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Biodiversity of Gelatinous Organisms in the Western Adriatic Sea and Identification of Their Echo Traces in Acoustic Data

Andrea De Felice [1,*], Ilaria Biagiotti [1], Giovanni Canduci [1], Ilaria Costantini [1], Antonio Palermino [1], Michele Centurelli [1], Samuele Menicucci [1,2] and Iole Leonori [1]

[1] National Research Council (CNR), IRBIM-Institute for Marine Biological Resources and Biotechnology, Largo Fiera della Pesca, 60125 Ancona, Italy; ilaria.biagiotti@cnr.it (I.B.); giovanni.canduci@cnr.it (G.C.); ilaria.costantini@cnr.it (I.C.); antonio.palermino@irbim.cnr.it (A.P.); michele.centurelli@irbim.cnr.it (M.C.); samuele.menicucci@irbim.cnr.it (S.M.); iole.leonori@cnr.it (I.L.)

[2] Alma Mater Studiorum, Department of Biological, Geological and Environmental Sciences (BiGeA), University of Bologna, Via Zamboni, 33, 40126 Bologna, Italy

* Correspondence: andrea.defelice@cnr.it

Abstract: The abundance of gelatinous organisms, such as salps and jellyfish, in the Adriatic Sea has significantly increased over the past decade. Environmental factors play a key role in driving this shift in abundance through rising temperatures and a consequent decrease in oxygen levels in the water, for which jellyfish have higher tolerance levels. Additionally, fisheries may contribute to the proliferation of jellyfish by diminishing their natural predators and food competitors. Pelagic trawl catch data from 2015 to 2023 acquired during MEDIAS acoustic surveys in the western Adriatic Sea were reviewed to extract information concerning the abundance and distribution of salps and jellyfish. These data were subsequently analyzed and compared with satellite environmental information to identify potential correlations. When considering environmental information related to the month of the survey, the results show two significant relationships: one between the abundance of *Aequorea aequorea* and average salinity and another one between the abundance of *Rhizostoma pulmo* and bottom temperature. Furthermore, when considering environmental data from the month preceding the survey, a relationship between the overall abundance of gelatinous organisms, salps and jellyfish together, and surface temperature was identified. Additionally, an analysis was conducted on specific hauls that almost exclusively yielded jellyfish, with the aim of identifying their echo traces. Although it was not possible to allocate one jellyfish species to a specific echo trace due to the frequent co-occurrence of more than one species, a general indication of typical backscatter for these species, with a higher response at 70 kHz, was consistently observed in all cases examined.

Keywords: jellyfish; salp; Adriatic Sea; acoustic survey; biodiversity

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1. Introduction

In the last few years, an increasing number of gelatinous organisms have been observed in several marine areas [1]. This type of phenomenon does not seem to be a general rule but rather a periodic event [2] and seems to depend on each area's characteristics [1,3]. The surge in jellyfish seems to be concomitant with the rise in sea temperature, which has been particularly pronounced in the past decade. The rise in temperature is often associated with a decrease in the amount of oxygen dissolved in the water, an environment that jellyfish can endure better than other marine organisms [4–6]. Local phytoplankton blooms, which are sometimes associated with pollution, may also favor subsequent anoxia conditions. This can result in abundant increases in salps and jellyfish. In other cases, jellyfish grazing on zooplankton can disrupt the regulation of phytoplankton abundance, leading to anoxia and the settling and decomposition of phytoplankton on the seabed [7,8]. Another factor that may positively influence the abundance of jellyfish is the decrease in their predators, such as turtles, tuna, swordfish, etc. [9,10], or the decrease in food competitors, such as small pelagic fish [9,11]; both events could be a consequence of fishery activities. For their part, jellyfish may regulate the biomass of other marine taxa, for example, small pelagic fish, through the direct predation of their eggs and early life stages [12]. Jellyfish could impact fishery and aquaculture activities, generally in a negative way, causing problems and economic losses [13]. The proliferation of jellyfish in the Adriatic Sea has increased in recent years, considering the higher number of sightings and the biggest jellyfish bycatch reported by fishermen [14,15]. The same evidence can be found by examining the samples caught during the Mediterranean International Acoustic Surveys (MEDIAS) [16] conducted in the western Adriatic Sea by means of a mid-water trawl; the number of individuals and related biomass of jellyfish and salps caught by the net has substantially increased since 2013. The proliferation of these organisms, possibly driven by climatic changes, has spurred several studies dedicated to them, even in the marine acoustics field. Quantifying the abundance levels of jellyfish species is something that could be carried out through acoustic monitoring once certain specific characteristics have been defined, such as the target strength (TS). Given the rise in the importance of jellyfish species in recent years, having an estimate of their biomass at sea could be very useful; moreover, correctly identifying jellyfish echo traces in acoustic data could also contribute to having more precise biomass estimates for other marine organisms, such as small pelagic fish, reducing the risk of erroneous allocations of echo traces. The target strength of several jellyfish species has been measured by several authors: Yoon et al. [17] studied the TS of *Aurelia aurita* and its movement in the water column, comparing the collected acoustic data with video camera images and showing the potential use of the collected information to distinguish this species from other marine organisms. Mutlu studied the same species with an ex situ experiment in the setting of an experimental enclosure [18], estimating the TS at 120 and 200 kHz. Brierley et al. reported the TS measurement results obtained for tethered and free-swimming individuals of *Chrysaora hysoscella* and *Aequorea aequorea* in Namibian waters [19], estimating their TS values at 18, 38, 120, and 200 kHz and finding agreement between ex situ and in situ experimental results. Apart from TS, knowledge of the typical aggregation patterns of jellyfish is fundamental to separating their signals from those of other marine organisms. Unfortunately, the literature on this specific aspect is very scarce. Colombo et al. studied the echo traces produced by gelatinous species [20], specifically considering Cnidaria, Ctenophora, and Salpidae species in the Argentine continental shelf, reporting some features of the observed aggregations.

