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Contribution to the Special Issue: "MEDiterranean International Acoustic Survey (MEDIAS)"

# Effects of sampling intensity and biomass levels on the precision of acoustic surveys in the Mediterranean Sea

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### Abstract

Acoustic surveys represent the standard fishery-independent method worldwide for evaluating the biomass and spatial distribution of small pelagic fish populations. Considering the peculiarities of the spatial behaviour of pelagic fishes, the efficiency of the survey design in determining their biomass and spatial distribution is related to the ability to capture the portion of the patches accounting for a larger part of the total biomass. However, the spatial structure of the patches could be strongly influenced by ecosystem characteristics as well as by changes in total biomass related to a density-dependent mechanism. This is of particular interest for anchovies and sardines, which are known for their wide fluctuations and high sensitivity to the environment. In this study, we analysed the efficiency of acoustic surveys targeting European anchovies (Engraulis encrasicolus) and European sardines (Sardina pilchardus) in 10 different areas of the Mediterranean Sea spanning three years of different biomass levels. Using the geostatistical coefficient of variation (CV<sub>geo</sub>) of the average occurrence probability of high/medium density values, we showed different patterns in terms of survey design efficiency among areas and species. Anchovies usually showed a lower CV<sub>een</sub> than sardines in the Alboran Sea. In 4 out of 20 cases, CV<sub>geo</sub> values showed a consistent decrease with increasing biomass, while in the remaining cases, the CV<sub>geo</sub> did not follow any clear pattern, suggesting the presence of important environmental effects. Higher survey design efficiency was found in highly productive sectors influenced by river run-off, allowing us to hypothesize that higher productivity along with the presence of well-localized enrichment mechanisms could favour a spatially consistent distribution and coherent organization of fish populations, leading to higher precision estimates with a given transect design. While most surveys displayed CV<sub>geo</sub> close to 10% or less even at low biomass, indicating generally good performances of the survey design, a few areas exhibited higher CV<sub>geo</sub>, yielding a potential need to decrease the intertransect distance, always keeping in mind that any survey should be as synoptic as possible.

Keywords: Acoustic survey; spatial sampling efficiency; density-dependent effects; Mediterranean Sea.

### Introduction

In the Mediterranean Sea, acoustic surveys targeting European anchovies (Engraulis encrasicolus) and European sardines (Sardina pilchardus) are routinely carried out in most European waters (Tugores et al., 2011; Giannoulaki et al., 2013; Saraux et al., 2014; Bonanno et al., 2014; 2016), i.e., the geographical subareas (GSA as defined by GFCM, 2009) 1, 6, 7, 9, 10, 16, 17, 18 (western side), 20 and 22 (Fig. 1). Since 2009, all acoustic surveys carried out in the EU waters of the Mediterranean Sea have been conducted under the EU umbrella as a specific action of the European Data Collection Framework (DCF), called MEDIAS (MEDiterranean International Acoustic Survey; www.medias-project.eu). Acoustic data are generally recorded along transects perpendicular to the bathymetric gradient following a parallel transect design. In specific sectors where the coastline morphology is complex and/or the continental shelf is too narrow to justify the adoption of parallel transects, an adaptive zigzag survey design is adopted (Simmonds & MacLennan, 2005). This latter approach is generally adopted in small sectors, regardless of the local biomass level. The precision of the biomass estimates in the case of parallel transect survey design is strongly related to the adopted intertransect distance, representing the degree of sampling intensity and thus an important aspect to consider when planning the survey design. Aglen (1983; 1989) related the precision of acoustic surveys to the degree of coverage, expressed as the ratio between the total length of the cruise track and the square root of the area of the surface covered by the survey (the larger the ratio, the higher the precision). However, the fish spatial distribution also strongly impacts the survey precision; the same intertransect distance may lead to higher precision if fishes are widely distributed rather than concentrated in isolated patches (Simmonds & MacLennan, 2005).

Populations of small pelagic fishes, according to eco-

system characteristics and species biology, are characterized by complex spatial behaviours, presenting different aggregation patterns at different spatial and temporal scales (Petitgas et al., 2001; Muiño et al., 2003). At a small spatial scale, individuals form schools during the daytime, while during the nighttime, individuals are scattered in the water column, forming loose aggregations. At larger spatial scales, schools are aggregated in patches (clusters of schools) extending from a few to tens of nautical miles. The spatial distribution and temporal stability of the patches may depend primarily on ecosystem characteristics (i.e., temporal dynamics of environmental processes) and on species biology following a seasonal pattern according to the spawning and recruitment season (Erisman et al., 2012; Boyra et al., 2016). The geometry of the study area, strongly influenced by continental shelf extension, could also represent an important driver in determining the spatial organization of small pelagics (Giannoulaki et al., 2006). Finally, the population dynamics could lead to modifications in the spatial distribution and density levels through a density-dependent mechanism. In this context, depending on the ecosystem characteristics, an increase in biomass could lead 1) to an increase in the presence area while keeping constant the average density (Iles & Sinclair, 1982); 2) to an increase in fish density at all observation points (Houghton, 1987; Hilborn & Walters, 1992) or in specific sectors (Petitgas, 1998) while keeping constant the area of presence; and 3) to an increase in both density and presence area (Ulltang, 1980; McCall, 1990; Swain & Wade, 1993). In this latter case, both parameters (density and presence area) can react to different processes and occur successively. For instance, McCall's basin hypothesis (1990) suggested that density should increase first in the most favourable areas until a certain density level, above which density-dependent processes counteract the benefits of favourable environments, and fish should then expand their presence area. In summary, environmental variability and ecosys-



*Fig. 1:* GSAs considered in the analysis of the survey design. The red polygons represent the areas considered for each survey. The selection was made in order to reduce possible biases in the analysis (see text).

tem characteristics represent important drivers for both spatial distribution and population dynamics (Gutiérrez *et al.*, 2012; Doray *et al.*, 2018; Patti *et al.*, 2020), which might affect the efficiency of survey design.

