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ACOUSTIC BACKSCATTER OF SMALL PELAGIC FISH SPECIES IN THE ADRIATIC SEA

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Summary

Acoustic pelagic trawl surveys are highly effective approach to estimate the biomass and distribution of pelagic species worldwide. But, in order to convert the backscattering volume strength, indicator of fish density, to numerical abundance estimates, the species-specific Target Strength (TS) function must be known. TS is a measure of the energy reflected by a single fish of a certain length in relation to several physiological and physical factors which determines its stochastic nature and challenges for its determination. Since TS is strongly related to the size of the backscattering object, it is commonly accepted the use of a species-specific conversion parameter b_{20} arising from the relation between TS and the 20log of Total Length (TL) of insonified fish, for the conversion of acoustic backscatter into fish abundance. In the Mediterranean Sea scientific acoustic surveys have been carried out since 1970's, focused on the study of a few species important for the fishery. The latter have been subjected to empirical TS experiments, while the TS of non-target species, less valuable for the fishery, has seldom been investigated in this basin. The aim of the present study is to apply a broad range of techniques to measure for the first time the TS of two ancillary species, *Scomber colias* and *Trachurus mediterraneus* and to increase the knowledge of acoustic properties of *Sprattus sprattus*, in the Adriatic Sea, where it is considered a key species in the pelagic ecosystem.

A novel study using tethered live fish but not involving hooks and anesthetic was tested on *T. mediterraneus* and *S. colias* through several *ex situ* experiments using a split-beam scientific echosounder operating at 38, 120, and 200 kHz. The proposed method removes some potential biases due to the unnatural behavior of anesthetized fish and echoes coming from the system, trying to retrieve the natural behavior of alive fish into natural environment. The mean TS was estimated for 29 live specimens of *T. mediterraneus* and *S. colias*, resulting in a conversion factor b_{20} value of -71.4 dB re 1 m^2 and -71.6 dB re 1 m^2 respectively which is ~3 dB lower than the current one in use in the Mediterranean Sea.

Successively, the acoustic data collected during two monospecific trawl hauls were analyzed through the application of *in situ* approach for the computation of TS values of *S. sprattus*. Due to the small dataset, we proposed two alternative approaches and three known methods to overcome problems concerning post-processing data analysis when only a small number of monospecific hauls are available. Different combinations of the aforementioned methods led to six b_{20} values for sprat (range, -68.8 dB re 1 m^2 to -65.6 dB re 1 m^2), all higher than the current known value of -71.7 dB re 1 m^2. Nevertheless, the results coming from the new methods proposed herein have to be still considered preliminary. Accordingly, we suggested a value of b_{20} between -67.5 dB re 1 m^2 and -68.8 dB re 1 m^2 for sprat in the Mediterranean Sea based on the conservative combinations of the aforementioned

approaches. The high difference up to 4.2 dB compared to the current value translates in a significant decrease of absolute sprat biomass along the time series un to 20%. However, the lack of confidence in the proper sprat's b_{20} value led to an overall uncertainty in absolute biomass estimates up to 20%.

Finally, 149 specimens of the three species were collected during the Mediterranean International Acoustic Survey (MEDIAS) acoustic surveys carried out in the Adriatic Sea, in 2020 and 2021. Digital images of the fish body and swimbladder were obtained from Computer Tomography (CT) and X-Ray scans for the reconstruction of 2D and 3D-models of fish body and swimbladder morphologies. With the 2D and 3D-models enabled the computation of theoretical prediction on the acoustic reflectivity from complex surfaces using two backscattering models (i.e. Kirchhoff-ray mode model (KRM) and Finite Element Method). KRM and FEM allow the adjustment of the main parameters affecting the TS which was computed as a function of tilt angle and frequency obtaining the multi-frequency and the broadband fingerprint of the three species. The results of KRM on conversion parameters for *S. colias* and *T. mediterraneus* are in agreement with empirical results when considering a negative tilt angle of 10° during model computations, as may be expected from fish swimming abnormally, otherwise they slightly diverge. Conversely, for *S. sprattus* the b_{20} found by the FEM model felt exactly in the range of values recommended from the empirical evidence found here.