This article aims to analyze the data generated by MEDIAS acoustic surveys conducted between 2015 and 2023 to investigate the abundance of the main species of salps and jellyfish in relation to environmental factors and identify the most abundant species. Moreover, this study presents an endeavor to acoustically identify *Rhizostoma pulmo*, *Aurelia aurita*, and *Aequorea aequorea* in echograms. This study seeks to characterize the typical range of their acoustic backscatter by selecting hauls that predominantly captured jellyfish species in recent acoustic surveys conducted in the Adriatic Sea.

2. Materials and Methods

Annual biomass estimates of European anchovy (*Engraulis encrasicolus*) and European sardine (*Sardina pilchardus*) are obtained from acoustic surveys conducted in several coastal areas of the Mediterranean Sea within the framework of the MEDIAS program and in accordance with the EU Data Collection Framework [16,21,22]. The data considered in this paper were collected in the western Adriatic Sea, Geographical Subareas (GSAs) 17 and 18 [23], by Italy's CNR IRBIM (formerly ISMAR) of Ancona.

The main frequency of the scientific echosounder in use for biomass calculation was 38 kHz, which is the standard international frequency employed to estimate the biomass of small pelagic fish. Other frequencies (70, 120, and 200 kHz) are used to differentiate between species in the water column by comparing them to corresponding 38 kHz data. Echo trace classification is based on visual scrutinization of the echograms by means of direct allocation and is based on representative fishing stations.

During the acoustic prospection performed at prefixed speeds (generally between 8 and 10 knots) along planned transects, pelagic trawl sampling is carried out to collect representative samples of the fish population, identify fish species composing the pelagic biomass, and infer the size distribution of the fish populations. Haul sampling intensity cannot be predetermined, as it relies on the patterns visualized on the echosounder screen, while also trying to cover different areas and different bathymetries to the fullest extent possible. Vessel speed during pelagic trawl sampling is typically 3.5–4.5 knots. Haul duration in most cases is half an hour; it could be more in case of echo traces of new occurrences. The cod-end of the pelagic net is 18 mm stretched mesh size. The net is cast at the depth of the echo traces to be identified, with the help of the acoustic equipment in use to monitor the net position. In general, the net is cast near the bottom during the day, where most of the fish are located, while it is cast at around 15–25 m depth during the night, following target fish species' movements in the water column. Pelagic trawling is conducted both during the day and at night, and the results can be pooled on the basis of the outcomes of the paper by Machias et al. [24]. The mid-water trawl in use during the survey proved to be a good tool to catch salps and jellyfish together with pelagic fish.

2.1. Echogram Analysis of Jellyfish Monospecific Hauls

The acoustic data acquired from fishing hauls characterized by a very high abundance of jellyfish (more than 80% in weight) were analyzed using Echoview Software (v.12) in the same manner as Palermino et al. [25]. Haul data from 2015 to 2023 were examined in order to verify if they met the above mentioned criterium. The stratum, delimited by net depth at headrope and footrope levels, was considered for the analyses. Background and impulse noises were removed using the Echoview post-processing-specific tools [26]. TS values were extracted from the selected echoes through the "single target detection split-beam Method 2", with the same settings as in [25] except for the target identification threshold that was set to −85 dB, which is a more suitable value given the target species considered in this study [17]. The single target detection parameters are listed in Table 1.

Table 1. Settings installed in Echoview software for the analysis of acoustic data logged during selected hauls through the operator "single target detection split-beam Method 2".

TS threshold	−85 dB
Pulse length determination level	6 dB
Minimum normalized pulse length	0.7
Maximum normalized pulse length	1.5
Two-way maximum beam compensation	4 dB
Maximum standard deviation minor-axis angle	0.6°
Maximum standard deviation major-axis angle	0.6°

We also applied a high-density filter algorithm [27,28], where the detection probability for multiple target echoes was determined from the estimated density of jellyfish. A high-density filter was applied after generating cells with a horizontal size of 5 pings and a vertical size of 1 m in the echograms of each haul [27]. We set the threshold of the total number of jellyfish in the reverberation volume Nv at 0.04 and a threshold of 0.7 for the ratio of multiple echoes M to account for the probability of detecting multiple targets. Single target TS values extracted through this procedure were also used to compute a multi-frequency algorithm on spatial matching criteria [29] without accounting for the target's angular position. The match ping time tool was previously applied to all single targets to implement the method at 38 and 120 kHz frequencies. After that, the target range detected at the two frequencies was the basic information needed to run the Echoview single target intersection operator algorithm, which only keeps single targets from one frequency (38 kHz) within a specified range of any single target detected at the other frequency (120 kHz).

2.2. Abundance of Salps and Jellyfish in Relation to Environmental Parameters

Data pertaining to salps and jellyfish were recorded for each haul during catch sorting in terms of species, whenever possible, and relative total weight. Since these organisms represent bycatch in MEDIAS surveys targeting small pelagic fish, the acquired information is not very detailed and has only been consistently recorded since 2015. In any case, this information was considered useful to analyze whether specific environmental conditions promote or inhibit the proliferation of salps and jellyfish. To achieve this objective, environmental data from the Copernicus website https://marine.copernicus.eu/ (accessed on 28 September 2023) were downloaded to be analyzed jointly with the abundance data for gelatinous organisms.

For the period between May 2015 and March 2021, daily temperature and salinity data were acquired from the Med MFC physical multiyear product that is generated by a numerical system composed of a hydrodynamic model. The model's horizontal grid resolution is 1/24° (ca. 4 km), and the unevenly spaced vertical levels are 141. For the period between March 2021 and August 2023, data were acquired from the physical component of the Mediterranean Forecasting System (Med-Physics), which is a coupled hydrodynamic-wave model implemented across the whole Mediterranean Basin, including tides. The models' horizontal grid resolution is 1/24° (ca. 4 km) and it has 141 unevenly spaced vertical levels. Three different types of temperature were downloaded: temperature at the bottom of the sea, temperature vertical profile from 0 to 200 m, and surface temperature. Salinity was downloaded as a vertical profile from 0 to 200 m.

The data on a-chlorophyll concentration and dissolved oxygen concentration between May 2015 and December 2019 were obtained from the Mediterranean Sea biogeochemical reanalysis at 1/24° of horizontal resolution (ca. 4 km) produced by means of the MedBFM3 model system. Daily data from January 2020 to August 2023 were acquired from the biogeochemical analysis and forecasts for the Mediterranean Sea at 1/24° of horizontal resolution (ca. 4 km); these data are produced by means of the MedBFM4 model system.

