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Acoustic correction factor estimate for compensating the vertical diel migration of small pelagic species

Angelo BONANNO[1], Marco BARRA[2], Andrea De FELICE[3], Marianna GIANNOULAKI[4], Magdalena IGLESIAS[5], Iole LEONORI[3], Ana VENTERO[5], Salvatore ARONICA[1], Ilaria BIAGIOTTI[3], Vjekoslav TIČINA[6], Giovanni CANDUCI[3] and Simona GENOVESE[1]

[1] National Research Council (CNR), Institute for the study of the anthropic impacts and sustainability in the marine environment (IAS) , SS Capo Granitola, Campobello di Mazara, TP, Italy
[2] National Research Council (CNR), Institute of Marine Sciences, SS Naples, Italy
[3] National Research Council, Institute for Marine Biological Resources and Biotechnologies, SS Ancona, Italy
[4] Hellenic Centre for Marine Research (HCMR), Institute of Marine Biological Resources and Inland Waters, Iraklion, Greece
[5] Instituto Español de Oceanografía. Centre Oceanogràfic de Balears. Palma de Mallorca, Spain
[6] Institute of Oceanography and Fisheries, Split, Croatia

Corresponding author: Marco BARRA marco.barra@cnr.it
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Abstract

Differences in acoustic estimates of small pelagic fish biomass, due to data acquisition during daytime and night-time surveys, have been recognized as a problem in acoustic surveys for many years. In the absence of a single rule for all species and for all locations, some expert groups have identified specific time intervals for acoustic data acquisition in relation to the schooling behavior of the target species. In the Mediterranean Sea, the research groups working in the MEDIAS (Mediterranean International Acoustic Survey) agreed on the necessity of acoustic sampling being conducted only during daytime. Only when available time does not permit to complete the survey during daytime, data collection might be extended. In this case, working on data collected during both daytime and night-time, a bias may occur in the biomass estimates. To evaluate and correct such bias, specific experiments were performed in some geographical subareas of the Mediterranean Sea. The data analysis allowed the estimation of a mean correction factor for the Strait of Sicily, where five surveys were carried out in different years. The correction factor was also estimated for the Adriatic Sea, Tyrrhenian Sea and northern Spain; the observed variability among areas highlighted the importance of the spatial and temporal coverage of the survey area to obtain reliable estimates of the correction factor. Further studies are necessary to improve the interpretation of the obtained estimates in relation to area-related peculiarities such as zooplankton composition and abundance along with small pelagic fish community structure.

Keywords: Day-night acoustic comparison; small pelagic species; Mediterranean Sea; MEDIAS.

Introduction

Acoustic data acquisition during daytime or night-time surveys is known to produce differences in acoustic estimates of small pelagic fish biomass (e.g., Rose, 1992; Fréon *et al.*, 1993a; Aglen, 1994; Domínguez-Contreras *et al.*, 2012). Changes in target strength due to the diel vertical migration of fish (Hjellvik *et al.*, 2004; Knudsen *et al.*, 2009), dead zone effects near the seabed (Ona & Mitson, 1996), acoustic shadowing through signal attenuation (Zhao & Ona, 2003; Hanchet *et al.*, 2000), lateral avoidance of the research vessel (Fréon *et al.*, 1993a; Hjellvik *et al.*, 2008) and changes in density distribution of the target species (Aglen, 1994; Vaz Velho *et al.*, 2010) are among the possible reasons causing such differences. The magnitude and sign of the day-night differences can be significant depending on the fish species and the area. For example, Godlewska (2002) observed that the difference between day and night estimates depended on the seasonal changes in food availability in the Dobczyve Reservoir. In addition to vertical migration, the authors observed also horizontal migrations, which led to higher night estimates than day estimates. Hydroacoustic surveys, performed in the Lake Laojianghe (China), showed that the average biomass estimate from the daytime transects was nearly 60% lower than that from the corresponding night transects (Ye *et al.*, 2013). Appenzeller & Leggett (1992) working on lacustrine pelagic fish abundance in Quebec, found significantly lower acoustic estimates during the day than at night, attributing the difference to acoustic shadowing. In contrast, higher acoustic relative densities were obtained for Cunene horse mackerel (*Trachurus trecae*) off Angola by day than by night (Vaz Velho *et al.*, 2010). The authors argued that these differences were likely caused by changes in packing density, or TS (Target Strength) between day and night, or a combination of both. Also for the Atlantic cod, higher abundance estimates were obtained during daytime surveys (Lawson & Rose, 1999; Hjellvik *et al.*, 2004). Fréon *et al.* (1993a) working on different acoustic surveys in different locations (i.e., Venezuela, Indonesia and Mauritania), observed that the highest estimates of fish abundance tended to be obtained from data collected at night, although this was not the case for all fish species. Variation in the schooling behavior and in the factors affecting the diel migration of small pelagic fish might further differentiate the degree of bias in acoustic sampling between night-time and daytime among different areas (e.g., Iglesias *et al.*, 2003; Zwolinski *et al.*, 2007).

In the absence of a single rule for all species and for all locations, some expert groups have identified specific time intervals for acoustic data acquisition in relation to the schooling behavior of the target species. For example, the planning group for pelagic acoustic surveys in the ICES (International Council for the Exploration of the Sea) Subareas VIII and IX, taking into account all available information on small pelagic fishes in such subareas, since 1998, decided that acoustic surveys should be carried out only during daytime in the ICES Subareas VIII and IX (ICES, 1998). At the Mediterranean level, a European program using acoustic techniques to estimate the abundance/biomass and spatial distribution of the main small pelagic fish species (*Engraulis encrasicolus* and *Sardina pilchardus*) has been active since 2009 under the EU Fisheries Data Collection Framework (EC 665/2008). The research groups of the involved EU Mediterranean Member States annually conduct the Mediterranean International Acoustic Survey (MEDIAS) and have adopted a common protocol (MEDIAS, 2019), which strongly recommends that if species identification depends on the recognition of schools based on the echograms, the survey must take place only during daytime and be interrupted during periods in the 24-hour cycle when small pelagic fish schools disperse.

Nevertheless, there are occasions in which a restricted time frame prevents coverage of the whole survey area due to limited availability of the research vessel, bad weather conditions or other reasons, forcing acoustic sampling to occur at night. In this case, echo allocation into species is not based on echo type identification. In the framework of the AcousMed project (AcousMed, 2012), appropriate daytime and night-time acoustic data have been collected and analysed to evaluate differences in acoustic estimates, regardless of the organisms present in the water column (fishes and/or plankton). The results indicated that night estimates can be higher or lower than daytime estimates, and the difference largely depends on the area characteristics in terms of local plankton and fish densities. However, the results showed that correction is possible, and it is advisable when night sampling is inevitable.

In the present study, acoustic data from daytime and night-time sampling, acquired during the AcousMed Project together with data collected in more recent acoustic surveys in the Mediterranean Sea, were analyzed to evaluate whether there were differences between day and night acoustic estimates related only to fish in different Geographical Sub-Areas (GSA as defined by the General Fisheries Commission for the Mediterranean - GFCM, 2009) of the Mediterranean Sea. Moreover, a correction factor was estimated for night-time acoustic data collected at 38 kHz, the frequency of scientific echosounders generally used for fish monitoring surveys (Simmonds & MacLennan, 2005).