Acoustic surveys in Mediterranean waters are generally carried out during the summer season, representing the spawning period for anchovies and the post recruitment period for sardines (Basilone et al., 2006; Basilone et al., 2021). In all the studied GSAs, the adopted survey design and the sampling intensity were chosen to best balance different constraints such as the study area characteristics (coastline morphology, continental shelf extension) and the available vessel time (Simmonds & MacLennan, 2005). However, questions remain regarding how the efficiency of the survey design might vary in the case of important biomass fluctuations or changes in the spatial structure driven by environmental variability. Such questions are especially important in the case of anchovies and sardines since both species show large interannual biomass variations and high sensitivity to environmental variability.

The assessment of the uncertainty associated with acoustic biomass estimates needs to account for the presence of spatial autocorrelation, and several studies have investigated the precision of acoustic biomass estimates focusing on different sources of variability and methodological aspects (Gimona & Fernandes, 2003; Walline, 2007; Woillez *et al.*, 2009; Tugores *et al.*, 2010; Tugores *et al.*, 2016).

In this work, we focus on evaluating the survey design efficiency in detecting patches of high values representing most of the total biomass, like the work carried out in previous working groups (ICES WKACUGEO report, 2011; Acousmed, 2012). In particular, the efficiency of the survey design was investigated in 10 different Mediterranean GSAs characterized by distinct productivity and environmental features. Three different biomass levels for anchovies and sardines were selected per GSA, allowing the evaluation of the current survey design precision under different population levels. Finally, for each survey, the estimated spatial structure of high/medium density patches was used to simulate the changes in the survey precision according to different intertransect distances, thus focusing on an important parameter in the establishment of acoustic surveys.

### **Materials and Methods**

### Survey selection and generation of pseudosurveys

All the surveys considered in this study were carried out according to the sampling protocol adopted by the MEDIAS working group (MEDIAS handbook, 2019). In particular, acoustic surveys (Fig. 1) carried out in GSAs 1 (Northern Alboran Sea), 6 (Northern Spain), 7 (Gulf of Lion), 9 (Ligurian and North Tyrrhenian Sea), 16 (South of Sicily), 17 (Northern Adriatic), 18 (Italian part of Southern Adriatic Sea) and 22 (Greek part of Aegean Sea: Thermaikos Gulf and Thracian Sea) were considered. To demonstrate differences in the survey design efficiency in years with different biomass levels, for each GSA and species, three years characterized by low, medium and high biomass were selected (Table 1). Only areas surveyed by a parallel transect survey design were considered (to avoid possible biases due to the zig-zag design adopted in cases of complex coastal morphology). Furthermore, in GSAs where modification in the surveyed area occurred from year to year (e.g., GSA16), only the common area among the considered years was selected, and only sectors characterized by homogeneous intertransect distance and transect direction were considered (Fig. 1). In the case of GSA22, two subareas were considered, namely, the Thermaikos gulf and Thracian Sea (Fig. 1). This choice was justified by different spatial aggregation patterns characterizing anchovy and sardine populations in these subareas (Barra et al., 2015).

### Acoustic data

Acoustic data collection was performed by means of scientific splitbeam echosounders working at 38 kHz and calibrated following standard techniques (Demer et al., 2015). Acoustic data were acquired at a speed of 8-10 knots. The size of the elementary distance sampling unit (EDSU, representing the length of the cruise track along which the acoustic data are averaged to obtain sampling points) was one nautical mile (nmi, 1.852 km). Mid-water pelagic trawl sampling was performed to identify and verify anchovy and sardine echo traces and to estimate length frequency distributions and length-weight relationships. Anchovy or sardine presence was then determined based on echo trace classification and echo-trawl association (Simmonds & MacLennan, 2005). Anchovy and sardine density (t/nmi<sup>2</sup>) for each EDSU was evaluated by merging biological and acoustic data (Simmonds & MacLennan, 2005). In particular, the acoustic energy at each EDSU was partitioned between species based on specific TS equations (Medias handbook, 2019), and biomass values were obtained by considering the estimated length frequency distribution and length weight relationships.

### Geostatistical analyses

The precision of the survey design was estimated by computing, for each survey, the coefficient of variation of the average probability ( $CV_{geo}$ ) to encounter high/medium density values representing most of the fish biomass (ICES WKACUGEO report, 2011; AcousMed, 2012). For each area and biomass level (i.e., year), anchovy and sardine densities (t/nmi<sup>2</sup>) in each EDSU were coded as binary variables according to a specific threshold. The threshold value was determined by sorting in decreasing order the density values and computing the contribution of each value to the cumulative sum of density up to 90%. All the values contributing less than 90% of the total biomass were coded as 0, and the others were coded as 1.

**Table 1.** Acoustic surveys considered in this study. The intertransect distance adopted for each survey and the total estimated biomass are reported. In the case of sardines in the Thermaikos Gulf, the biomass in the selected polygon was higher in the low biomass year than in the medium biomass year.

			Anchovy	,		Sardin	e
Area	Inter-transects (nmi)	Year	Biom. lev.	Biomass (metric tons)	Year	Biom. lev.	Biomass (metric tons)
		2013	Low	76	2016	Low	1710
GSA1	4	2017	Medium	1200	2017	Medium	4723
		2015	High	3058	2018	High	15593
		2010	Low	22306	2014	Low	6215
GSA6	8	2013	Medium	44874	2015	Medium	25627
		2018	High	139821	2013	High	41871
		2016	Low	22739	2017	Low	43827
GSA7	12	2018	Medium	32342	2015	Medium	67140
		2017	High	60631	2012	High	80537
		2014	Low	23814	2017	Low	20116
GSA9	9	2018	Medium	50660	2018	Medium	27260
		2017	High	102036	2016	High	48861
		2014	Low	3115	2013	Low	8069
GSA16	6	2016	Medium	7740	2012	Medium	13596
		2012	High	10419	2015	High	29852
		2016	Low	106723	2016	Low	107859
GSA17W	10	2015	Medium	232261	2015	Medium	275434
		2018	High	278927	2011	High	401099
		2014	Low	20805	2016	Low	89208
GSA17E	10	2016	Medium	29451	2014	Medium	113088
		2018	High	57931	2018	High	194057
		2016	Low	18689	2016	Low	1109
GSA18	10	2015	Medium	33164	2015	Medium	6885
		2018	High	39730	2018	High	16181
CSA22 Themasilian		2019	Low	2392	2019	Low	1692
oulf	10	2014	Medium	7599	2016	Medium	3004
		2016	High	15655	2014	High	7886
CS 4 22		2014	Low	9378	2014	Low	6911
USA22 Thracian Sea	10	2013	Medium	27764	2013	Medium	18343
		2016	High	42982	2016	High	29829