In general terms, the present work proposes the acoustic backscatter characterization of *S. colias*, *S. sprattus* and *T. mediterraneus* in the Mediterranean Sea. The empirical results reported in the present study, validated by the theoretical computations, have the potential to become new reference values for the biomass assessment of the three species in the Mediterranean Sea. Moreover, we gave the first insights into the use of broadband and relative frequency response methods as diagnostic tools for the correct identification of the species that can be implemented during post-processing acoustic data analysis. The present work stands for the first acoustic backscatter characterization of *S. colias* and *T. mediterraneus* worldwide and the second never carried out on sprat in the Mediterranean Sea.

1. Introduction

1.1 Fisheries acoustic background

1.1.1 History

The use of echosounder for fish detection goes back in time to 1929 when Kimura published the first echogram on greyscale paper marked two sprat fish schools in Norwegian waters (Anon, 1934; Kimura, 1929; Sund, 1935). Soon after World War II, advances in acoustics instruments resulting from military application, led to their use in fisheries research to describe the horizontal and vertical distribution of pelagic fish (Cushing, 1952; Trout et al., 1952). New equipment specifically designed for fish detection assisted by multi-colored echograms was developed (Simmonds and MacLennan, 2005). This marked a turning point for fisheries biologists who started to investigate the potential estimation of fish abundance through the simple calculation of single echoes or the summation of echo amplitudes called echo counting method (Mitson and Wood, 1962; Richardson et al., 1959). This method is still applied in particular condition, however, a data processing milestone was the introduction of echo integration (Scherbino & Truskanov, 1966). This methodology was successfully used in several survey cruises in the late 1960's and the 1970's for estimating fish abundance (Midttun & Nakken, 1977). Echo integration played a key role in transforming acoustic measurements from qualitative to quantitative data. Ever since the beginning of the acoustic surveys, the method has demonstrated a high potential to detect and monitor acoustic targets such as small pelagic fish, but in the early days, due to the imprecision of calibration method and uncertainty linked to the equipment performance, the results were characterized by large bias (Demer at al., 2015). Echosounder systems have evolved from single-beam and single-frequency analogue systems to split-beam multi-frequency digital systems change through the use of the dual-beam echosounder. The most recent split-beam transducer allows the acquisition of the precise location of the target in the beam making possible the characterization of single targets (Foote, 1987). By knowing the position of a target in the transducer beam, the beam pattern loss could be estimated, affecting directly the measurement of fish target strength (TS). This kind of transducer is divided in four quadrants and the target direction is determined by the differences in echo phase among each quadrant (Figure 1.1.1). The evolution from single-frequency (narrow band) to multiple-frequency systems along with the transition from analogic to digital technologies has improved the ability to characterize the scattering targets facilitating the processing of echo data from the 1970s (Jech and Michaels, 2006). Digital signal processing systems were introduced followed by the development of software to assist in data storage, retrieval, visualization, scrutinizing, and quantification (Simmonds and MacLennan, 2005).