Materials and Methods

Day-night experiments

The experimental design foresaw the acquisition of acoustic data along the same transects in daytime and night-time intervals in some geographical subareas of the Mediterranean Sea (Fig. 1; Table 1). In particular, one experiment was carried out in the Tyrrhenian Sea (GSAs 9-10), two in the Northern Adriatic Sea (GSA 17), and two in the Southern Adriatic Sea (GSA 18). In GSA 6 (Northern Spain), acoustic data were collected along a single transect (Fig. 1; Table 1). In the Strait of Sicily (GSAs 15 and 16), five experiments were performed in the period of 2009-2015 (Table 1); the overlap of the different transects insonified during the experiments is shown in figure 1. In GSAs 17 and 18 specific experiments were repeated three times: once overnight and twice on two consecutive days (e.g., “Day 1”, “Night” and “Day 2”). The daytime interval for acoustic data acquisition was from 05:00 UTC to 17:00 UTC, while the night-time interval was from 20:00 UTC to 03:00 UTC.

Even if the dominant pelagic fish species in almost all the considered areas were *Engraulis encrasicolus* and *Sardina pilchardus*, biological sampling with a pelagic net showed some differences in terms of secondary species (Table 1).

In all cases, the echosurvey adopted a parallel transect design perpendicular to the coastline over the continental shelf. The Simrad EK60 scientific echosounder, equipped with different split beam transducers, permitted the acquisition of acoustic data during all surveys. For standardization purposes, it was decided to analyse data collected at 38, 120 and 200 kHz, which were common acquisition frequencies used during all surveys. Each system was calibrated according to standard techniques (Foote *et al.*, 1987; Demer *et al.*, 2015). Acoustic data were recorded along all transects at a speed of 8-10 knots, and all collected datasets were postprocessed using the Myriax Echoview software. For the aims of this study, special attention was given to estimating the acoustic backscatter data at 38 kHz linked to the presence of fish within the insonified water column. Acoustic data acquired at 120 and 200 kHz were used to build *ad hoc* filters to separate fish from zooplankton.

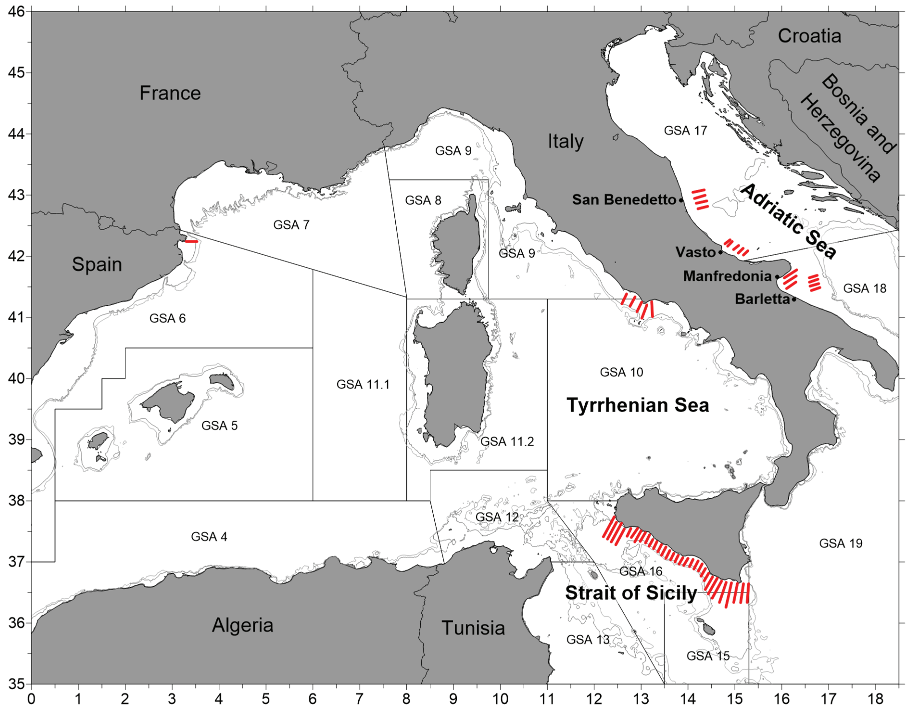

Fig. 1: Position of the transects surveyed in daytime and night-time intervals. The transects in the Strait of Sicily are obtained as an overlap of all transects surveyed in the period of 2009-2015. The map also shows the limits of the geographical subareas (GFCM, 2009) in the Mediterranean Sea.

Table 1. Information on the surveys carried out at daytime and night-time intervals. The most abundant (primary) and less abundant (secondary) fish species in each study area are also presented.

Echosurvey area	GSA	Period of the survey	Number of day-night pairs of transects	Number of day-night pairs of transects perpendicular to the coastline	Pelagic fish assemblage	
					Primary species	Secondary species
Northern Spain	6	June 2018	1	1	*Engraulis encrasicolus, Sardina pilchardus*	*Sardinella aurita, Sprattus sprattus, Trachurus sp., Scomber colias, Boops boops*
Tyrrhenian Sea	10	August 2019	9	5	*Sardina pilchardus, Engraulis encrasicolus*	*Trachurus trachurus, Spicara maena, Sardinella aurita, Scomber colias*
Strait of Sicily	16	July 2009	10	5	*Engraulis encrasicolus, Sardina pilchardus, Sardinella aurita*	*Trachurus trachurus, Scomber colias, Boops boops, Spicara maena*
Strait of Sicily	16	July 2011	21	10		
Strait of Sicily	16	June 2012	37	19		
Strait of Sicily	16	July 2014	6	4		
Strait of Sicily	16	July 2015	26	12		
Vasto	17	August 2009	9	5	*Engraulis encrasicolus, Sardina pilchardus, Sprattus sprattus*	*Spicara maena, Boops boops, Alosa fallax, Sardinella aurita*
San Benedetto	17	September 2009	7	4	*Engraulis encrasicolus, Sardina pilchardus*	*Sprattus sprattus, Scomber colias, Boops boops*
Manfredonia	18	August 2009	5	3	*Engraulis encrasicolus, Sardina pilchardus*	*Scomber colias, Trachurus mediterraneus, Spicara maena, Boops boops, Alosa fallax, Aphia minuta*
Barletta	18	July 2010	7	4	*Engraulis encrasicolus (larvae and adults)*	*Sardina pilchardus, Trachurus trachurus*

Acoustic data processing

The typical workflow for acoustic data processing, reported in the MEDIAS Handbook (MEDIAS, 2019), was adopted for analyzing daytime data. The main steps of the workflow for daytime data are shown in the left panel of Figure 2. In particular, the method proposed by De Robertis & Higginbottom (2007) was used to remove background noise from the echograms collected at 38, 120 and 200 kHz (step c in Fig. 2). The use of lines, regions and mathematical operators (e.g., the school detection module in Echoview®) permitted the separation of fish from plankton. For night-time data, in which scattered fishes were present in the water column, the first three steps of the workflow (right panel of Fig. 2) were similar to those for analyzing daytime data, while the step "d" was modified to try to separate fishes from planktonic organisms according to the procedure proposed by Swartzman *et al.* (1999). In brief, a filter was applied on the Sv (Volume backscattering strength; MacLennan *et al.*, 2002) echograms at 120 kHz and 200 kHz to remove Sv values outside the range between -72 dB and -54 dB; then, the echogram "Sv200 – Sv120" was estimated by differencing the two Sv echograms (at 200 kHz and 120 kHz). Morphological filters were applied on the resultant binary echogram with Sv values having positive differences greater than or equal to 2 dB. Such filters are image-processing methods used to separate well-defined objects from background noise. For the adopted procedure, dilation and erosion operations were applied on the echograms. These morphological filters fill small holes, emphasize the boundaries of the patches, break small isthmuses between patches, and eliminate small patches (more details in Swartzman *et al.*, 1999). As a result, a binary mask for plankton patch removal was identified and applied to the echograms at 38 kHz. In this context, it is important to highlight that the procedure proposed by Swartzman *et al.* (1999) was directly applied to the acoustic datasets without performing any experiment or mathematical modelling to identify the optimal parameter values to be used. Consequently, this procedure was able to remove only part of the signals backscattered by zooplanktonic organisms present in the water column. Where necessary, specific regions were defined on the echograms to remove zooplankton aggregations or jellyfish layers from the analysis (e.g., in Fig. 3). Furthermore, the use of different Sv thresholds (between -60 dB and -54 dB) allowed us to gradually reduce the presence of smaller targets, likely associated to zooplankton organisms (Fig. 3).