The CV<sub>geo</sub> was computed using linear geostatistics under the intrinsic hypothesis (Matheron, 1971). The so-called variogram (Gringarten & Deutsch, 2001) was the structural tool used to model the spatial autocorrelation. It describes how the variance between pairs of sample values changes while the distance between them increases. The variogram was obtained according to the following equation:

$$\gamma(h_k) = \frac{1}{2N(h_k)} \sum_{1}^{N(h_k)} \left[ \left\{ z_{x_{i+h}} - z_{x_i} \right\}^2 \right]$$

where

k is a vector of directions;

 $h_k$  is a vector of distances separating sampling points in a specific direction (k);

 $\gamma(h_{\nu})$  is the variance of the measured values at the given

separation vector  $h_{\mu}$ ;

N is the number of pairs separated by  $h_k$ ;

 $z_{xi}$  is the sample value at location  $x_i$ , and  $z_{xi+h}$  is the sample value at location  $x_{i+h}$ .

For each case (i.e., area, species, and biomass level), the presence of spatial autocorrelation was analysed by considering a maximum distance equal to the width of the continental shelf (Tugores *et al.*, 2016) or the distance for which the data variance reached the experimental variogram plateau. Furthermore, to evaluate the presence of spatial anisotropy, the experimental variograms were estimated by considering the along and across transects (i.e., along isobaths) direction, thus testing for possible changes in the degree of autocorrelation with direction (i.e., the presence of spatial anisotropy). Whenever no spatial anisotropy was present, isotropic experimental variograms were considered. The model describing the

spatial continuity was then obtained by fitting the experimental variograms by choosing the appropriate mathematical function (exponential or spherical) according to the shape observed in the experimental variogram. Important features of the variogram model are the so-called range and nugget, the former indicating the distance at which no spatial correlation exists, while the latter indicate the presence of a short-scale variability that has no spatial correlation (Gringarten & Deutsch, 2001). The fitting was performed by using an automatic fitting procedure based on iterative least squares fitting (Desassis & Renard, 2013), weighting the variance values according to the number of pairs and distance (inversely). In cases where using a 90% biomass threshold led to a pure nugget-effect variogram, a higher threshold (99%) was considered.

Under the intrinsic hypothesis, the estimation variance, depending on the geometry of the spatial domain V, on sampling location I and the underlying spatial structure can be computed as (Rivoirard *et al.*, 2000):

$$\sigma_{geo}^2 = 2\overline{\gamma}(I,V) - \overline{\gamma}(V,V) - \overline{\gamma}(I,I)$$
  
The CV<sub>reo</sub> is then obtained as:

$$CV_{geo} = \frac{\sqrt{\sigma_{geo}^2}}{\mu}$$

If the underlying spatial model is known, the survey design efficiency can be evaluated with respect to different pseudosurveys (i.e., alternative survey designs characterized by a different intertransect distance), thus allowing us to characterize the changes in survey efficiency based on a different sampling scheme (Rivoirard et al., 2000). In particular, for each area, pseudosurveys were created according to a vector of interttransect distances ranging from 4 to 16 nmi (considering only multiples of 4, *i.e.*, four alternative surveys with intertransect distances of 4, 8, 12 and 16 nm). Each pseudosurvey closely mimics the original survey design with small changes in the direction of the transects to accommodate the coastline morphology. The main assumption in this process is that the estimated variogram model is unbiased with respect to the survey design (ICES WKACUGEO report, 2011; AcousMed, 2012).

All computations were carried out in the R statistical environment (R Core Team, 2020) by using the RGeo-Stats package (MINES ParisTech/ARMINES, 2019).

## Testing differences among areas, species, and biomass levels

The decrease in the sampling efficiency of high-density patches with respect to the decrease in the sampling effort was first analysed considering simulation results. In particular, the  $CV_{geo}$  values obtained for each pseudosurvey were regressed against the respective intertransect distance. This allowed us to describe the increase in  $CV_{geo}$ due to increasing intertransect distance in terms of intercept and slope. ANCOVA was applied to test for differ-

ences among  $\mathrm{CV}_{\mathrm{geo}}$  regressions with respect to the considered factors. The homogeneity of variance was tested by Levene's test. Although the homogeneity of variance assumption was not met, Levene's test showed no significant deviation from the homoscedasticity assumption when working on log transformed  $CV_{geo}$  and intertransect distance. Possible interactions among the considered factors and intertransect distance were also considered. Diagnostic plots and normality of residuals (Shapiro-Wilk test) were evaluated at each step of the model selection. In a first step, the full model (*i.e.*, the one considering all fixed terms and possible interactions) was evaluated. The obtained results showed significant interactions among intertransect distance and all the other factors except for species, but significant deviation from normality in the residuals was evidenced. Consequently, the best model was developed by evaluating step-by-step the addition of each interaction term. Three- and four-way interactions were also considered, but when significant, strong deviations were found in the residual qq-plot and were confirmed by the Shapiro-Wilk test. Differences among the groups of a specific factor (e.g., area and species) were assessed by contrasting the  $CV_{geo}$  marginal means (as computed from the ANCOVA model), representing the average  $CV_{geo}$ over all the remaining factors and according to a specific intertransect distance. In particular, the marginal means were computed considering an intertransect distance of 10 nmi, the intertransect distance used in most of the considered GSAs. All comparisons were made by using the "emmeans" package (Lenth R. V., 2020) in the R statistical environment.