Geographical boundaries ranged from latitude 40.5° N to 46° N and from longitude 12° E to 20° E, practically encompassing the whole Adriatic Sea; environmental data in netCDF format were extracted and successively cropped into a polygon corresponding to the area being surveyed by MEDIAS in the western Adriatic Sea (GSAs 17 and 18). The processing of environmental data was conducted using the statistical software R version 4.3.3. [30], and the primary packages employed were specifically "ncdf4" [31] and "raster" [32].

Monthly means for the selected environmental parameters were calculated from the cropped dataset using R. The biomass of salps and jellyfish (in kg) registered during the acoustic surveys was compared with monthly means of environmental parameters using distance-based linear model (DISTLM) analysis [33,34]. For this purpose, data were transformed to ln(x + 1), and the analysis was based on Euclidean distances. The results of this analysis determined the suite of environmental variables that describe significant and independent proportions of variations in the salps and jellyfish biomass. Environmental variables that were strongly correlated ($r > 0.8$) were removed prior to the analysis. Since temperature was the most represented information in the dataset and was presented in three ways, exclusion was primarily based on one or two temperature variables whenever possible.

3. Results

3.1. Jellyfish Echo Trace Identification

Three case studies were selected from the hauls carried out during MEDIAS acoustic surveys in 2022 and 2023, since the occurrence of monospecific hauls for jellyfish or salps has become increasingly frequent in recent years. The small number of case studies is due to the fact that the hauls selected had to be highly monospecific for jellyfish species, while most MEDIAS surveys target small pelagic fish. There were no hauls organized specifically to catch jellyfish. The geographical positions of the selected hauls are provided in Figure 1.

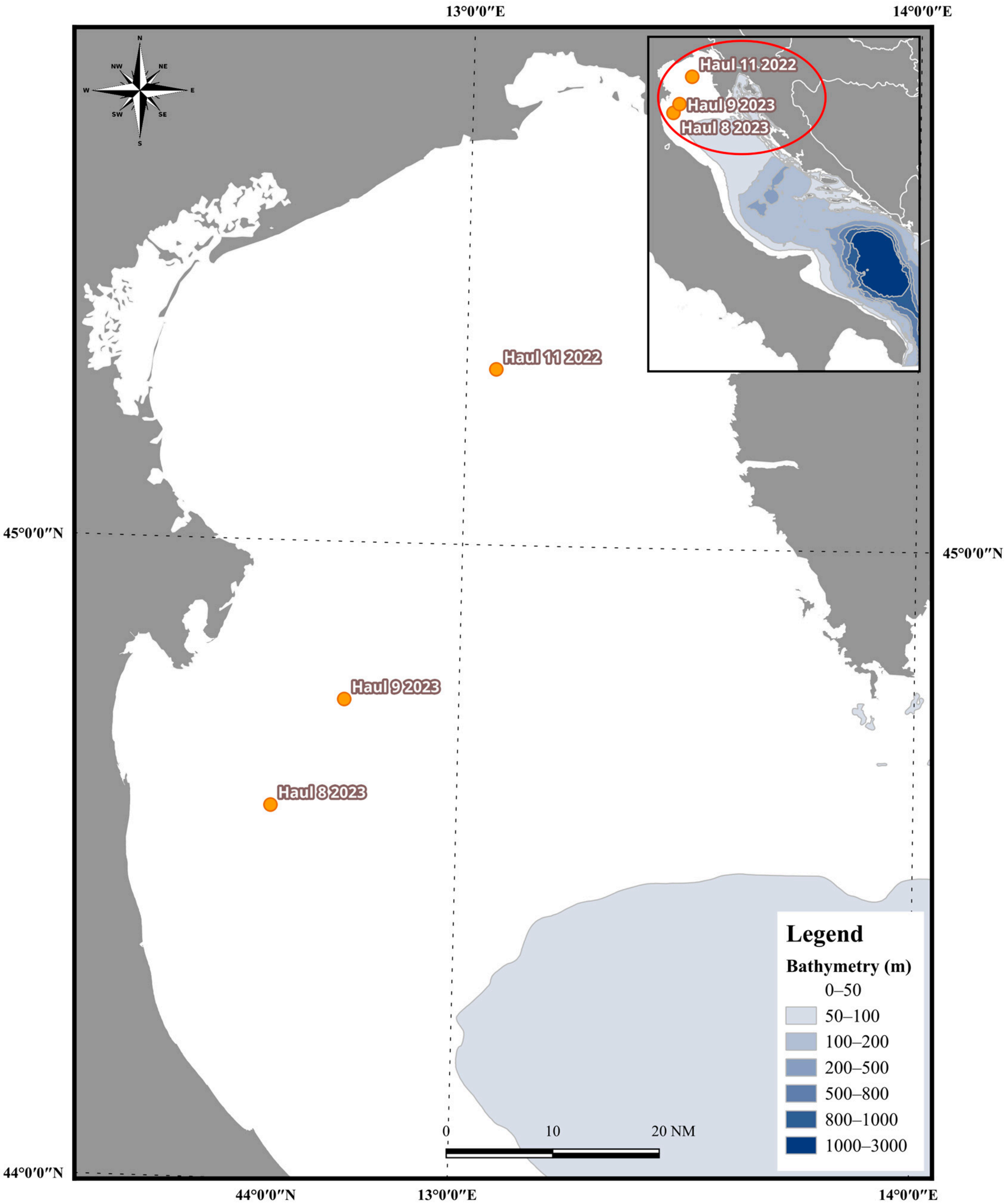

Figure 1. Geographical positions of the hauls, carried out in northern Adriatic Sea, catching more than 80% of jellyfish species in weight, and selected to identify typical jellyfish echo traces.

Haul No. 11 (20 June 2022)

This haul was conducted during the day, from 10:07 to 10:28. Echograms recorded at 38, 70, 120, and 200 kHz during the sampling are reported in Figure 2.