For the Adriatic Sea data, the resultant binary mask was obtained by selecting Sv values in the range (-7 dB; +7 dB), which was inspired by the results of the SIMFAMI project report (Fernandes *et al.*, 2006). This interval should discriminate the plankton leaving as invariant as possible the acoustic density of swim-bladdered pelagic fish species as a whole; it was defined from local experience on the acoustic data and was also validated through SIMFAMI project results modulated joining together the outcomes for *Engraulis encrasicolus*, *Sardina pilchardus* and *Trachurus trachurus*.

In each elementary distance sampling unit (EDSU = 1 nm) of each transect, the acoustic nautical area scattering coefficient (NASC; MacLennan *et al.*, 2002) associated to fish was estimated at 38 kHz by setting different values for the minimum Sv threshold: on daytime echograms, the threshold was set only at -60 dB, while on night-time echograms, different thresholds, ranging between -60 dB and -54 dB (by 1 dB), were used. This method led us to estimate, in each EDSU, the fish NASC values associated with each abovementioned Sv thresholds (i.e., fish $NASC_{D-60}$, fish $NASC_{N-60}$, fish $NASC_{N-59}$, fish $NASC_{N-58}$, fish $NASC_{N-57}$, etc.). The choice to not consider Sv threshold values higher than -54 dB in night-time echograms was linked to the aim of reducing the effects of plankton try-

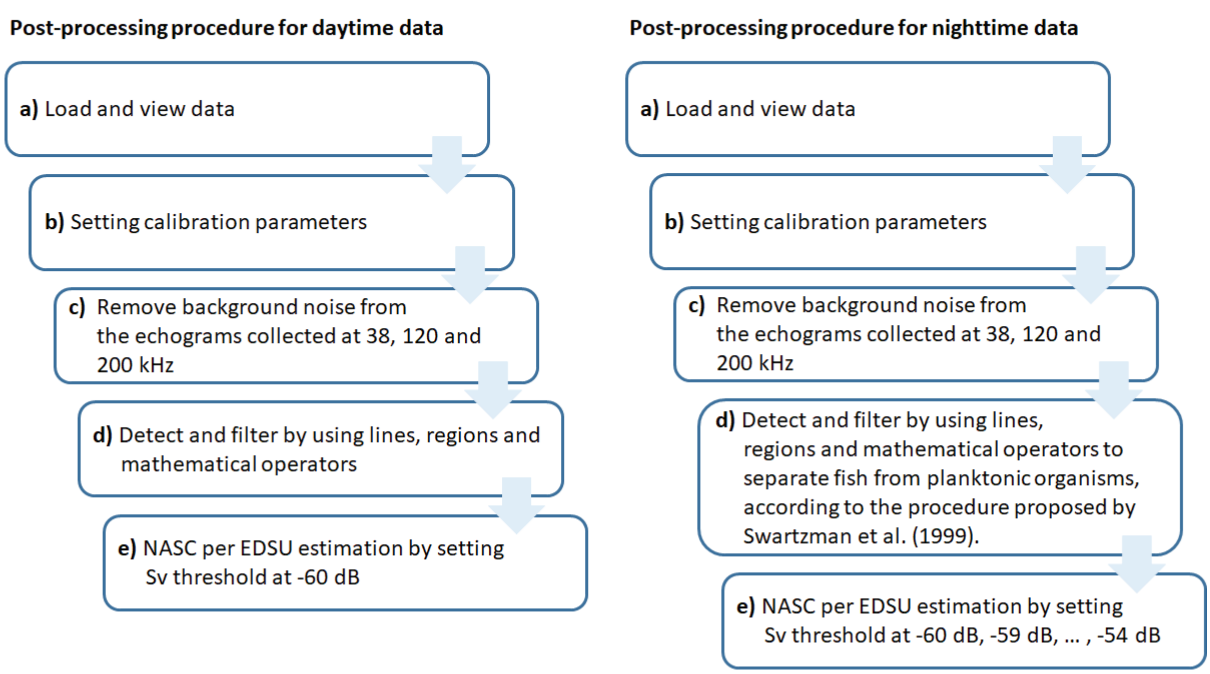

Fig. 2: Steps of the processing procedures adopted for analysing daytime data (left panel) and night-time data (right panel).