Considering the nested structure of the dataset and that it was not possible to include in the model some interaction terms, separate ANCOVA models were fitted to test for differences among different biomass levels for the same species and area.

### Results

#### Biomass variation in the selected surveys

Biomass levels for selected surveys showed large variations among the different GSAs (see Table 1). Biomass ranged between 76 (GSA1) and 278927 (GSA17W) metric tons for anchovies and between 1109 (GSA18) and 401099 (GSA17W) metric tons for sardines (Table 1). In general, sardines presented more patchy aggregations and a more coastal distribution in most of the considered areas compared to anchovies (e.g., Fig. S2, S6, S9 and S10). This behaviour was more pronounced in the years of low biomass.

For some GSAs, only specific sectors with parallel survey design were selected for geostatistical analysis, and the biomass levels in the selected sectors (polygons in Fig. 1) were approximated as the product of the average biomass density and polygon surface and were found to be coherent with those observed in the entire study area (Table S1). The only exception was the case of sardines in the Thermaikos gulf (GSA22), where considering the selected polygon (Table S1), the biomass in the low-biomass year (1495 metric tons) was higher than that in the medium biomass year (1253 metric tons). The Thermaikos Gulf in GSA22 is an area where the open part (surveyed with parallel transects) is adjacent to a narrow productive zone (receiving the outflow of three rivers, surveyed by a zig-zag) where a large part of the area biomass is located. The difference in biomass between the inner and outer parts of the Thermaikos gulf can be even more pronounced depending on the annual variability of the environmental conditions.

### Geostatistical analysis

Geostatistical analyses allowed us to characterize the spatial structure in the distribution of the two species for each considered case (area, species, and biomass levels; see Supplementary material). In GSA1, GSA17E and GSA22, it was not always possible to identify a spatial structure when focusing on the high/medium density values, and thus, a higher threshold (99% of the total biomass) had to be considered. Only in 23 cases out of 60 was the presence of spatial anisotropy evidenced, and variogram models were fitted accordingly.

The percentage of nuggets in the case of anchovies ranged from 23% to 80% (Table S2) and from 4.9% to 89.5% for sardines (Table S3). In general, both species showed a high nugget (*i.e.*,  $\geq$ 50%). This was the case for 67% and 60% of sardine and anchovy cases, respectively. In years of low biomass, the nugget was  $\geq$ 50% in 7 out

of 10 cases for anchovies and in 6 out of 10 cases for sardines. In high biomass years, nuggets were  $\geq$ 50% in 4 out of 10 cases for anchovies and in 5 out of 10 cases for sardines. Finally, in the case of medium biomass, sardine aggregations showed nuggets  $\geq$ 50% for 9 out of 10 cases and 7 out of 10 cases for anchovies.

For anchovies, anisotropy was found in 13 cases, with anisotropy coefficients ranging between 0.18 and 0.6 (Table S2). For anisotropic variograms, the across- and along-transect variogram ranges were in the intervals of 8.5 nmi to 48.1 nmi and 3.2 to 16.9, respectively, while the isotropic variograms spanned between 2 nmi and 34.8 nmi (Table S2). In the case of sardines, spatial anisotropy was identified in 10 cases, and anisotropy coefficients ranged between 0.26 and 0.83 (Table S3). For anisotropic variograms, the across- and along-transect variogram ranges were in the intervals of 10 nmi to 63 nmi and 3.4 nmi to 25.1 nmi, respectively, while for isotropic variograms, they spanned between 4.3 nmi and 45 nmi (Table S3).

### Survey precision

The obtained CV<sub>geo</sub> values were in the range of 3.2% to 34.9% for anchovies (Table 2a; average value 10.7%) and 4.8% to 44.3% for sardines (Table 2a; average value 13.6%). Considering the average CV<sub>geo</sub> values by species and area (Table 2b), anchovies showed lower CV<sub>geo</sub> (and thus better survey precision) than sardines, except for GSA1, where anchovies showed higher values. In GSA1,

**Table 2.**  $CV_{geo}$  values (%) obtained for anchovies and sardines in the considered surveys (a). The average  $CV_{geo}$  values by area and species obtained in this study and during the AcousMed project are also reported (b).

a)											
										GSA22	GSA22
Species	Abund. lev.	GSA1	GSA6	GSA7	GSA9	GSA16	GSA17W	GSA17E	GSA18	Thermaiko	s Thracian
										gulf	Sea
	Low	34.9	8.2	11.8	7.9	12.7	4.9	3.2	12.2	22.5	10.5
Anchovy	Medium	23.9	5.7	11	13	8.7	5.7	4.3	12.5	13.7	7.8
	High	15.8	6.4	6.7	6.3	10	5.5	4.1	8.6	13.3	8.9
	Low	5.9	12.1	13.6	7.3	12.6	6.7	7.4	14.5	38.2	19
Sardine	Medium	14.1	9	11.7	13.8	9.4	8.3	7.6	15.6	44.3	20.2
	High	8	6.8	10.7	7.9	13.7	4.8	8.1	15.7	20.6	21.8

b)

