using different sampling tools, while no significant differences were detected among different areas explored with the same sampling tool (Table 4).

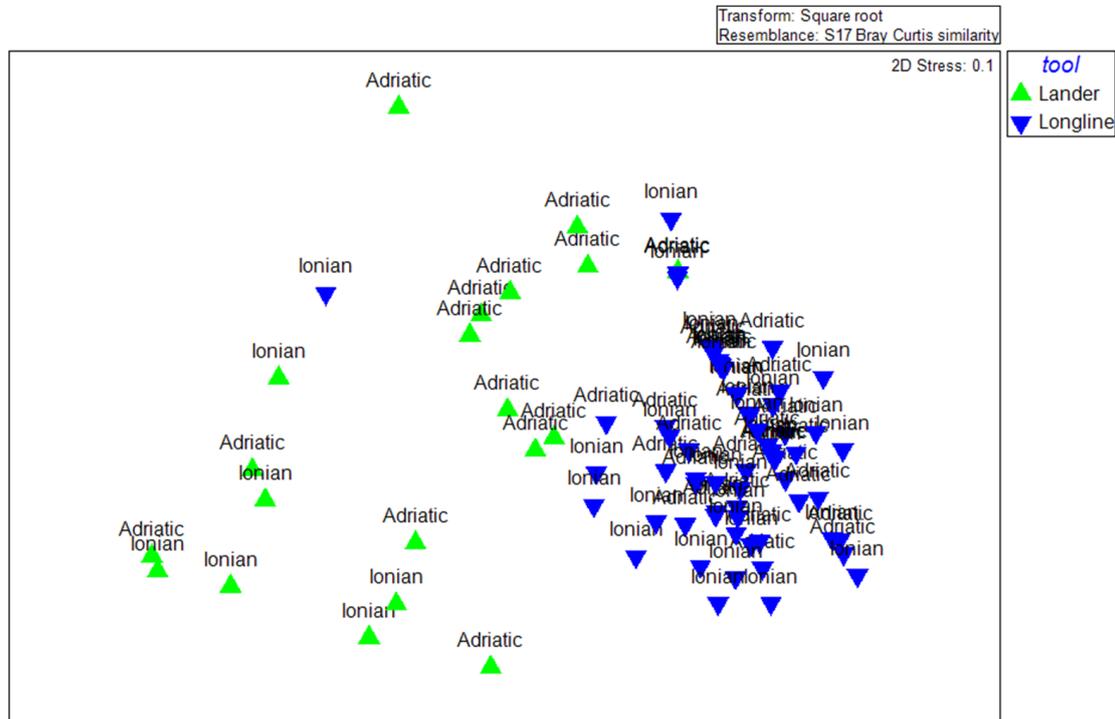


Figure 3. Non-metric multidimensional scaling (nMDS) plot of the abundance distinguishing among the areas and tools.

Table 4. Results of a permutational analysis of variance investigating the differences among the geographic areas and tools examined in this study, based on Bray–Curtis dissimilarity matrices of abundance indices of cartilaginous fish species. The table gives the degrees of freedom (df), the sums of squares (S), the mean squares (MS), the values of the pseudo-F statistics, the p-values, and the number of permutations (perms).

		df	S	MS	Pseudo-F	p-Value	Perms
Tools	Tools	1	54721	54721	37475	0.001	997
	Res	83	1.212 e+5	1460.2			
	Total	84	1.7592 e+5				
Area	Area	1	2334.2	2334.2	11.161	0.328	998
	Res	83	1.7358 e+5	2091.4			
	Total	84	1.7592 e+5				

The SIMPER analysis found that the species *G. melastomus* and *E. spinax* contributed the most to the dissimilarity within groups (Table 5). The INDVal analysis showed that *G. melastomus* is representative for the assemblage sampled in the southern Adriatic and north-western Ionian area using longline, whereas *D. licha* and *H. griseus* characterized the assemblage recorded using baited lander in the former and latter basin, respectively (Figure 4).

Table 5. Results of similarity percentages analysis (SIMPER) of the differences in species composition between areas and sampling tools.

Species	Av. dissim	Contrib. %	Cumulative %
<i>Galeus melastomus</i>	60.14	74.65	74.65
<i>Etmopterus spinax</i>	6.67	8.28	82.93
<i>Hexanchus griseus</i>	3.23	4.01	86.94
<i>Centrophorus granulosus</i>	3.18	3.94	90.88
<i>Dalatias licha</i>	2.89	3.59	94.47
<i>Pteroplatytrygon violacea</i>	1.30	1.61	96.08
<i>Dipturus oxyrinchus</i>	1.05	1.31	97.38
<i>Scyliorhinus canicula</i>	0.89	1.10	98.49
<i>Leucoraja fullonica</i>	0.43	0.53	99.02
<i>Chimaera monstrosa</i>	0.38	0.47	99.49
<i>Somniosus rostratus</i>	0.18	0.22	99.71
<i>Leucoraja circularis</i>	0.12	0.15	99.86
<i>Prionace glauca</i>	0.11	0.14	100.00