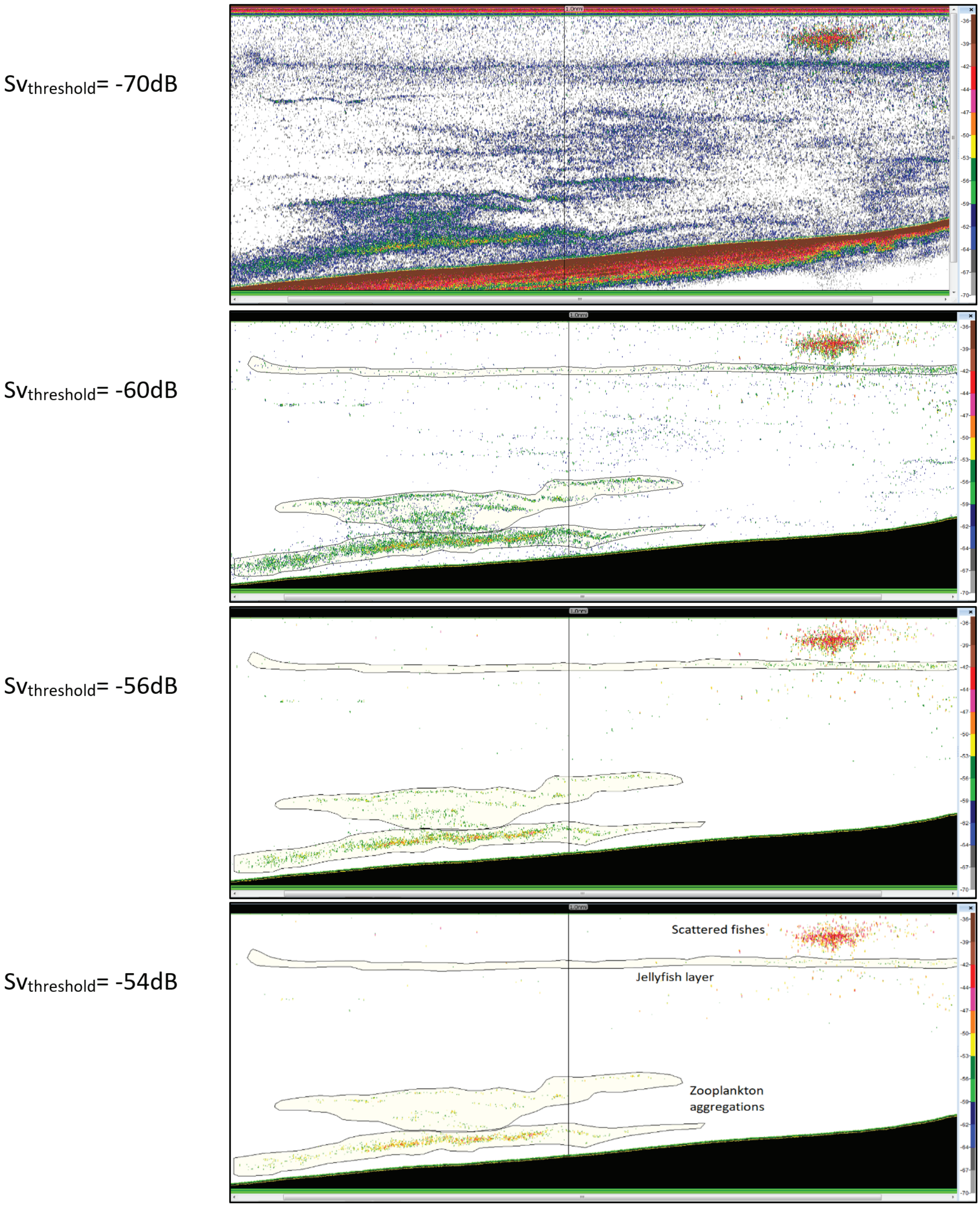

Fig. 3: Night-time echogram acquired in summer 2017 in the Strait of Sicily. Each panel shows the same echogram with a specific Sv threshold. The use of a pelagic trawl net permitted the catching of larger size targets (anchovies and jellyfishes) in the upper water layer.

ing to remove as few fish specimens as possible (Fig. 1S in the Supplementary Materials section).

Furthermore, the total NASC values associated to all organisms (fish and plankton) present in the water column were estimated for both daytime and night-time data from the 38 kHz echograms obtained after removing background noise; in this case, the Sv threshold was set at -70 dB in both daytime and night-time Sv echograms, thus estimating the values of the total $NASC_{D-70}$ and total $NASC_{N-70}$ for each EDSU.

Correction factor computation

The comparison between acoustic data collected in daytime and night-time intervals was made based on the mean fish NASC values estimated in each study area. The mean fish NASC and standard deviation (SD), estimated per area, were based on the Jolly & Hampton (1990) formula (similar to O'Driscoll *et al.*, 2009):

$$\overline{NASC} = \frac{\sum_{i=1}^{N}\left(\overline{NASC_i}\cdot n_i\right)}{\sum_{i=1}^{N} n_i} \;\&\; SD\;(\overline{NASC}) = \sqrt{\frac{N}{N-1}\frac{\sum_{i=1}^{N}\left(\overline{NASC_i}-\overline{NASC}\right)^2 n_i^2}{\left(\sum_{i=1}^{N} n_i\right)^2}}$$

where $\overline{NASC_i}$ is the mean NASC for the i-*th* transect, n_i is the number of EDSUs for the i-*th* transect, and N is the number of transects. SD values are estimated to highlight the variability of NASC values within each survey.

For each experiment, the correction factor for night-time fish NASC was computed by considering the $NASC_N/NASC_D$ ratio. Furthermore, to investigate the possible influence of the area coverage (i.e., the percentage of day-night paired transects with respect to the total number of transects) on the variability of the correction factor, a simulation study was carried out based on the survey conducted in the Strait of Sicily during summer 2012, where 19 parallel transects, out of 30 identifying the entire survey area, were insonified during both day and night. Specifically, the simulation study was performed by computing the correction factor based on a different number of transects and by randomly selecting transects for the computation from the available pool. Thus, for each considered number of transects (from 2 to 18), all the possible combinations of transects were tested, allowing us to obtain the frequency distribution of the correction factors.

Finally, the availability of acoustic datasets collected in the Adriatic Sea in surveys also performed on two consecutive days, allowed us to assess possible effects associated to small-scale movements of small pelagic fishes from/to the surveyed area.

Results

Some examples of the echograms obtained by applying the procedure for analyzing daytime and night-time acoustic data are shown in figures 2S, 3S, 4S and 5S of the Supplementary Materials section. All these figures show how the use of higher Sv thresholds gradually reduces the presence of smaller TS values, likely associated to smaller targets.

In the Strait of Sicily, higher mean fish NASC values at 38 kHz were estimated during night-time than during daytime (Sv threshold = -60 dB for daytime) in all five surveys (Table 2a); such a finding was confirmed for all the adopted Sv thresholds in night-time data (Table 2a). Additionally, the mean total NASC (Sv threshold = -70 dB, which allows keeping many backscatter echoes possibly related to mesozooplankton) was higher in the night-time estimates in all surveys, except for the 2009 one. A synthesis of the results obtained for the Strait of Sicily is presented in Figure 4a, showing the mean NASC values (and standard deviations) for the 2009-2015 period. The $NASC_N/NASC_D$ ratios, estimated at the same Sv threshold for the five surveys in the Strait of Sicily, showed values > 1 (Table 3a). The higher variability associated with the lower Sv thresholds, as observed in Table 3a, suggests the Sv threshold at -54 dB ($NASC_{N-54}/NASC_{D-60}$ = 1.2291; SD = 0.0603) was the most reliable correction factor for the Strait of Sicily.

The analysis of the data collected along the transect located in the northern Spain area showed results like those obtained in the Strait of Sicily, with higher mean fish NASC and total NASC values during the night-time (Fig. 4a; Table 2a) and $NASC_N/NASC_D$ ratios greater than 1 (Table 3a). Completely different results were obtained regarding the Tyrrhenian Sea survey, where the mean fish NASC values estimated during the daytime were slightly higher than the night-time estimates (at least for the higher Sv thresholds until -58 dB). Therefore, for this survey the $NASC_N/NASC_D$ ratios were lower than 1 for the higher Sv thresholds (Table 3a). The total NASC values in the daytime and night-time were comparable (Fig. 4a; Table 2a).

Four acoustic datasets collected in the Adriatic Sea (San Benedetto, Vasto, Manfredonia and Barletta areas) allowed both the day-night comparison of NASC values and the comparison of data acquired on two consecutive days in the same transects. In the San Benedetto area, higher mean fish NASC values were obtained during both daytime surveys than at night (Fig. 4b; Table 2b). However, the day and night total NASC estimates showed comparable values (Table 2b). Furthermore, the mean fish NASC and total NASC values were higher during the "Day 2" survey than during the "Day 1" survey. For this area, very low values were estimated for the $NASC_N/NASC_D$ ratios for both daytime surveys (Table 3b). For the surveys carried out in the Vasto area, the daytime NASC estimates were lower than the night-time estimates, leading to $NASC_N/NASC_D$ ratios greater than 1 (Table 3b). Only in this area, mean fish NASC and total NASC values estimated during the "Day 1" and "Day 2" surveys were similar (Fig. 4b; Table 2b). The surveys performed in the Manfredonia area showed similar mean fish NASC estimates between night-time survey and the "Day 1" survey (Fig. 4b; Table 2b). Higher mean fish NASC and total NASC values were estimated during the "Day 2" survey in this area, thus leading to completely different $NASC_N/NASC_D$ ratios for the two surveys carried out in the daytime (Table 3b). During the survey in the Barletta area, higher NASC values were recorded during the night-time both for fish NASC and for total NASC (Fig. 4b; Table 2b). It is worth noting that the mean fish NASC estimates in the "Day 2" survey revealed higher values than those in the "Day 1" survey (Fig. 2b); this result highlights the possible effects of small-scale horizontal movements of small pelagic fishes in this area. As a result, different values of the $NASC_N/NASC_D$ ratios were obtained for the two surveys carried out in the daytime (Table 3b).