0)										
	GSA1	GSA6	GSA7	GSA9	GSA16	GSA17W	GSA17E	GSA18	GSA22 Thermaikos gulf	GSA22 Thracian Sea
Anchovy avg (this study)	24.9	6.8	9.8	9.1	10.5	5.4	3.9	11.1	16.5	9.1
Anchovy avg (AcousMed)		15.8	12.1		12.3	5.2			24.5	30.4
Sardine avg (this study)	9.3	9.3	12.0	9.7	11.9	6.6	7.7	15.3	34.4	20.3
Sardine avg (AcousMed)		16.0	18.4		11.6	10.9			39.4	47.9

the Thermaikos gulf and the Thracian Sea (GSA22), the absolute differences between the average  $\mathrm{CV}_{_{\mathrm{geo}}}$  of the two species (Table 2b) were higher, ranging between 11.2% and 17.9%, while in the other areas, the differences ranged between 0.6% and 4.2%. A coherent and consistent decrease in  $CV_{geo}$  values with increasing biomass level was observed in GSA1 and GSA7 for anchovies and in GSA6 and GSA7 for sardines (Table 2a). In GSA17E and 17W  $\text{CV}_{\text{geo}}$  values showed low variability among the three different biomass levels, especially in the case of anchovies. In GSA18,  $CV_{geo}$  related to the medium-biomass year for anchovies was close to the one estimated for the low-biomass year, followed by a decrease in the high-biomass year. In the case of anchovies in GSA6, the Thermaikos Gulf and the Thracian Sea (GSA22) and sardines in GSA18 and the Thracian Sea (GSA22), the CV related to medium-biomass estimates was close to the high-biomass estimates. Both anchovies and sardines in GSA16 presented the lowest  $CV_{geo}$  estimate in the year of medium biomass. The opposite was found for GSA9 (both for anchovies and sardines) and sardines in the Thermaikos Gulf (GSA22), where the highest value for  $CV_{reo}$  was related to a medium-biomass year.

### ANCOVA results

An ANCOVA model was developed to test for differences among the obtained  $CV_{geo}$ -intertransect regressions in terms of species and area (Table 3). The obtained best model (adjusted R-squared: 0.95) evidenced a significant effect of intertransect distance on  $CV_{geo}$  values (Table 4). No significant two-way interactions were found between the intertransect distance and the considered factors, while a significant interaction was found between area and species as well as between area and biomass level (Table 4). No patterns or deviations from normality were apparent in the residual plot, as also confirmed by the Shapiro–Wilk test. Considering an intertransect distance of 10 nmi,  $CV_{geo}$  averaged over species and biomass level

Table 3. CV<sub>geo</sub> regression coefficients computed at the area level (pooled anchovy and sardine) and by area and species.

	Area	Area level		hovy	Sardine		
Area	Intercept	Slope	Intercept	Slope	Intercept	Slope	
GSA1	7.95	2.30	11.5	1.86	4.40	1.25	
GSA6	2.51	0.67	2.45	0.49	2.58	0.83	
GSA7	1.66	0.75	0.76	0.77	2.56	0.76	
GSA9	0.96	1.05	0.63	0.94	1.3	1.05	
GSA16	3.36	1.27	2.53	1.41	4.2	1.19	
GSA17E	1.93	0.38	0.96	0.30	2.90	0.46	
GSA17W	1.78	0.42	1.7	0.36	1.86	0.48	
GSA18	4.35	0.83	3.85	0.69	4.85	0.96	
GSA22 Thermaikos gulf	3.64	2.4	1.30	1.70	5.98	3.10	
GSA22 Thracian Sea	5.09	0.97	2.45	0.67	7.73	1.27	

**Table 4.** ANCOVA results showing the significance of fixed terms and interactions between the covariate (itr: intertransect distance) and considered factors, namely, area, biomass level (biom.lev) and species (sp).

	Sum. Sq	Df	<b>F-value</b>	p-value
(Intercept)	17.042	1	721.242	2.20E-16
log(itr)	32.079	1	1357.591	2.20E-16
area	21.776	9	102.396	2.20E-16
sp	6.076	1	257.124	2.20E-16
biom.lev	0.607	2	12.85	5.64E-06
area:sp	14.261	9	67.057	2.20E-16
area:biom.lev	4.83	18	11.356	2.20E-16
Residuals	4.702	199		

Area	Cv (%)	se	95% CI
GSA1*	27.2	0.85	25.39-28.75
GSA6	9.23	0.291	8.68-9.82
GSA7	9.08	0.286	8.53-9.66
GSA9	10.74	0.338	10.09-11.43
GSA16	16.24	0.511	15.27-17.28
GSA17W	6.09	0.192	5.72-6.48
GSA17E*	5.65	0.178	5.31-6.01
GSA18	12.91	0.406	12.13-13.73
GSA22 Thermaikos gulf*	24.94	0.785	23.44-26.53
GSA22 Thracian Sea*	14.08	0.443	13.23-14.98

**Table 5.**  $CV_{geo}$  estimated marginal means along with its standard error and 95% confidence interval for a 10 nmi intertransect distance at the area level (disregards species and biomass levels). \* The analysis focused on 99% biomass threshold.

varied between 5.65 and 27.20% depending on the area (Table 5). No significant differences were found in terms of  $CV_{geo}$  between GSA18 and the Thracian Sea (GSA22), GSA1 and Thermaikos gulf (GSA22), GSA17E and GSA17W or between GSA6 and GSA7, while significant differences (p < 0.05) were evidenced for all the other cases (Table S4). Fitting the simulated  $CV_{geo}$  vs. intertransect distance by area and species (Fig. 2) showed good agreement with the observed average CV<sub>geo</sub> values (Table 2b; black dots in Fig. 2). The differences between the two species were assessed at the area level by considering an intertransect distance of 10 nmi and averaging  $\text{CV}_{\text{geo}}$  over the biomass level factor. The obtained results showed significant differences (p < 0.05) between the two species in all the considered GSAs except GSA16 and GSA9 (Table S5). Finally, to test for differences related to biomass levels by species and area (Fig. 3 and 4), ANCOVA was carried out for each considered case. Significant differences in CV<sub>geo</sub> were found in each area and for both species except for sardines in GSA17E and GSA18 (Table S7). No significant interaction between intertransect distance and biomass level was found for anchovies in GSA1, GSA16, GSA17E and GSA18 (Table S5) or for sardines in GSA16 (Table S6), highlighting similar slopes among biomass levels; in such areas, for the same intertransect distance, the difference in  $CV_{geo}$  between abundance levels was equal to the difference between intercepts. It is worth noting that, according to the pattern evidenced in the observed  $CV_{geo}$  values (Table 2a), a clear progression in slope or intercept values among the three biomass levels was found in a few cases, while in all the other cases, the regression lines of medium biomass years did not follow a clear pattern.