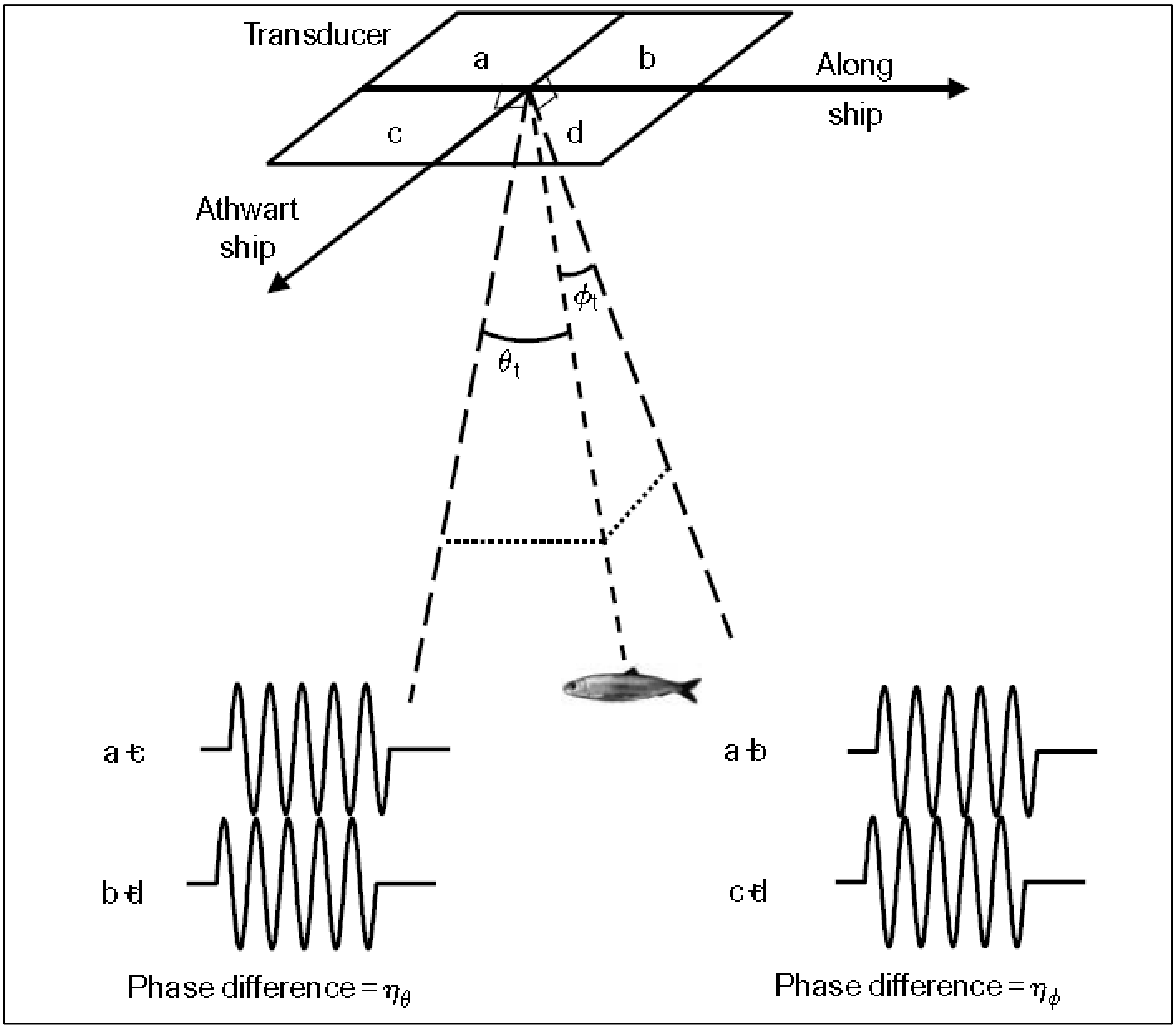

Figure 1.1 1 Split beam echosounder. Source: Simmonds and MacLennan, 2005

The man–machine interface principle was enunciated in order to maintain operator control of all critical decisions through the digital process. This principle was adhered to the 1990s, establishing the standard for subsequent commercial echo data post-processing systems (Foote et al., 1991). Following the fast development of fisheries acoustics the ICES Fish Capture Committee brought the foundation of a dedicated working group of the International Council for the Exploration of the Sea, the Working Group on Fisheries Acoustics Science and Technology (WGFAST) in 1984 (ICES, 2021). It gathers scientists from all over the world to discuss ongoing developments in acoustic surveys, being the major international working group in fisheries acoustics. Presently, a great deal of monitoring studies on small pelagic fish is done through acoustic surveys worldwide.

In the last fifty years, active acoustic methods have been routinely used worldwide in fisheries research with the goal to estimate single-species abundance indices for direct input into stock assessments (Misund, 1996). The study of fish population dynamics by acoustics methods provides data that are not easy to obtain using other fishery-independent approaches such as diver visual census, trawls and optical tools involving camera sledges (Leonori et al., 2021). Acoustic measurements are remote, less invasive and non-extractive and have the advantages of providing high-resolution horizontal as well as vertical data over large spatial and temporal scales (Chu, 2011). Acoustic instruments are unique recording continuously fish and plankton throughout the water column (Holliday et al., 2009; Leonori et al., 2017), in contrast to fish capture methods over specific depths as accomplished by trawls, nets and similar tools. Acoustics measurements, combined with direct observation (*e.g.* trawl sampling to identify target size and species composition), allow estimating the abundance and biomass of biological communities by species or species groups.