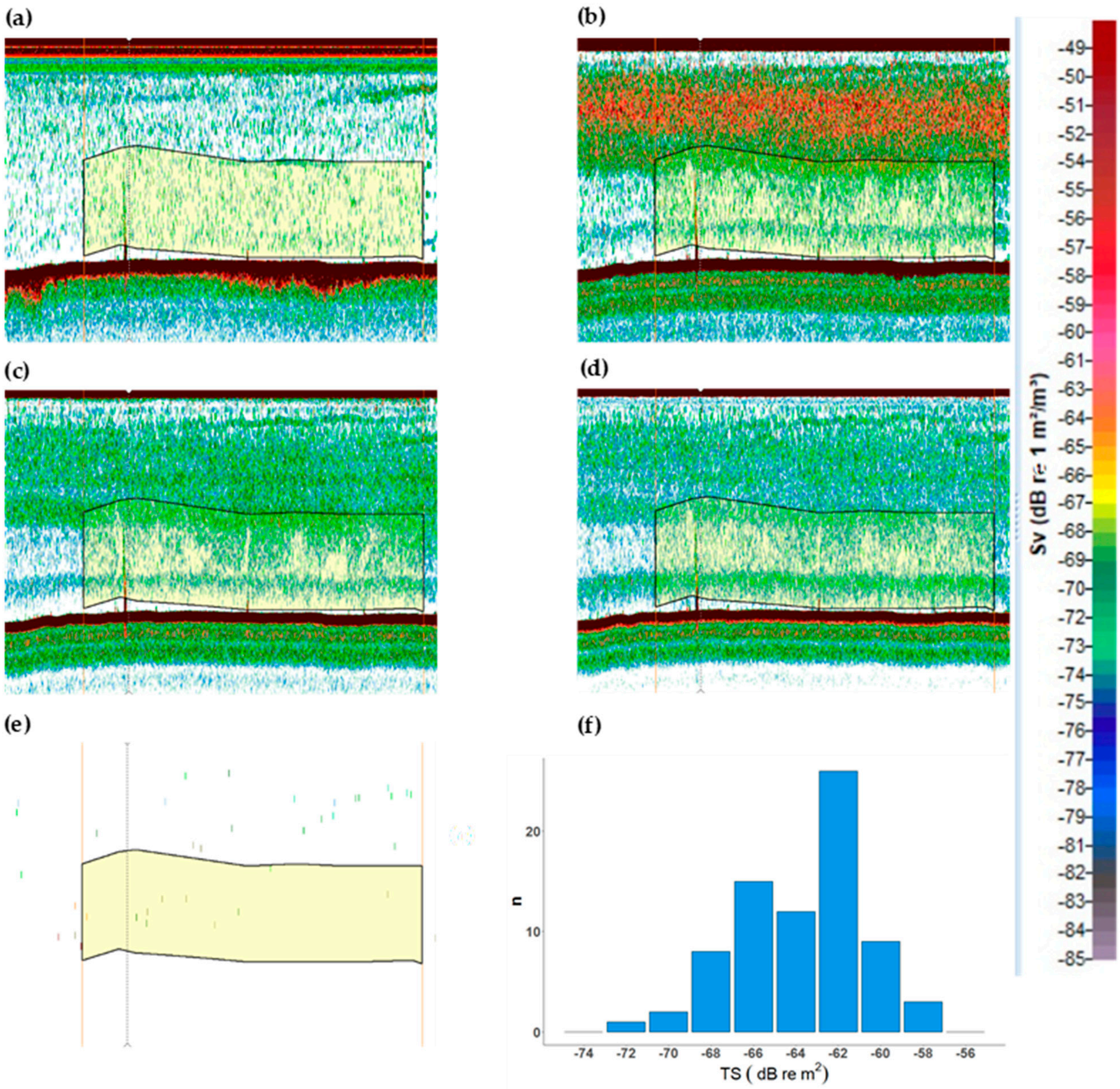

Figure 2. Echograms at four frequencies during haul no. 11 of the MEDIAS 2022 acoustic survey: (**a**) 38 kHz, (**b**) 70 kHz, (**c**) 120 kHz, and (**d**) 200 kHz; (**e**) single target echogram showing all the targets identified through the operator "single target detection split-beam Method 2" with the fishing stratum highlighted in yellow; (**f**) histogram of the single targets in the fishing stratum. The scale of the echograms was adjusted to display the entire trawling operation. Echogram color bar reference is reported on the right side of the figure.

Table 2 depicts the weight of each species in grams and as a percentage relative to haul no. 11.

Table 2. Composition by species in terms of weight and as a percentage of the overall catch weight for haul no. 11 carried out during 2022 MEDIAS survey.

Species	Total Catch (g)	Total Catch %
Rhizostoma pulmo (jellyfish)	9500	56.63%
Aequorea aequorea (jellyfish)	6900	41.13%
Chrysaora hysoscella (jellyfish)	348	2.07%
Merlangius merlangus (fish)	22	0.13%
Loligo vulgaris (squid)	7	0.04%
Total	16,776	100.00%

This haul is characterized by the almost exclusive presence of gelatinous organisms, mostly *R. pulmo* and *A. aequorea*. The echograms of this haul present two distinct layers at two different depths, but they are almost entirely invisible at 38 kHz. The shallower layer was partially affected by fishing operations, while the deeper layer was wholly within the fishing stratum. However, it was possible to conclude that fishing activities were being conducted in both strata. An interesting element was that the shallower layer reflected a higher acoustic energy at 70 kHz compared to the other frequencies, while the deeper one had the highest response at 200 kHz. *R. pulmo* individuals were visually observed at the surface, indicating their definitive presence in the first few meters of the water column. The average TS registered was −63.7 dB re 1 m^2, with a standard deviation of 39.0; the TS range was from −72 to −58 dB re 1 m^2.

Haul No. 8 (4 June 2023)

This haul was conducted during the day, from 8:58 to 9:30. Echograms recorded at 38, 70, 120, and 200 kHz during the sampling are reported in Figure 3.