### Discussion

Acoustic biomass estimates are routinely used as tuning indices in stock assessment models, making the

precision of the estimates an important aspect. In this work, the precision of the acoustic survey was evaluated by considering the ability of the survey to adequately capture the high/medium density patches (accounting for 90% of the total biomass) under different biomass levels. In 4 out of 10 areas (GSA1, GSA17E and GSA22 in both the Thracian and Thermaikos Gulfs), the analysis relied on patches accounting for 99% of the total biomass, as for some years, it was not possible to obtain proper experimental variograms. Where the analysis focused on the 90% biomass threshold, the average  $CV_{geo}$  values (Table 2b) were lower than 15%. This result highlights that in such areas, the intertransect distance was correctly calibrated according to the study area characteristics and fish aggregation patterns. In particular, in highly productive areas driven by river run-off, such as the Gulf of Lion and the western Adriatic, the use of 10/12 nmi as the intertransect distance generally provides CV<sub>rec</sub> lower than 12% regardless of the biomass level, while in other areas, the higher sampling effort (lower intertransect distance) leads to  $CV_{geo}$  values close to or lower than 10%. In contrast, where it was necessary to focus on an higher biomass threshold implicitly highlighted, along with the higher CV<sub>geo</sub> values, that reducing the intertransect distance could help to improve the survey design efficiency (AcousMed report, 2012). Nonetheless, it must be considered that a reduction in the intertransect distance will increase the amount of time needed to cover a specific sector. Considering that the available vessel time is often limited and that one of the desirable characteristics of the survey is to provide a synoptic view of the spatial distribution and biomass of the targeted species, a reduction of the intertransect distance must always be carefully evaluated in terms of cost/benefit, accounting also for the relative importance (in terms of biomass) of the considered sectors. Furthermore, the analysis of pseudosurveys evidenced that, depending on the area, species, and changes in the spatial arrangement of fish populations, increasing the intertransect distance could lead to CV<sub>veo</sub> values well



*Fig. 2:* Linear fitting of  $CV_{geo}$  vs. inter-transects distance by area and species, disregard the biomass level. The average  $CV_{geo}$  observed for the current survey design (see table 2b) is reported in black. Shaded regions represent the 95% confidence intervals.

above 15% (Fig. 3 and 4) for one or both species; thus, under a precautionary approach, it is advisable to keep the current intertransect distance.

When CV<sub>geo</sub> was compared among GSAs where the analysis focused on the 90% biomass threshold and by considering the same intertransect distance (Table 5), a higher efficiency in detecting high-medium density patches was found in GSA6, GSA7, GSA9 and GSA17W. These areas are strongly influenced by river runoff affecting the primary production and habitat suitability of small pelagic fish species (Santoianni et al., 2006; Brosset et al., 2015; Feuilloley et al. 2020). Thus, it is possible to hypothesize that the presence of massive and well-localized enrichment mechanisms seems to favour a spatially consistent and coherent organization of fish populations captured well by the existing survey design. The obtained results thus evidenced that the selection of the "optimal" intertransect distance should start by considering the productivity of the area, the specific environmental and enrichment processes and, if available, information about the density-related spatial dynamics of target populations.

Only in a few cases (4 out of 20, anchovies in GSA1 and GSA7, sardines in GSA6 and GSA7) was a coherent and consistent decrease in  $CV_{geo}$  with increasing bio-

mass observed, whereas in GSA17W (for anchovies) and GSA17E,  $\mathrm{CV}_{_{\mathrm{geo}}}$  values were almost comparable among the different biomass levels. In all the other cases, survey design efficiency did not present a clear pattern with respect to biomass. For sardines in GSA6 and anchovies in GSA1, a coherent progression in  $CV_{geo}$  values was observed with decreasing biomass. In both cases, the spatial distribution of high-density values showed a coherent expansion with biomass level (Fig. S1 and S2). A coherent pattern between biomass levels and survey efficiency was also found in GSA7 for both anchovies and sardines, but here, an expansion of the high-density values with increasing biomass was not evident (Fig. S3), as in the abovementioned cases. It is important to highlight that in GSA7, a density-dependent mechanism on the occupied area was reported for sprats but not for anchovies and sardines. For these species, a positive relation was reported between packing density and biomass (Saraux *et al.*, 2014); thus, the lower  $CV_{geo}$  obtained here with increasing biomass levels could depend on changes in the internal structure of the patches, leading to a higher probability of detecting anchovy and sardine schools. Fish populations modify their spatial distribution depending on ecosystem characteristics, considering species biolo-



Fig. 3: Linear fitting of  $CV_{geo}$  vs inter-transects distance for anchovy by area and biomass level.

gy and the variation in biomass levels (MacCall, 1990; Hilborn & Walters, 1992; Petitgas, 1998; Shepherd & Litvak, 2004; Barange et al., 2005; 2009). Based solely on the density-dependent hypothesis, a higher efficiency of the survey design in years of high biomass values would be expected. In contrast, if patch size decreases with biomass or fish are concentrated only in specific locations, survey efficiency should decrease when fish biomass drops and fish become scarcer, *i.e.*, when information might be the most critical for managers. In this context, a consistent evaluation of the survey efficiency, along with a deep knowledge of the environmental factors influencing the spatial distribution and structure of fish populations, could help to develop adaptive sampling strategies, thus allowing us to better manage the survey efficiency during critical times of low biomass and avoiding the increase in survey time due to a reduction of the intertransect distance over the whole survey area. During low-biomass periods, specific adaptive sampling strategies could be developed and tested through simulation studies, considering different autocorrelation levels and intertransect distances, allowing the evaluation of different scenarios in terms of the cost-benefit ratio.