1.1.2 Transducers and sound propagation

The scientific echosounder system used in fisheries acoustic is composed of: (a) display - (b) processor unit - (c) Ethernet switch - (d) transceiver - (e) transducers as shown in Figure 1.1.2. The transducer which is made from piezo-electric ceramic materials, is usually placed on the vessel keel (Figure 1.1.3) or on platforms (Figure 1.1.4). It is linked to the transceiver which converts voltage into sound signal (sound waves) and vice versa, transmitting to the processor unit that amplifies, filters, removes noise and digitalizes the signal making the echogram on the display (Furusawa,

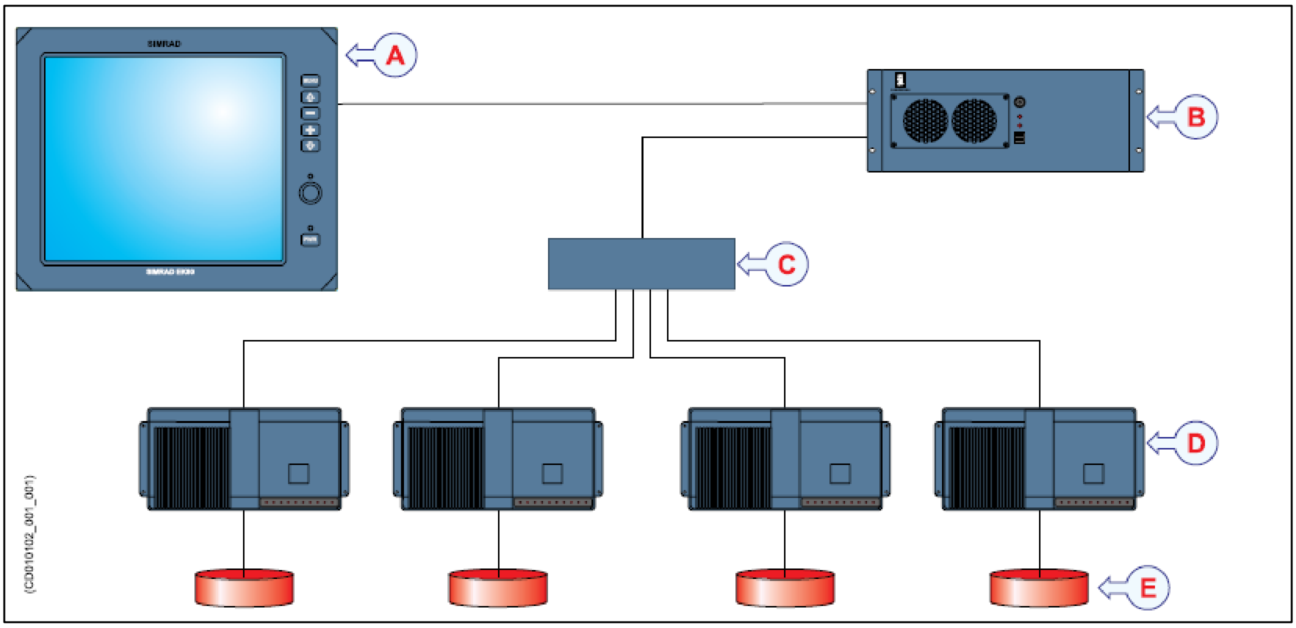

Figure 1.1 2. Echosounder diagram: E = transducers, D = Transceiver, C= switch, B = processor unit, A = Display. Source: SIMRAD EK80 Reference Manual

1991).

Figure 1.1 3. Blister mounted on the keel of R/V G. Dallaporta with four transducers: 38, 70, 120, 200 kHz (photo: G. Canduci)

Through the employment of transducers operating at different frequencies, it is possible to send at the same time pulse at several wavelengths:

$$\lambda = cf \qquad \text{(Eq. 1.1.1)}$$

in which c is the sound speed in the water and f is the frequency: e.g. with a 38 kHz transducer, considering a typical sound speed in salt water of 1500 m/s, λ is about 3.9 cm. The pulse transmitted by the sonar comprises a few cycles of pressure waves which lasts for a finite time depending on the pulse duration. The travelling waves transport energy from one place to another. The acoustic intensity depends on the energy flux per unit of time I passing through a unit of area span by the wave-front J. Where J is the integral of I with respect to time. By definition, the total energy carried out by the wave E, equals the integral of J per unit of area over the surface of the wave-front. Namely, it should be described as the total energy transmitted over the finite pulse duration because it may vary among cycles, especially in the case of short pulse (Craig, 1983). The instantaneous intensity is the product of the pressure and the particle velocity. In a plane wave, the intensity is proportional to the pressure squared:

$$I = p^2 /\rho c \qquad \text{(Eq. 1.1.2)}$$

The echo energy is the fundamental principle in the propagation of sound for fisheries acoustic because it is proportional to the observed number of fish and it is directly related to the characteristic of the target, leading to the basis of the echo integration. Greater is the discontinuity in acoustic impedance across boundaries of a target, strongest is the scattered wave: $impedance = pc$ where p is the sound pressure and c is the sound speed (Foote, 1980).