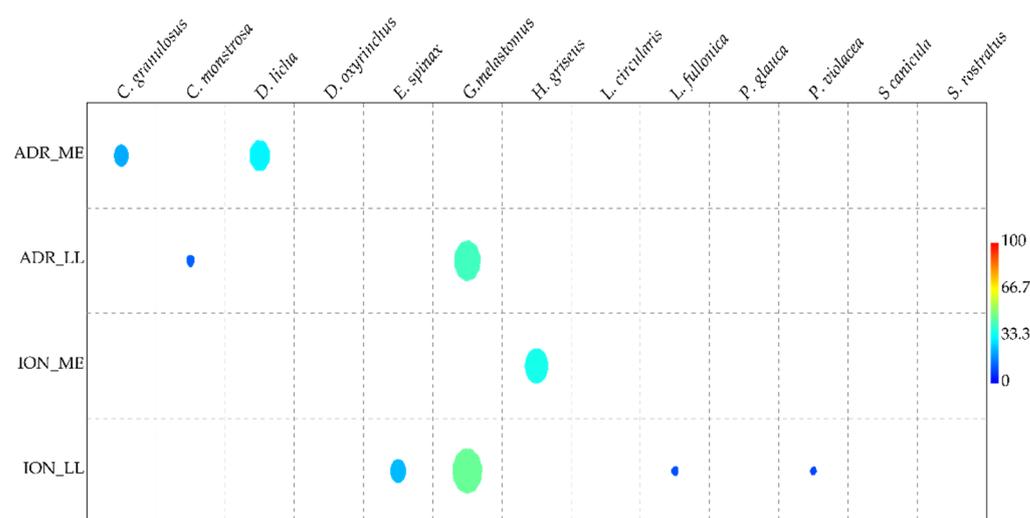


Figure 4. Indicator species analysis (IndVal) plot with representative species of Adriatic (ADR) and Ionian (ION) area and MEMO lander (ME) and longline (LL).

4. Discussion

The simultaneous application of two different low impact sampling tools allowed the collection of new information on abundance and distribution of deep-water cartilaginous fishes living in the southern Adriatic Sea and north-western Ionian Sea.

Using the longline, the most abundant species collected in both areas was *G. melastomus* (Table 2). Data on distribution and abundance of demersal chondrichthyans in the Mediterranean Sea collected during MEDITS bottom trawl surveys [72] during the period 2012–2015 showed that *G. melastomus* and *E. spinax* are the most abundant in both the southern Adriatic Sea and the north-western Ionian Sea [11]. The lower abundance of *G. melastomus* recorded with baited lander could be explained by its high visual acuity and sensibility to the light that can disturb the animal [73]. Using MEMO lander, the most observed species were *D. licha* and *G. melastomus* in the southern Adriatic Sea and *H. griseus* in the north-western Ionian Sea. This shark was never collected using longline in both areas. This can be explained by its ability to break hooks and longline snoods thanks to its body dimension and sharp teeth, as suggested by [29]. As a result, in the case of large bodied species, baited lander can be more efficient than longline in collecting information on abundance and distribution. In the course of MEDITS bottom trawl surveys carried out in the period 2012–2015 in north-western Ionian Sea, *H. griseus* was never sampled by trawl net [11], confirming the importance of the application of alternative low impact sampling

tool. The higher abundance of *D. licha* recorded in the southern Adriatic Sea using baited lander can be explained by its feeding strategy and its limited scavenger behaviour that make this species less attracted by longlines: kitefin shark in fact feeds mainly on teleost fishes and small sharks such as *G. melastomus* and *E. spinax* [74].

In the southern Adriatic Sea, four of the seven collected species were sampled using both sampling tools, whereas in the north-western Ionian Sea, only three species were sampled using both baited lander and longline. ANOSIM and PERMANOVA showed significant differences in the assemblage recorded in the same area using different sampling tool, while no significant differences were detected among the two areas explored with the same method. This might be explained by the different selectivity of the techniques used in this study as well as by the homogeneity of the areas explored with longline and baited lander. The southern Adriatic Sea and north-western Ionian Sea are indeed connected by a complex deep-water circulation flowing along the Apulian margin and are both characterized by the presence of several geomorphological structures [48,49,52,53]. The differences in the species composition and abundance between the two sampling tools might be explained by their different selectivity in relation to species ecology and feeding habits. Moreover, the two techniques also differed in the type and quantity of bait used: each longline had 500 individual baits spreading a broad odour plume, whereas MEMO lander is a single baited system [21,29]. As baited lander is equipped with lights, differences in species composition might be also explained by physiological characteristics of the species involving the visual system.

The samples were sufficient to describe the species distribution sampled with two different tools, although differences in their efficiency have been detected by the cumulative curves. These results suggest good efficiency of baited lander to collect information on species diversity of cartilaginous fishes, adding information on the abundance of species that could not be collected with longline. Given the differences between the two sampling tools in their efficacy and the need for a different sampling effort, baited lander and longline should be used simultaneously to obtain a good level of information about the diversity of cartilaginous fishes with low or even zero impact on threatened species living in fragile habitats.