In contrast to the abovementioned cases, in GSA17W and GSA17E,  $CV_{geo}$  values showed limited variability

among the different biomass levels, indicating almost no effect of biomass on survey precision. This could be associated with the fact that the Adriatic Sea is a highly productive basin, generally characterized by a shallow extended continental shelf allowing fish populations to distribute over a wider area.

In other areas, a lack of a consistent pattern was observed. In particular,  $CV_{geo}$  values for medium-biomass years varied depending on the area, lower than in high-biomass years or higher than in low-biomass years. Anchovies and sardines in GSA16 presented the lowest CV<sub>geo</sub> estimate in the year of medium biomass. In the Strait of Sicily (GSA16), the main enrichment factor is related to the presence of upwelling, presenting high interannual variability in relation to the Atlantic Ionian Stream path (Bonanno et al., 2014) and strongly influencing the spatial distribution of small pelagics. Here, anchovy and sardine distributions also present two distinct patterns, as sardines are mainly distributed in the narrow zone between the Adventure and Maltese banks, while anchovies are also found over the two banks (Barra et al., 2015). The opposite, with respect to GSA16, was found for GSA9 (both for anchovies and sardines) and sardines in the Thermaikos Gulf (GSA22), where the highest value for CV<sub>geo</sub> was related to the medium-biomass year.



Fig. 4: Linear fitting of CV<sub>peo</sub> vs inter-transects distance for sardine by area and biomass level.

Concerning the Thermaikos Gulf, this is misleading, as in this area, a large part of sardine biomass is distributed within the inner part of the Gulf, outside the parallel transect area (the one evaluated here). In contrast to the above mentioned areas, where  $\mathrm{CV}_{\mathrm{geo}}$  in medium biomass years was the highest or the lowest, in GSA18, CV related to the medium-biomass year for anchovies was close to the one estimated for the low-biomass year, followed by a decrease in the high-biomass year. Finally, in the case of anchovies in GSA6, the Thermaikos Gulf and Thracian Sea (GSA22), and sardines in GSA18 and the Thracian Sea (GSA22), the CV<sub>gco</sub> related to medium-bio-mass estimates was close to the high-biomass estimates. The lack of clear patterns highlights the important role of the ecosystem in shaping the spatial structure of fish populations, providing evidence of a complex link among environmental characteristics, population dynamics and the efficiency of survey design. In this context, it is important to note that the size and location of the patches are influenced by physical features (Bertrand et al., 2008), such as the extension of upwelling areas (e.g., Strait of Sicily, GSA16), the presence of gyres or river plumes determining the degree of suitability of specific sectors (e.g., GSA9, Thermaikos Gulf, Thracian Sea) and the extension of the continental shelf (Giannoulaki et al.,

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2006). The obtained results thus evidenced the peculiarity of the Mediterranean Sea, characterized by highly diverse and patchy habitats in terms of productivity and topography. Furthermore, considering the case of sardines in the Thermaikos Gulf, the occurrence of a threshold effect that triggers spatial changes (Ciannelli *et al.*, 2012) due to population increases could also be the case in some of the considered areas. In this context, to better understand the link among survey design efficiency, spatial distribution, environmental variability and population dynamics, more than three years are needed for each study area along with a wide range of biomass to be considered taking into account environmental regimes.

Another consideration to keep in mind is that most acoustic surveys are multispecies, and their design has to accommodate more than one species. Here, we looked at how the efficiency varied according to species for sardines and anchovies, the two most targeted small pelagic species in the Mediterranean Sea. In certain GSAs, the efficiency of the survey design was found to be higher for anchovies than for sardines, even if the observed difference was generally lower than 5%. Summer coincides with the spawning period of anchovies (Basilone *et al.*, 2013; Giannoulaki *et al.*, 2013), while for sardines, this is largely the beginning of the recruitment period (Tugores *et al.*, 2011; Basilone *et al.*, 2021). Thus, anchovies are expected to concentrate around the most favourable and more productive areas for spawning, leading to a higher probability of encountering high- to medium-density patches. In contrast, in the case of sardines, generally characterized by denser aggregations than anchovies (D'Elia *et al.*, 2014), two different life stages coexist in the considered period (adults and juveniles), and the different spatial behaviour of the two life stages could enhance the picture of patchiness.

The evaluation of the survey design for some of the areas considered in this work had already been carried out in the framework of AcousMed project (AcousMed, 2012) but using a slightly different threshold biomass value (80% of the total biomass, thus focusing on high density patches) and on surveys carried out prior to the adoption of a common protocol (MEDIAS handbook, 2019).  $CV_{geo}$  values obtained in the AcousMed project were in the range of 4.7% to 42.3% for anchovies and 6.4% to 52% for sardines and showed generally higher  $CV_{geo}$  values compared to the one obtained in this work (Table 2b), thus evidencing the effect of considering only the patches accounting for 80% of the total biomass.

It must be noted at this point that acoustic biomass estimates are the result of a two-stage sampling (e.g., Simmonds & MacLennan, 2005); thus, several sources of uncertainty should be considered to evaluate the precision of biomass estimates. In particular, along with the error due to acoustic spatial sampling, the uncertainty due to biological sampling should also be considered. Biological sampling is a crucial aspect in acoustic surveys, as it allows to perform biological measurements and to link observed echoes and species. In this context, in mixed-species ecosystems, echo trace identification problems could also represent an important source of error (Petitgas et al., 2003). Even if the estimation of the area characterized by high-density values and the related precision represents a useful tool to monitor the efficiency of the survey, confidence intervals for biomass estimates should take into account all the above-mentioned sources of variability.

### Conclusion

Acoustic surveys carried out in the Mediterranean Sea under the EU umbrella showed  $CV_{geo}$  values generally lower than 15%, thus indicating in most of the cases that the chosen interttransect distance is adequate to characterize the sectors where most of the biomass is concentrated. Furthermore, even though a significant difference between anchovies and sardines was found in terms of survey design efficiency, the observed average differences were generally not important in terms of magnitude and in most of the considered areas were lower than 5% (Table 2b), highlighting that the adopted intertransects distance is suitable for both species.