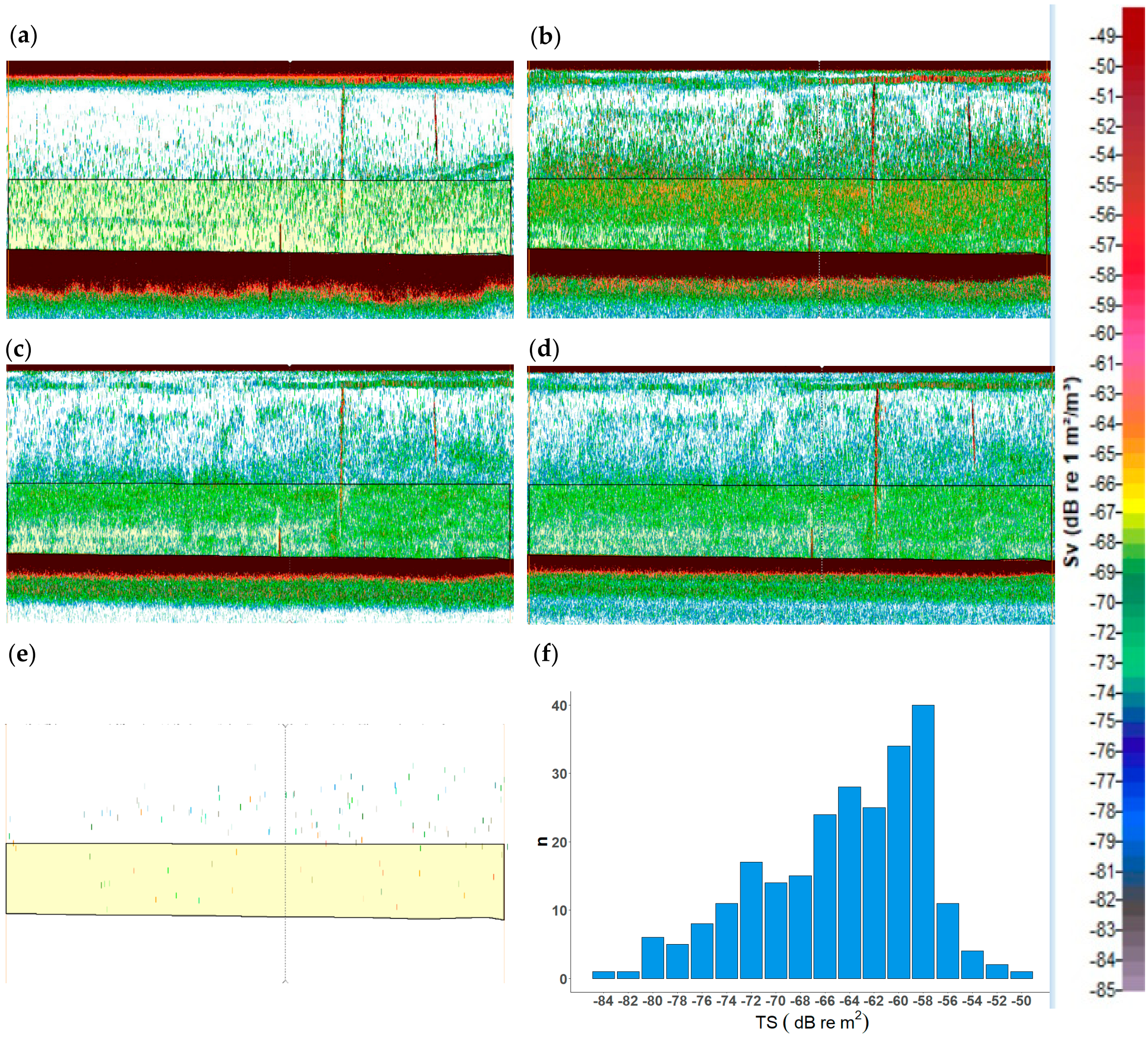

Figure 3. Echograms at four frequencies during haul no. 8 of the MEDIAS 2023 acoustic survey: (**a**) 38 kHz, (**b**) 70 kHz, (**c**) 120 kHz, and (**d**) 200 kHz; (**e**) single target echogram showing all the targets identified through the operator "single target detection split-beam Method 2" with the fishing stratum highlighted in yellow; (**f**) histogram of the single targets in the fishing stratum. The scale of the echograms was adjusted to display the entire trawling operation. Echogram color bar reference is reported on the right side of the figure.

Table 3 provides the results of the fishing operations, presenting weight and weight percentages by species.

Table 3. Composition by species in terms of weight and as a percentage of the overall catch weight for haul no. 8 carried out during 2023 MEDIAS survey.

Species	Total Catch (g)	Total Catch %
Alloteuthis media (squid)	6	0.01%
Trachurus mediterraneus (fish)	38	0.05%
Merlangius merlangus (fish)	34	0.04%
Engraulis encrasicolus (fish)	1982	2.42%
Salpa maxima (salp)	616	0.75%
Aequorea aequorea (jellyfish)	13,640	16.65%
Sprattus sprattus (fish)	87	0.11%
Aurelia aurita (jellyfish)	65,031	79.39%
Chrysaora hysoscella (jellyfish)	480	0.59%
Total	81,914	100.00%

This haul is characterized by a high prevalence of gelatinous species, with *Aurelia aurita* accounting for almost 80% of the weight and *Aequorea aequorea* comprising around 17%, accompanied by a minor presence of fish, squids, salps, and other jellyfish species. Fish echo traces are distinctly identifiable in echograms as very dense aggregations (represented in red). The rest are distributed in a particularly dense cloud form at 70 kHz and, to a lesser extent, at 120 and 200 kHz, while they are not so evident at 38 kHz. The most abundant jellyfish species in this haul, *A. aurita* and *A. aequorea*, should mostly comprise this layer, but it is very difficult to distinguish their specific echo traces. The recorded TS values vary over a wide range, suggesting that, even if a large part of the catch consisted of *A. aurita*, the presence of other species is not negligible.

Both the surface layer and mid-water layer are particularly reflective at 70 kHz, confirming the behavior previously observed in haul no. 11 of 2022. Our target jellyfish species probably constitute a relevant portion of the mid-water layers in both hauls, while other scattering layers, weaker at 70 kHz, may be characterized by other planktonic organisms.

The average TS registered was −64.6 dB re 1 m^2, with a standard deviation of 42.4; the TS range was from −84 to −50 dB re 1 m^2.