Baited lander is a low impact non-extractive sampling method for collecting data on fish abundance and its passive nature has become a fundamental aspect when targeting rare and threatened species that can be negatively affected by fishing gears and in the study of sensitive and vulnerable habitats, where other sampling tools would damage the benthic fauna and habitats unacceptably [28,33–36]. The application of baited lander could avoid biases owing to mesh or hook selectivity that affect the conventional extractive sampling method [21,28]. Moreover, videos generated by BRUVSs enable observation of specie behavior and interactions and, although the identification of species can in some cases be doubtful, they represent a permanent record that can be examined and checked by different observers, allowing impartial and repeatable data collection [28,34,38,62].

Experimental longlines with a small number of hooks seem to be the least harmful fishing gear, especially in a structurally complex habitat [38]. This cost-effective and relatively easy-to-use sampling method provides high quality data on species that could not be easily identified on video and allows the collection of tissue sample and the application of conventional and electronic tags [21,27]; moreover, longline can provide information on population biology, diet, reproduction, age, and growth [21–24].

Mediterranean Sea is considered a key hotspot of extinction risk for cartilaginous fish, with highest proportion of threatened species [42–44]. Of the 13 species sampled in this study, only 5 are classified by IUCN as least concern; 2 as near threatened (*Chimaera monstrosa* and *Dipturus oxyrinchus*); 5 as threatened (*Dalatias licha* is classified as Vulnerable and *Prionace glauca*, *Leucoraja circularis*, *Leucoraja fullonica*, and *Centrophorus granulosus* are classified as critically endangered); and 1 species, *Somniosus rostratus*, recorded exclusively with baited lander, is classified as data deficient.

Conservation strategies depend on the available information on their distribution, biology, ecology, and abundance, as well as on the identification and protection of habitats that can act as spawning and nursery area for these species [1,16]. Although *G. melastomus* is a deep-water shark affected by commercial fishing, its higher level of abundance could be due to a deeper distribution than that of fishing and the availability of refuge areas not affected by this activity [75]. This may also be true for *E. spinax*, whose distribution is still deeper [14]. Although bathymetric distribution of *C. granulosus* and *D. licha* is quite comparable to those of *G. melastomus* and *E. spinax* [14], their abundances are lower because they might be more sensitive to fishing activities as top predators [76]. Data from MEDITS bottom trawl surveys carried out in the period 2012–2015 confirm their different abundances. The frequencies of occurrence in both areas were greater than 70% and 60% for *G. melastomus* and *E. spinax*, respectively, whereas they were 11.1% in the southern Adriatic Sea and 7.6% in the north-western Ionian Sea for *D. licha* and less than 1% in both areas for *C. granulosus* [11]. Female of the latter species has low fecundity with only one embryo and a reproductive cycle that could last two years, making this species particularly vulnerable to human impact [77,78]. The highest resilience shown by *G. melastomus* could be explained not only by its wide distribution, but also by its reproductive traits (i.e., egg deposition occurring throughout the year and deposition of multiple egg case) and its feeding strategy [16,75,79,80]. *G. melastomus*, in fact, is an opportunistic meso-predator and scavenger or generalist feeder with a broad spectrum of prey; it is capable of adapting its diet to seasonal fluctuations of prey and taking advantage of discarded material from fishing activities [16,76,80]. During the MEDITS trawl surveys carried out from 2012 to 2015, the frequency of occurrence recorded for *L. circularis* was 8.3% in the southern Adriatic Sea and 1.7% in the north-western Ionian Sea, whereas, in the same period, *L. fullonica* was never collected in both areas [11]. The batoid species, such as *L. circularis* and *L. fullonica*, are particularly vulnerable to bottom trawling as bycatch owing to their strictly benthic habitus. Moreover, the recovery of their population is hindered by the limited capacity of skates to realize seasonal movements owing to their philopatric behaviour [81,82].

When a species has a bathymetric distribution that overlaps with that of fishing, its conservation status also depends on the availability of refuge areas in the same depth range. Cold-water coral areas are characterized by the presence of three-dimensional complex habitats, which represent a refuge for several vertebrate and invertebrate species that use this habitat for shelter, feeding, spawning, and as nursery areas, thus providing an essential fish habitat (EFH) for species harvested by fishing activities carried out on the muddy bottoms surrounding cold-water coral areas [47,51,83].

Cold-water coral habitats are also included on the list of vulnerable marine ecosystems (VMEs) for which conservation constitutes a global priority (FAO 2009). A fisheries restricted area (FRA), in which towed dredges and bottom trawl nets have been prohibited, has been established for the Santa Maria di Leuca cold-water coral province in 2006 [84]. Currently, negotiations are ongoing for the implementation of a FRA in the Bari Canyon system [85] that would ensure a better governance of the Apulian cold-water coral ecosystems and thus a better conservation status of cartilaginous species that use this habitat.

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Institutional Review Board Statement: Ethical review and approval were not necessary for this study because all animals sampled were already dead when arriving on board. Therefore, the scientific activity in the context of this study is not subject to the European Commission recommendations (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010) or to

Italian National Law (Decree Law No. 26 of 4 March 2014) regarding the protection of animals used for scientific experiments.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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