Effects of area coverage during specific day-night acoustic experiments

The estimate of the $NASC_N/NASC_D$ ratios, useful for correcting night-time acoustic data collected at 38 kHz, was highly variable mainly in zones where acoustic experiments covered a small part of the echosurvey area. To evaluate the effect of the area coverage during specific day-night acoustic experiments, the data collected during

Table 2. Mean NASC and standard deviation (SD) estimates at 38 kHz per area, based on the Jolly & Hampton (1990) formula. Section b) of the table shows the estimated values for the echosurvey repeated three times (in one night and on two consecutive days) in the Adriatic Sea.

a)

Area - Year		Sv (dB) Threshold	Mean NASC (m^2/nm^2)	SD (m^2/nm^2)	Area - Year		Sv (dB) Threshold	Mean NASC (m^2/nm^2)	SD (m^2/nm^2)
Strait of Sicily - 2009	Day	-60	42.06	14.01	Strait of Sicily - 2014	Day	-60	60.74	25.69
	Day	-70	398.08	41.04		Day	-70	372.08	125.42
	Night	-54	52.35	8.31		Night	-54	71.05	19.48
	Night	-55	59.89	7.21		Night	-55	78.48	19.78
	Night	-56	69.56	6.64		Night	-56	87.98	20.85
	Night	-57	81.66	7.50		Night	-57	99.47	23.21
	Night	-58	96.28	10.05		Night	-58	113.41	27.20
	Night	-59	113.40	13.89		Night	-59	129.58	32.67
	Night	-60	133.18	18.61		Night	-60	147.75	39.35
	Night	-70	385.75	75.63		Night	-70	534.50	188.58
Strait of Sicily - 2011	Day	-60	98.90	20.78	Strait of Sicily - 2015	Day	-60	58.08	14.83
	Day	-70	381.04	52.90		Day	-70	368.99	40.77
	Night	-54	118.82	17.35		Night	-54	77.01	9.42
	Night	-55	127.97	16.61		Night	-55	88.08	9.55
	Night	-56	137.43	15.87		Night	-56	101.45	10.36
	Night	-57	147.20	15.13		Night	-57	117.59	12.13
	Night	-58	157.55	14.51		Night	-58	136.74	15.17
	Night	-59	168.56	14.15		Night	-59	158.71	19.10
	Night	-60	180.34	14.13		Night	-60	183.24	23.50
	Night	-70	526.43	82.74		Night	-70	628.84	97.99
Strait of Sicily - 2012	Day	-60	81.50	12.99	Tyrrhenian sea - 2019	Day	-60	125.59	42.61
	Day	-70	364.78	63.41		Day	-70	371.99	68.69
	Night	-54	98.12	17.43		Night	-54	75.77	27.32
	Night	-55	104.68	18.97		Night	-55	83.01	30.02
	Night	-56	110.95	20.30		Night	-56	91.56	32.65
	Night	-57	116.94	21.40		Night	-57	101.06	35.54
	Night	-58	122.64	22.25		Night	-58	112.51	38.56
	Night	-59	128.13	22.96		Night	-59	126.59	41.74
	Night	-60	133.56	23.51		Night	-60	142.92	44.64
	Night	-70	408.72	84.89		Night	-70	372.10	59.66
Northern Spain - 2018	Day	-60	54.69	-					
	Day	-70	93.58	-					
	Night	-54	87.79	-					
	Night	-55	96.40	-					
	Night	-56	105.23	-					
	Night	-57	114.03	-					
	Night	-58	123.26	-					
	Night	-59	132.37	-					
	Night	-60	142.01	-					
	Night	-70	242.18	-					

Continued

Table 2 continued

b)

Area - Year		Sv (dB) Threshold	Mean NASC (m^2/nm^2)	SD (m^2/nm^2)	Area - Year		Sv (dB) Threshold	Mean NASC (m^2/nm^2)	SD (m^2/nm^2)
Southern Adriatic Sea (Barletta) - 2010	Day1	-60	97.66	29.66	Southern Adriatic Sea (Manfredo-nia) - 2009	Day1	-60	154.09	133.42
	Day1	-70	535.63	118.36		Day1	-70	422.79	154.96
	Day2	-60	199.03	41.75		Day2	-60	546.26	191.63
	Day2	-70	529.12	60.13		Day2	-70	800.10	231.20
	Night	-54	374.75	172.92		Night	-54	152.00	70.02
	Night	-55	438.02	185.58		Night	-55	159.54	73.44
	Night	-56	498.51	194.94		Night	-56	165.96	76.40
	Night	-57	554.91	201.44		Night	-57	171.37	78.88
	Night	-58	604.86	205.79		Night	-58	175.97	81.03
	Night	-59	648.31	208.56		Night	-59	179.87	82.86
	Night	-60	684.49	210.36		Night	-60	183.23	84.45
	Night	-70	1444.08	437.58		Night	-70	584.26	116.25
Northern Adri-atic Sea (San Benedetto) - 2009	Day1	-60	352.59	147.58	Northern Adriatic Sea (Vasto) - 2009	Day1	-60	207.29	114.50
	Day1	-70	632.84	208.38		Day1	-70	480.84	139.00
	Day2	-60	629.79	316.53		Day2	-60	205.60	58.65
	Day2	-70	863.41	326.55		Day2	-70	469.19	69.69
	Night	-54	98.28	53.95		Night	-54	339.98	60.26
	Night	-55	109.85	61.21		Night	-55	400.02	61.30
	Night	-56	121.25	68.14		Night	-56	463.10	59.75
	Night	-57	132.37	74.89		Night	-57	528.05	56.34
	Night	-58	144.03	81.12		Night	-58	591.57	51.52
	Night	-59	155.52	86.98		Night	-59	652.89	46.08
	Night	-60	167.07	92.24		Night	-60	708.12	40.80
	Night	-70	703.74	139.63		Night	-70	1242.75	76.28

the survey performed in the Strait of Sicily in 2012 were analyzed. The $NASC_{N-54}/NASC_{D-60}$ ratios were estimated using an increasing number of transects insonified during both daytime and night-time intervals. Fig. 5 shows the estimated mean value of the $NASC_{N-54}/NASC_{D-60}$ ratios, the associated standard deviation (SD) and coefficient of variation (CV = mean value/SD). It is worth noting that the CV decreased as the number of transects increased. In particular, considering that the survey design was composed of 30 parallel transects, it was necessary to acquire daytime and night-time acoustic data in approximately 50% of the survey area to obtain a CV value lower than 10% (Fig. 5).