Haul No. 9 (4 June 2023)

This haul was conducted during the night, from 21:43 to 22:15. Echograms recorded at 38, 70, 120, and 200 kHz during the sampling are reported in Figure 4.

Table 4 depicts the weight of each species in grams and as a percentage relative to haul no. 9.

Table 4. Composition by species in terms of weight and as a percentage of the overall catch weight for haul no. 9 carried out during 2023 MEDIAS survey.

Species	Total Catch (g)	Total Catch %
Alloteuthis media (squid)	48	0.30%
Aphia minuta (fish)	1145	7.22%
Merlangius merlangus (fish)	14	0.09%
Engraulis encrasicolus (fish)	641	4.04%
Aequorea aequorea (jellyfish)	13,300	83.86%
Sprattus sprattus (fish)	159	1.00%
Aurelia aurita (jellyfish)	537	3.38%
Sepiola sp (cuttlefish)	13	0.08%
Lesueurigobius friesii (fish)	2	0.01%
Total	15,859	100.00%

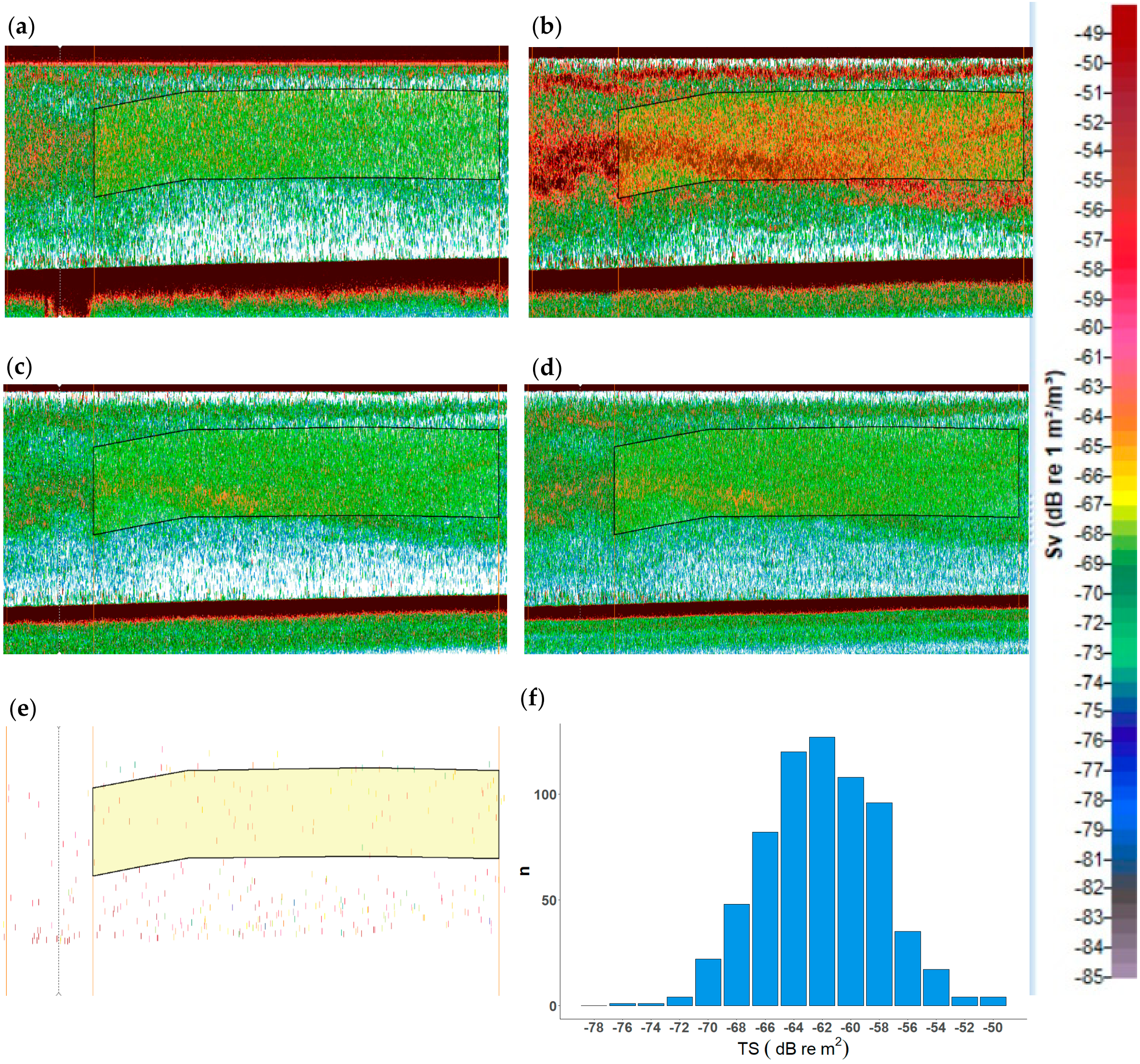

Figure 4. Echograms at four frequencies during haul no. 9 of the MEDIAS 2023 acoustic survey: (**a**) 38 kHz, (**b**) 70 kHz, (**c**) 120 kHz, and (**d**) 200 kHz; (**e**) single target echogram showing all the targets identified through the operator "single target detection split-beam Method 2" with the fishing stratum highlighted in yellow; (**f**) histogram of the single targets in the fishing stratum. The scale of the echograms was adjusted to display the entire trawling operation. Echogram color bar reference is reported on the right side of the figure.

This haul is mainly dominated by the species *A. Aequorea*, accounting for 83.9% of the total catch weight, while *A. aurita* represents just 3.4%. The rest of the catch is mainly composed of transparent goby (*Aphia minuta*), anchovy, and sprat. In this case, since we were fishing at night, fish and other marine organisms tend to form diffuse layers, and, as observed in the other hauls, it is not easy to differentiate between them. In any case, the layer in the fishing stratum, as evidenced by the yellow background, should be characterized by a strong presence of *A. aequorea* and *A. minuta*. The latter species should produce strong echoes particularly at the 38 [35] and 70 kHz frequencies, given the presence of a gaseous bubble in their body. The contribution of *A. minuta* to the catch may seem low due to its small dimensions, but it is meaningful in terms of the number of individuals. Another rather dense layer is visible above the fishing stratum at approximately 7–10 m. This aggregation could consist of *A. aurita*. In fact, its presence in the catch is low and may have been an accidental capture in this case. There is a short geographical distance between the two selected hauls conducted in 2023, but they were carried out under different light conditions. From an analysis of the results, it seems that the *A. aurita* is following circadian day–night cycles, positioning itself very close to the surface at night, while *A. aequorea* seems to be found a little bit deeper. The average TS recorded was −62.1 dB re 1 m^2, with a standard deviation of 65.9; the TS range was from −76 to −50 dB re 1 m^2.