In the sectors where higher  $CV_{geo}$  were found and the analysis relied on the 99% biomass threshold rather than on the high/medium density patches, the evaluation of

whether to decrease the intertransect distance should be made carefully, according to the relative importance (in terms of biomass) for the stock of the specific sectors analysed here and bearing in mind that the survey should be as synoptic as possible.

Only in a limited number of cases was a positive effect of increasing biomass on the precision evidenced, highlighting the important role of environmental factors, but this aspect should be further investigated to better understand the mechanisms behind the observed differences. In particular, a wider range of biomass levels, longer time series and specific analyses considering the environmental importance in shaping the spatial structure of fish populations should be considered.

A consistent evaluation of survey precision, along with a deep knowledge of the environmental factors contributing to shaping the spatial structure of fish populations, could help to develop adaptive sampling strategies, allowing to better manage survey precision during critical times of low biomass.

Finally, the regression parameters relating intertransects distance and  $CV_{geo}$  obtained for a wide range of Mediterranean Sea areas could represent a reference for setting up acoustic surveys in new areas, obtaining different possible scenarios in terms of  $CV_{geo}$  according to different intertransect distances.

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### **Supplementary Data**

The following supplementary information is available online for the article:

Table S 1. Biomass estimated by multiplying the average fish density by the polygon surface in the GSAs where only a specific sector was considered (See polygons in Fig. 1). For sardine in the Thermaikos Gulf, the biomass found in the polygon in the low biomass year (related to the whole area as reported in Tab. 1) was higher than the one found in the medium abundance year.

Table S 2. Variogram model parameters related to European anchovy. For each area and year, the nugget, the model type, the anisotropy coefficient, the sill and range are reported.

Table S 3. Variogram model parameters related to European sardine. For each area and year, the nugget, the model used, the anisotropy coefficient, the sill and range are reported.

**Table S 4.** Comparisons by area among  $CV_{geo}$  estimated marginal means considering an inter-transect distance of 10 nmi. **Table S 5.** Comparisons of the estimated  $CV_{geo}$  marginal means between species (ANE: anchovy; PIL: sardine) in each area considering an inter-transect distance of 10 nmi.

Table S 6. p-values for the fixed and interaction terms in the ANCOVA models used to test for differences among high, medium, and low biomass levels for anchovy in each considered area. For each area was first evaluated the presence of interaction between the inter-transect distance (itr) and the biomass level factor.

Table S 7. p-values for the fixed and interaction terms in the ANCOVA models used to test for differences among high, medium, and low biomass levels for sardine in each considered area. For each area was first evaluated the presence of interaction between inter-transect distance (itr) and biomass level factor.

Figure S 1: European anchovy and sardine maps, experimental variograms and fittings in GSA1. The red points in each map represent the EDSU accounting for the 99% of the total biomass. In the case of variograms accounting for spatial anisotropy, the red lines and dots represent the across-transect direction while the black ones the along-transect direction. All the variogram models were fitted by using a weighted automatic fitting procedure.

Figure S 2: European anchovy and sardine maps, experimental variograms and fittings in GSA6. The red points in each map represent the EDSU accounting for the 90% of the total biomass. In the case of variograms accounting for spatial anisotropy, the red lines and dots represent the across-transect direction while the black ones the along-transect direction. All the variogram models were fitted by using a weighted automatic fitting procedure.

Figure S 3: European anchovy and sardine maps, experimental variograms and fittings in GSA7. The red points in each map represent the EDSU accounting for the 90% of the total biomass. In the case of variograms accounting for spatial anisotropy, the red lines and dots represent the across-transect direction while the black ones the along-transect direction. All the variogram models

were fitted by using a weighted automatic fitting procedure.

**Figure S 4:** European anchovy and sardine maps, experimental variograms and fittings in GSA9. The red points in each map represent the EDSU accounting for the 90% of the total biomass. In the case of variograms accounting for spatial anisotropy, the red lines and dots represent the across-transect direction while the black ones the along-transect direction. All the variogram models were fitted by using a weighted automatic fitting procedure.

**Figure S 5:** European anchovy and sardine maps, experimental variograms and fittings in GSA16. The red points in each map represent the EDSU accounting for the 90% of the total biomass. In the case of variograms accounting for spatial anisotropy, the red lines and dots represent the across-transect direction while the black ones the along-transect direction. AllGSA16 the variogram models were fitted by using a weighted automatic fitting procedure.

**Figure S 6:** European anchovy and sardine maps, experimental variograms and fittings in GSA17W. The red points in each map represent the EDSU accounting for the 90% of the total biomass. In the case of variograms accounting for spatial anisotropy, the red lines and dots represent the across-transect direction while the black ones the along-transect direction. All the variogram models were fitted by using a weighted automatic fitting procedure.

Figure S 7: European anchovy and sardine maps, experimental variograms and fittings in GSA17E. The red points in each map represent the EDSU accounting for the 99% of the total biomass.All the variogram models were fitted by using a weighted automatic fitting procedure.

**Figure S 8:** European anchovy and sardine maps, experimental variograms and fittings in GSA18. The red points in each map represent the EDSU accounting for the 90% of the total biomass. In the case of variograms accounting for spatial anisotropy, the red lines and dots represent the across-transect direction while the black ones the along-transect direction. All the variogram models were fitted by using a weighted automatic fitting procedure.

**Figure S 9:** European anchovy and sardine maps, experimental variograms and fittings in GSA22 Thermaikos gulf. The red points in each map represent the EDSU accounting for the 99% of the total biomass. All the variogram models were fitted by using a weighted automatic fitting procedure.

Figure S 10: European anchovy and sardine maps, experimental variograms and fittings in GSA22 Thracian Sea. The red points in each map represent the EDSU accounting for the 99% of the total biomass. All the variogram models were fitted by using a weighted automatic fitting procedure.