Effects of day-night acoustic data acquisition on fish NASC estimates

To evaluate the bias introduced by considering a mixed day-night survey, we compared the sum of fish NASC values based on 30 daytime transects of the Strait of Sicily survey in summer 2012 with that obtained in a mixed survey consisting of daytime and night-time transects. The sum of the fish NASC values for the 30 daytime transects was 28,898.77 m^2/nm^2; in this case, the sum was considered unbiased since the whole survey was performed in daytime intervals. If a mixed survey consisting of 11 daytime transects and 19 night-time transects (without applying any correction) is considered, the sum increases up to 32,425.30 m^2/nm^2, thus leading to an overestimation of approximately 12%. In the case that a correction factor, estimated for the Strait of Sicily based on data from all years, was applied (i.e., night-time fish NASC values were divided by 1.2291), the abovementioned sum was reduced to 28,531.86 m^2/nm^2, resulting in a small underestimation of approximately -1.27% with respect to the daytime survey estimate.

Furthermore, a sensitivity analysis in terms of the number of transects was carried out (Table 4). The obtained results showed that the mean value of the sum of fish NASC tended to be unbiased during the daytime value as the number of night-time transects decreased, while the difference between the maximum and minimum values tended to decrease with a lower number of transects or with a higher number of transects.

Table 3. Ratios between mean NASC estimates in night-time (i.e., $NASC_{N-60}$, $NASC_{N-59}$, $NASC_{N-58}$, $NASC_{N-57}$, $NASC_{N-56}$, $NASC_{N-55}$ and $NASC_{N-54}$) and mean NASC estimates in daytime ($NASC_{D-60}$) per area: a) values estimated in the Strait of Sicily, in the Tyrrhenian Sea and in the Northern Spain area; b) values estimated in the four surveys in the Adriatic Sea.

a)	Strait of Sicily							Tyrrhenian Sea	Northern Spain
Sv (dB) Threshold	$NASC_N/NASC_{D-60}$ 2009	$NASC_N/NASC_{D-60}$ 2011	$NASC_N/NASC_{D-60}$ 2012	$NASC_N/NASC_{D-60}$ 2014	$NASC_N/NASC_{D-60}$ 2015	Mean $NASC_N/NASC_{D-60}$	SD	$NASC_N/NASC_{D-60}$	$NASC_N/NASC_{D-60}$
-54	1.2446	1.2014	1.2040	1.1697	1.3259	1.2291	0.0603	0.6034	1.6053
-55	1.4238	1.2939	1.2845	1.2920	1.5164	1.3621	0.1039	0.6610	1.7627
-56	1.6537	1.3896	1.3614	1.4484	1.7466	1.5199	0.1707	0.7290	1.9241
-57	1.9413	1.4884	1.4349	1.6376	2.0244	1.7053	0.2656	0.8047	2.0850
-58	2.2888	1.5930	1.5048	1.8670	2.3541	1.9216	0.3894	0.8959	2.2538
-59	2.6959	1.7044	1.5722	2.1332	2.7324	2.1676	0.5405	1.0080	2.4204
-60	3.1661	1.8235	1.6389	2.4324	3.1547	2.4431	0.7176	1.1380	2.5967

b)	Southern Adriatic Sea				Northern Adriatic Sea			
	Barletta - 2010		Manfredonia - 2009		San Benedetto - 2009		Vasto - 2009	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Sv (dB) Threshold	$NASC_N/NASC_{D-60}$	$NASC_N/NASC_{D-60}$	$NASC_N/NASC_{D-60}$	$NASC_N/NASC_{D-60}$	$NASC_N/NASC_{D-60}$	$NASC_N/NASC_{D-60}$	$NASC_N/NASC_{D-60}$	$NASC_N/NASC_{D-60}$
-54	3.8374	1.8828	0.9864	0.2783	0.2787	0.1561	1.6401	1.6536
-55	4.4853	2.2007	1.0354	0.2921	0.3116	0.1744	1.9298	1.9457
-56	5.1047	2.5046	1.0770	0.3038	0.3439	0.1925	2.2341	2.2525
-57	5.6822	2.7880	1.1121	0.3137	0.3754	0.2102	2.5474	2.5684
-58	6.1937	3.0390	1.1420	0.3221	0.4085	0.2287	2.8538	2.8774
-59	6.6387	3.2573	1.1673	0.3293	0.4411	0.2469	3.1496	3.1756
-60	7.0092	3.4391	1.1891	0.3354	0.4738	0.2653	3.4160	3.4442

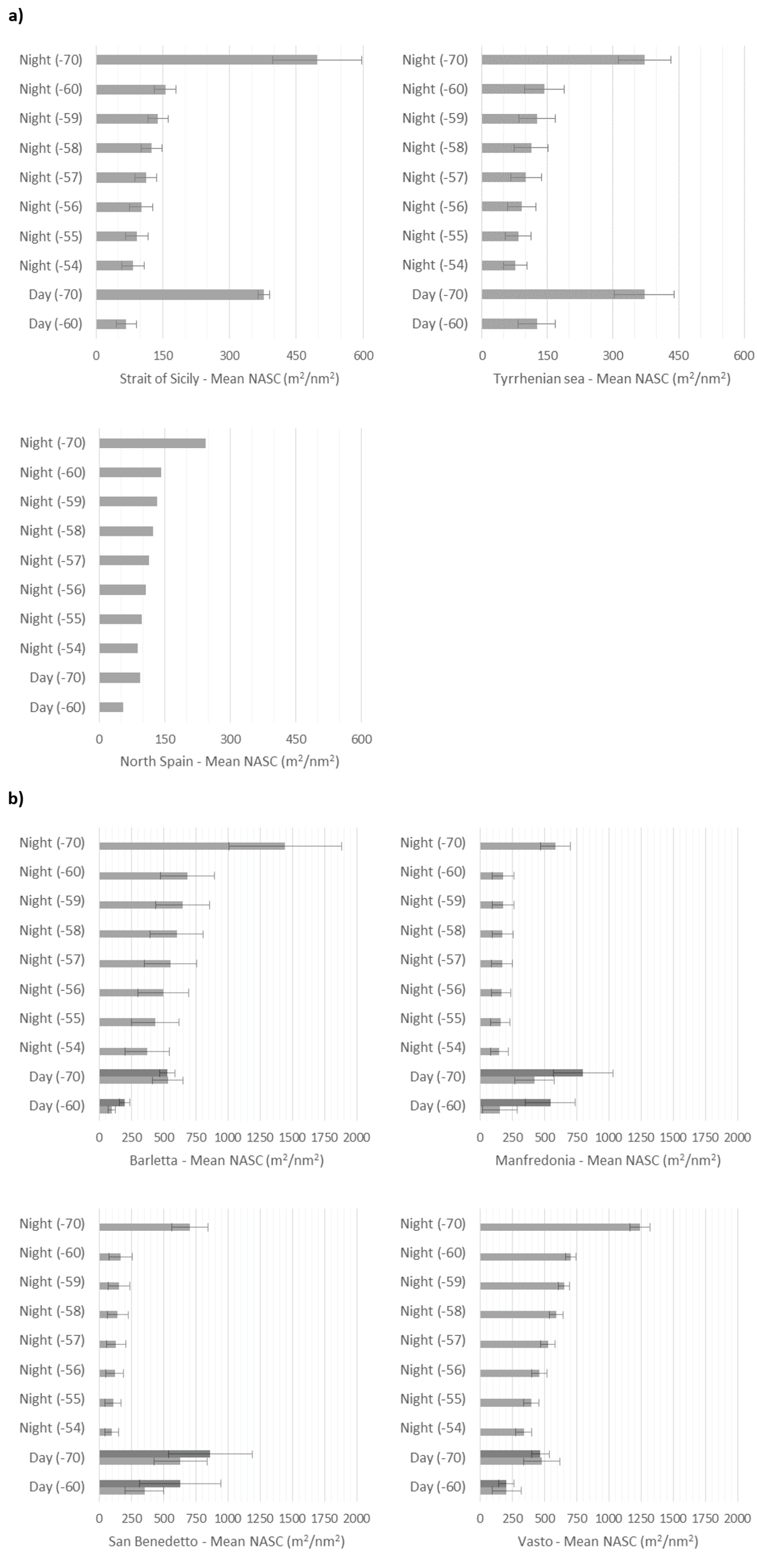

Fig. 4: Mean NASC and standard deviation (SD) in daytime and night-time per area: a) values estimated in the Strait of Sicily (mean values are obtained from the five surveys), in the Tyrrhenian Sea and in the Northern Spain area; b) values estimated in the four surveys in the Adriatic Sea (grey bars refer to the estimated values at night and in the "Day 1" surveys, while darker grey bars are obtained in the "Day 2" surveys).