3.2. Abundance of Salps and Jellyfish in Relation to Environmental Parameters

The first type of analysis performed to compare salps and jellyfish abundance data from the survey and environmental parameters was carried out considering the average values of the latter parameters for the survey month. Data on the abundance of salps and jellyfish were divided by species plus two more categories: total gelatinous organisms and unidentified jellyfish. Salps were considered collectively due to the absence of specific details about the species level in the records, even though the two most abundant species in the catch were *Salpa maxima* and *Thalia democratica*. Data were ln(x + 1) transformed, and Euclidean distance analysis was chosen. Based on the survey's monthly average values, *A. aequorea* and *R. pulmo* showed significant relationships, respectively, with salinity (explained proportion of 0.58) and bottom temperature (explained proportion of 0.47) (Tables 5 and 6). In this case, surface temperature data and dissolved oxygen data were excluded due to autocorrelation issues.

Table 5. DISTLM results for *Aequorea aequorea* with averaged environmental parameters of the survey month, excluding surface temperature and dissolved oxygen data due to an autocorrelation issue. Variables are listed in order of importance. Values in bold indicate significance ($p < 0.05$). SS = sum of squares; pseudo-F = pseudo-Fisher ratio; prop = explained proportion.

Conditional (Sequential) Tests					
Variable	**SS (Trace)**	**Pseudo-F**	***p***	**Prop**	**Cumulative**
Smean (psu)	10.7062	9.5938	0.0180	0.5782	0.5782
Tmean (°C)	1.5752	1.5155	0.2788	0.0851	0.6632
Chlmean (mg/m^3)	0.6626	0.5944	0.4618	0.0358	0.6990
BottomT (°C)	0.6667	0.5434	0.4966	0.0360	0.7350

BottomT = temperature at the bottom; Tmean = average temperature value in the water column; Smean = average salinity value in the water column; Chlmean = average chlorophyll concentration value in the water column.

Table 6. DISTLM results for *Rhizostoma pulmo* with averaged environmental parameters of the survey month, excluding surface temperature and dissolved oxygen data due to an autocorrelation issue. Variables are listed in order of importance. Values in bold indicate significance ($p < 0.05$). SS = sum of squares; pseudo-F = pseudo-Fisher ratio; prop = explained proportion.

Conditional (Sequential) Tests					
Variable	**SS (Trace)**	**Pseudo-F**	***p***	**Prop**	**Cumulative**
BottomT (°C)	11.6175	6.1320	0.0398	0.4670	0.4670
Chlmean (mg/m^3)	2.1992	1.1927	0.3696	0.0884	0.5553
Tmean (°C)	0.4699	0.2218	0.5804	0.0189	0.5742
Smean (psu)	0.0616	0.0234	0.8978	0.0025	0.5767

BottomT = temperature at the bottom; Tmean = average temperature value in the water column; Smean = average salinity value in the water column; Chlmean = average chlorophyll concentration value in the water column.

A second analysis considered average values from the previous month with respect to survey execution. In this case, only the aggregate gelatinous organisms resulted in an almost significant correlation with surface temperature (explained proportion of 0.42); bottom temperature and average temperature in the water column (0–200 m) were discarded due to autocorrelation issues (Table 7).

Table 7. DISTLM results for the aggregate gelatinous organisms, using averaged environmental parameters from the month prior to the survey. The data for bottom temperature and mean temperature in the water column (0–200 m) were excluded due to autocorrelation issues. Variables are listed in order of importance. Values in bold indicate significance ($p < 0.05$). SS = sum of squares; pseudo-F = pseudo-Fisher ratio, prop = explained proportion.

Conditional (Sequential) Tests					
Variable	**SS (Trace)**	**Pseudo-F**	***p***	**Prop**	**Cumulative**
Tsup (°C)	3.6761	5.1232	0.0564	0.4226	0.4226
Smean (psu)	1.9794	3.9025	0.1036	0.2276	0.6501
Oxmean (mmol/m^3)	0.2726	0.4920	0.5236	0.0313	0.6815
Chlmean (mg/m^3)	0.2884	0.4648	0.5408	0.0332	0.7146

Tsup = sea surface temperature; Smean = average salinity value in the water column; Chlmean = average chlorophyll concentration value in the water column; Oxmean = average dissolved oxygen value in the water column.

4. Discussion

The analysis of the acoustic data acquired during monospecific hauls for jellyfish has shown that it is challenging to identify a typical aggregation pattern for each represented species, given that the general appearance of these organisms in echograms is a diffuse pelagic layer in each case. A common characteristic across all three of the selected hauls is a stronger backscatter at 70 kHz that could be a distinctive aspect of the acoustic data pertaining to jellyfish; to our knowledge, previous studies on jellyfish species cannot confirm this, since data at 70 kHz were not collected [17–19,36], at least for the species considered in this paper. In any case, more data are needed to confirm this preliminary finding. The average response at 38 kHz, in relation to the TS of the most abundant jellyfish species found in the analyzed hauls, was prevalently concentrated around the interval of −70 to −60 dB re 1 m^2. These results are similar to those reported in previous studies [17–19,36] on *Aurelia aurita*, *Chrysaora hysoscella*, and *Aequorea aequorea*, with some differences that may be attributed to the average size, swimming trajectory, and the co-occurrence of other targets. Brierley et al. [37] reported higher values for *C. hysoscella* in comparison to our data, mainly because the sampled individuals were significantly larger. However, the data for the considerably smaller *A. aequorea* were once again within the range observed in our study. Moreover, the authors demonstrated that no evident acoustic patterns are visible on echograms where a monospecific catch of *A. aequorea* has been obtained, highlighting the difficulty in the identification of specific echo traces for this species. However, in the Argentine continental shelf [20], *A. aequorea* individuals were detected forming sound-scattering layers either on the bottom or in the water column, depending on the time of day. The behavior by *A. aequorea* in the Argentine continental shelf seems similar to what we have found in our monospecific hauls, were the largest amount of individuals of this species was found during the night time haul, while during the day their abundance level was much lower, probably because individuals were concentrated near the bottom. This suggests that this species could present different aggregation patterns depending on the area and sampling time. In summary, it is difficult to precisely identify typical aggregation patterns for gelatinous organisms in the water column, at least at the species level. The analysis of TS values recorded in conjunction with a monospecific haul may be of assistance; in any case, it is important to consider the relative scarcity of such hauls and the variability of TS caused by many factors, including the characteristic movement of jellyfish in the water, which can contribute to increased variability and should be accurately modeled [38].