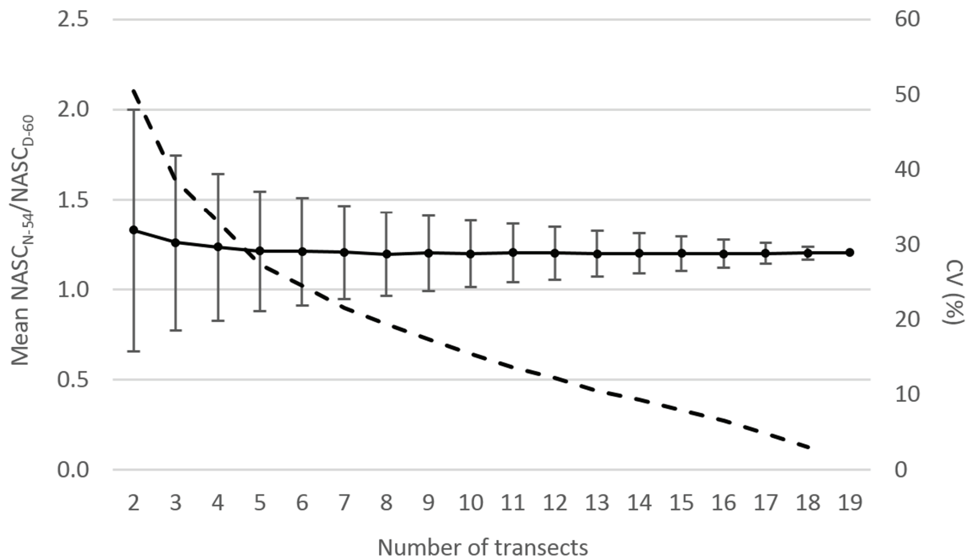

Fig. 5: Mean $NASC_{N-54}/NASC_{D-60}$ ratio as a function of the number of transects chosen among the ones insonified both in daytime and at night. Intervals for each value are ±SD, while the dashed line is the coefficient of variation (CV = SD/Mean).

Table 4. Minimum, medium and maximum values of the sum of NASC values associated with pelagic fish for the survey conducted in summer 2012 in the Strait of Sicily; the sum is estimated for all the possible combinations of night transects with corrected NASC values. The unbiased sum of the fish NASC values, computed using the 30 daytime transects, is 28,898.77 m^2/nm^2.

Number of night transects	Min (m^2/nm^2)	Mean (m^2/nm^2)	Max (m^2/nm^2)
19		28531.86	
18	26952.66	28551.17	29620.55
17	26372.87	28570.48	30639.23
16	25972.80	28589.79	31170.19
15	25644.06	28609.10	31633.67
14	25321.57	28628.41	31945.85
13	25156.88	28647.72	32229.85
12	25014.57	28667.03	32412.85
11	24931.24	28686.35	32578.28
10	24853.30	28705.66	32596.87
9	24833.76	28724.97	32577.33
8	24852.34	28744.28	32499.39
7	25017.78	28763.59	32416.06
6	25200.78	28782.90	32273.75
5	25484.78	28802.21	32109.06
4	25796.96	28821.52	31786.57
3	26260.44	28840.84	31457.82
2	26791.39	28860.15	31057.76
1	27810.08	28879.46	30477.97

Discussion

One of the most important issues in acoustic surveys for small pelagic fish is the time of acoustic sampling. Some studies in the literature pointed out advantages and disadvantages in adopting acoustic sampling in daytime or night-time intervals (e.g., Fréon *et al.*, 1993a; 1993b; Draštík *et al.*, 2009; Hanchet *et al.*, 2000; Domínguez-Contreras *et al.*, 2012). The diurnal schooling behavior of small pelagic fishes (Massé, 1996; Fréon & Misund, 1999; Giannoulaki *et al.*, 1999; Szczucka, 2000; Zwolinski *et al.*, 2007) makes the scrutinization of acoustic data more efficient due to the presence of more easily identifiable fish aggregations in the insonified water column. Additionally, acoustic monitoring of daytime aggregations can sometimes be affected by the sea bottom acoustic dead zone, thus generating a degree of bias in the echo densities used for abundance estimates (Hjellvik *et al.*, 2004). However, night-time sampling has raised several contradictions regarding the introduction of errors in the abundance estimates (e.g., ICES, 1998; Iglesias *et al.,* 2003; Zwolinski *et al.,* 2007). At night, fish schools tend to disperse, and individuals also move towards the surface (Blaxter & Hunter, 1982; Iglesias *et al.*, 2003). In this case, part of the biomass might not be considered in the acoustic estimates due to the acoustic transducer physical limits (Simmonds & MacLennan, 2005). Furthermore, biomass estimates can be affected by TS fluctuations during fish vertical migration (Hjellvik *et al.*, 2004; Knudsen *et al.*, 2009).

Acoustic data analysis for fish abundance monitoring is largely based on acoustic classification of biological targets applied to daytime acoustic data, i.e., when small pelagics tend to aggregate (Reid, 2000; Tsagarakis *et al.*, 2012; D'Elia *et al.*, 2014; Korneliussen, 2018; Aronica *et al.*, 2019). In contrast, during night-time it is very difficult to acoustically recognize which species each individual fish target belongs to; in this case, scientists must allocate echo energy according to the species proportion found in the fishing stations (e.g., Petitgas *et al.*, 2003). Pelagic trawls are known to be selective, and the samples collected are generally subject to some variability (Simmonds & MacLennan, 2005; Machias *et al.*, 2013), strongly affecting biomass estimates (Massé & Retiere, 1995; Petitgas *et al.*, 2003).

Taking into account that daytime acoustic estimates provide a more reliable evaluation of small pelagic fish biomass in the water column, it is a common suggestion in acoustic surveys that sampling should stop when the schools disperse (e.g., ICES, 1998; 2006; MEDIAS, 2019). Nevertheless, in the case of reduced survey time, due to bad weather conditions or unexpected technical problems, acoustic data acquisition may be extended to night-time, as acknowledged by the MEDIAS handbook (MEDIAS, 2019). In this case, the final acoustic dataset will be made of both daytime and night-time data, and a bias is expected in the biomass estimate of small pelagic species. The results obtained in this study, by analyzing acoustic data acquired in some areas of the Mediterranean Sea, indicated that it is possible to evaluate a correction factor for fish NASC values collected at night.