Our analyses yielded some interesting results in terms of the possible influence of environmental parameters on jellyfish. *A. aequorea* exhibited a significant relationship with salinity; a similar result was also found by [39], who discovered that preferential ranges of bottom temperature (8–20 °C) and bottom salinity (30.3–34.6 psu, with a peak at 33) facilitated the proliferation of these organisms. *Aequorea* spp. also exhibited a positive association with temperature and salinity in generalized additive models in the California Current [40], even though other parameters correlated with *Aequorea* spp. abundance as well, i.e., latitude, distance from shore and chlorophyll a, confirming the hypothesized connections between jellyfish populations and regional climate conditions. Other examples of the correlation between salinity and abundance of jellyfish species, even if not attributed specifically to *Aequorea* genus, could be found in Heim-Ballew and Olsen [41], where salinity and temperature were found to be the most influential factors for the occurrence of some Scyphozoan jellyfish species, with abundance peaks at high estuarine salinities for *A. aurita*, but different tolerance ranges for each of the studied species. Other scientists found that temperature and salinity have an important role in the seasonal patterns of *A. aurita* in the Yellow Sea [42], by regulating strobilation; these two abiotic factors, together with substrate and prey availability, have strong effects on abundance fluctuations, leading to jellyfish blooms in certain years. Moreover, it should be taken into account that jellyfish proliferation, especially in terms of asexual reproduction, seems to be sensitive to temperature and salinity conditions and that even a quite strongly related species could behave differently in respect to the influence of these abiotic factors [43]. Generally speaking, the effect of salinity, along with other factors such as light, on the asexual reproduction of jellyfish seems to be relevant [44].

The relationship found between the abundance of *R. pulmo* and bottom temperature can be more easily explained since a greater amount of research has been dedicated to investigating this parameter's influence on jellyfish compared to other factors. Leoni et al. [45] found different kinds of interactions in three Mediterranean lagoons, with optimal temperature and salinity ranges varying among different developmental stages from ephyra to adulthood. Some differences in this aspect were also observed across the studied areas. The authors also reported an ideal temperature range of 15–22 °C for *R. pulmo* in the Mediterranean, which is compatible with the temperatures found in the water column in late spring–early summer during the MEDIAS acoustic surveys in the western Adriatic Sea. Jellyfish face significant challenges in surviving at temperatures below 15 °C. However, climate change may alleviate this issue by potentially extending the duration of their life cycle throughout the year, particularly during the winter season. Ideal temperature conditions seem to also vary by species, as illustrated in Purcell et al. [46]. The authors reported that the survival and strobilation processes with the subsequent production of *ephyrae* of *A. aurita*, *R. pulmo*, and *C. tuberculata* are affected differently by temperatures. This may explain why there are distinct peaks in their abundance, starting with *A. aurita* in April–May, followed by *R. pulmo*, and finally *C. tuberculata* in mid-summer. The MEDIAS survey biological samplings also yield similar findings, wherein *R. pulmo*, *A. aurita*, and *A. aequorea* are commonly found in discrete quantities in the northern part of the study area. *C. tuberculata* was sighted many times during the surveys but has never been caught. Its absence in our trawl samples could be due to its distribution in the water column. Based on the environmental data from the month before the survey was conducted, the only association that was found to be almost significant was related to the whole group of gelatinous organisms, salps and jellyfish together. This result aligns with previous results obtained from surveys on environmental information collected over the course of a month and emphasizes the importance of temperature for the survival and proliferation of these organisms [39,47,48], as well as the relatively high tolerance of jellyfish to withstand rising temperatures [41]. However, jellyfish seem to respond quite quickly to environmental changes, potentially making it difficult to establish connections using environmental data that has been shifted by one month [49,50].

All the above examples demonstrate that temperature and salinity could exert an influence on the jellyfish life cycle, which may vary depending on the species, developmental stage, and area.

5. Conclusions

Collecting and reporting information on jellyfish species has become increasingly crucial, particularly in recent years, due to the increased collateral impacts of global warming on the world's oceans. Although the data gathered during the MEDIAS acoustic surveys for salps and jellyfish species lack sufficient detail, as these organisms were not the target species, these data still provided valuable insights into the environmental factors that potentially influence the abundance of gelatinous organisms in the Adriatic Sea. In general, these findings are consistent with the situations observed in other basins. As regards the identification of distinct acoustic echo traces in echograms, it is currently difficult to allocate a single species to a specific echo trace with a high degree of certainty. This difficulty arises from the frequent occurrence of mixed species, with at least two jellyfish types often intermingling with fish and other targets. Nevertheless, a consistent trait that has been observed is that the highest acoustic response occurs at 70 kHz in the presence of salps and jellyfish, as opposed to other frequencies. This recurrent trait could be a specific characteristic of gelatinous organisms that could help in their identification and isolation in echograms with the aim of either discarding their echo traces or purposely retaining their acoustic density and converting it to estimate the biomass using acoustic methodology.

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Institutional Review Board Statement: This study was exempted from ethical review and approval since the collection of fish specimens was authorized by the MEDIAS project as part of annual research surveys, which all involve lethal sampling. Our procedures did not involve any form of animal experimentation. The care and use of collected animals complied with animal welfare guidelines, laws, and regulations set by the Italian government.

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