In the case of the Strait of Sicily, higher night-than-day fish NASC values were consistently observed over time. Data collected in five dedicated surveys allowed us to estimate a mean correction factor ($NASC_{N-54}/NASC_{D-60}$ = 1.2291) and highlighted the limited interannual variability among the estimated values. The adoption of such a correction factor permitted us to reduce the bias in the sum of fish NASC values of the survey carried out in 2012 in the Strait of Sicily, from 12% (using data of daytime transects and uncorrected night-time transects) to -1.27% (using daytime transects and corrected night-time transects). As far as the other areas analyzed in this study are concerned, only one survey per area was performed, thus obtaining a single value of the correction factor for each surveyed area. The obtained results showed a wide range of correction factor values ($NASC_{N-54}/NASC_{D-60}$), from 0.1560 in the San Benedetto survey (Day 2), in the Northern Adriatic Sea to 3.8374 in the Barletta survey (Day 1), in the Southern Adriatic Sea. The five areas considered in this study (Strait of Sicily, Northern Adriatic Sea, Southern Adriatic Sea, Tyrrhenian Sea and Northern Spain) are quite different in terms of zooplankton abundance and composition (Piontkovski *et al.*, 2011; Mazzocchi *et al.*, 2014; Rumolo *et al.*, 2018; Malavolti *et al.*, 2018), pelagic fish species community structure (Iglesias *et al.*, 2003; Leonori *et al*.; 2012; Santos *et al.*, 2013; Bonanno *et al.*, 2016; 2018), and school dispersion into shoals (Azzali *et al.*, 2002; Cingolani *et al.*, 1996; Iglesias *et al.*, 2003; D'Elia *et al.*, 2009; 2014; Barra *et al.*, 2015; Aronica *et al.*, 2019). All these aspects can affect the estimation of a correction factor. The procedure for separating fish from planktonic organisms adopted here (proposed by Swartzman *et al.*, 1999) has been previously applied to data acquired in the Strait of Sicily (e.g., Patti *et al.*, 2011). It is important to note that the application of such a procedure may produce different results according to study area peculiarities in terms of zooplankton abundance and/or community structure. Alternatively, a specific zooplankton filtering procedure, able to take into account the area peculiarities, should be designed and implemented per area.

Similarly, different fish assemblages in terms of species proportions in different areas could affect the NASC differences between day and night. Thus, the effects related to species composition and school dispersion require further studies since the available datasets did not allow the investigation of this question. Another aspect that should be considered are the effects due to rising fish from the blind zone near the bottom (Ona & Mitson, 1996). Most of the pelagic fish species present in the study areas form schools positioned very close to the bottom during the daytime (Iglesias *et al.*, 2003; D'Elia *et al.*, 2014) and thus cannot be discriminated from the bottom echo and therefore integrated. During night-time, when pelagic fishes are scattered in the water column, the effects of blind zones close to the bottom may be less important, thus introducing a possible bias between daytime and night-time estimates.

The results obtained in the Strait of Sicily, where day-night experiments occurred in different years, suggest that acoustic surveys in each area should be repeated to obtain a more reliable estimate of the correction factor and to assess possible presence of interannual variability. Moreover, further sources of variability were revealed by three out of four surveys carried out in the Adriatic Sea (i.e., Barletta, Manfredonia and San Benedetto). Each of these surveys was carried out once during the night and twice during two consecutive days; the estimated fish NASC values on consecutive days and the associated correction factors showed wide variability for such small areas, leading us to hypothesize that small-scale movements of small pelagics can strongly affect the correction factor estimation. Peraltilla & Bertrand (2014) estimated for Peruvian anchovy schools a cruising speed of approximately 0.6 m/s that, under the hypothesis of constant moving during daylight hours, could permit them to cover theoretical linear distances of ~25.92 km per day. Thus, wider study areas should be considered in the Adriatic Sea before planning and carrying out future day-night experiments to assess the correction factor. The same consideration could probably also be valid for the areas investigated in the Tyrrhenian Sea and in the Northern Spain area, where very low coverage was adopted in comparison to the entire area surveyed by the MEDIAS. In this context, to propose an approach to define the most suitable coverage for estimating the correction factor for a survey area, a simulation study was carried out by considering the data collected in the Strait of Sicily in summer 2012. As expected, the coefficient of variation associated to the correction factor estimate decreased as the percentage of transects increased. Although the best approach is to run the entire survey twice (one during daytime and one during night-time), a possible choice to reduce the effort of acoustic data acquisition could be to keep the CV less than 10%. In this case, the number of transects to be insonified during both daytime and night-time will be approximately 50% of the total number of transects. In addition, the observed area-related variability of the correction factor (values higher or lower than 1 depending on the considered area) highlights that its computation should also consider the presence of specific subareas. When large areas, such as the Adriatic or the Tyrrhenian Seas, are considered, it is possible that specific relatively large sectors could present differences in zooplankton or pelagic fish communities, thus affecting the correction factor computations. This could probably be the case in the Adriatic Sea, where evident differences were observed in the NASC values among areas. In such cases, a best practice for the computation would be to repeat at least 50% of the survey in each subarea during the day and during the night.

Furthermore, if multiple surveys are carried out in the same area but in different seasons, the correction factor should be estimated seasonally to capture possible differences in zooplankton synthesis and abundance and/or pelagic fish communities (e.g., Hagan & Able, 2003; Jung & Houde, 2003; Siokou-Frangou *et al.*, 2010; Pitchaikani & Lipton, 2015; Albo-Puigserver *et al.*, 2017).

Considering the complexity in the planning and management of acoustic surveys, in which it is sometimes necessary to address problems related to vessel available time, bad weather conditions and technical issues, it is important to keep in mind that the development of validated correction factors of night-time acoustic estimates could represent a valid tool in case night sampling cannot be avoided.

Conclusions

In the present study, day-night differences in fish NASC values were estimated even in different areas within the same region. For the Strait of Sicily, the high consistency observed over the years permitted us to estimate a mean correction factor for night-time acoustic data. For the other areas, the results showed that further day-night acoustic data are necessary. Based on the results of the present study, the following conclusions can be made:

Day-night differences exist, but their order of magnitude seems to depend on the area and ecosystem characteristics;

Further work with data from additional areas is required to understand the effects of zooplankton and pelagic fish communities on correction factor estimation;

Since the presence of zooplankton represents an important source of bias, specific studies should be carried out to estimate *ad hoc* filtering procedures;

The correction factor estimation should be based on appropriate area coverage (at least 50% of the survey area) to take into account horizontal fish movements;

Correction factor estimation should take into account the possible effects of interannual variability.

Even if the best option is acoustic data acquisition during the daytime only, in some specific situations, day-night sampling cannot be avoided, and the obtained estimates will be biased due to a number of effects; the availability of a correction factor could help to compensate for such bias. Our results showed that general rules cannot be applied and area-specific research is needed to properly address the day-night acoustic sampling issue.

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Supplementary Data

The following supplementary information is available online for the article:

Figure 1S: Scattered targets (fishes and plankton) in the upper part of the water column and frequency distribution of TS values by setting different Sv thresholds (from -60dB to -52dB). As it is possible to observe, the use of higher Sv thresholds gradually reduces the presence of smaller TS values, likely associated to smaller targets.
Figure 2S: Acoustic data collected along two transects in the Strait of Sicily.
Figure 3S: Acoustic data collected along two transects in the Tyrrhenian Sea.
Figure 4S: Acoustic data collected in the Northern Spain area.
Figure 5S: Acoustic data collected along two transects in south-western Adriatic Sea.