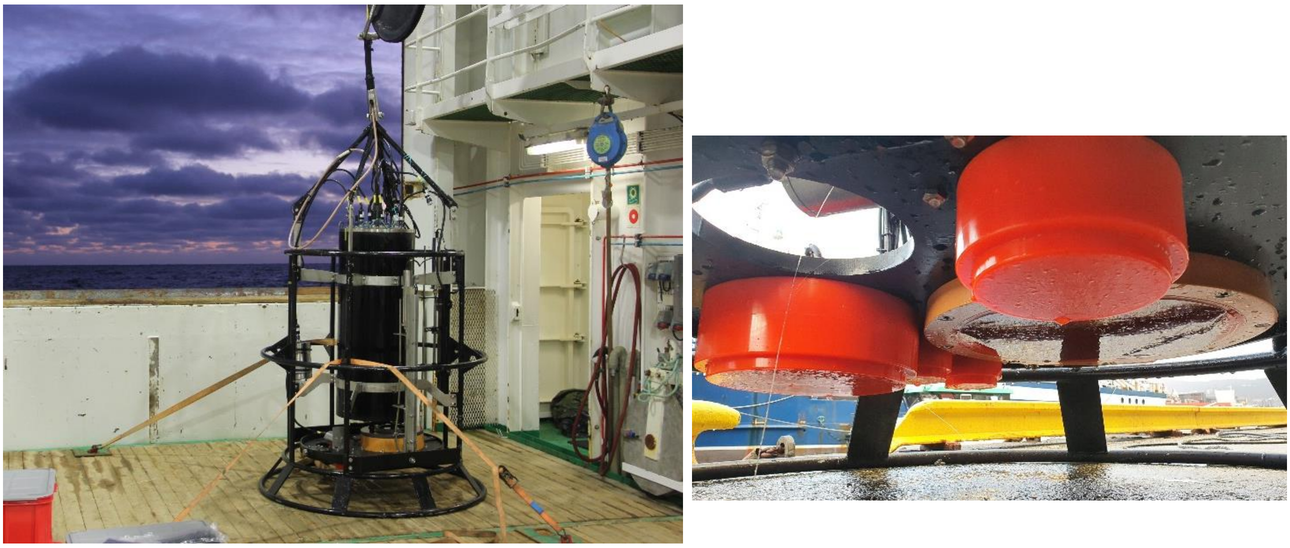

Figure 1.1 4. TS probe of Institute of Marine Research, Bergen, Norway, equipped with five echosounders: 38, 70, 120, 200, 333 kHz shown on the right (photo: R. Kubilius and A. Palermino 2021)

"A target is an object with a boundary across which there is a discontinuity in the acoustic impedance" (Simmonds and MacLennan, 2005). The wave-front backscatter from a small target with respect to wavelength is nearly spherical and E is finite. When acoustic waves match a small target, some of the incident energy is scattered, generating a secondary wave which propagates in all directions away from the target. The energy reflected back towards the sound source is said to be backscattered. When the acoustic wave propagates towards a large smooth surface, the secondary wave is confined to one direction at an opposite angle with respect to the incident wave (Figure 1.1.5). Depending on the material and the dimensions of the surface, the total energy may be almost entirely reflected. The intensity of scatter is variable depending on the dimensions compared to the wavelength: small targets contract and expands in response to the oscillation of sound pressure acting as a source of scattered waves which propagate spherically in all directions (Figure 1.1.6). Nevertheless, the direction of the backscatter is heavily affected by the direction of the target. Consequently, the scattering depends on the volume and matched surface rather than the shape. When the size (L), expressed in cube root of the volume, is $<< \lambda$, the scattered energy is proportional to $(L/\lambda)^4$ and the Rayleigh scattering is appropriate, while when $L >> \lambda$, the scattering is said geometric and it is the shape rather than the volume of the target that determines the scattering (Clay and Heist 1984). When $L \sim \lambda$, the geometric structure and the material properties of the target determine the scattering in a more complex way. In the geometric region the scattering strength of a gas bubble at higher frequencies is almost constant while in the Rayleigh scattering region there are one or more resonance frequencies, which cause a rapid change in the intensity of scattering with frequency (Simmonds and MacLennan, 2005).

However, the wavelengths commonly used in fisheries acoustics are related to the size of discrete scatterers such as swimbladder dimensions (Love, 1971).

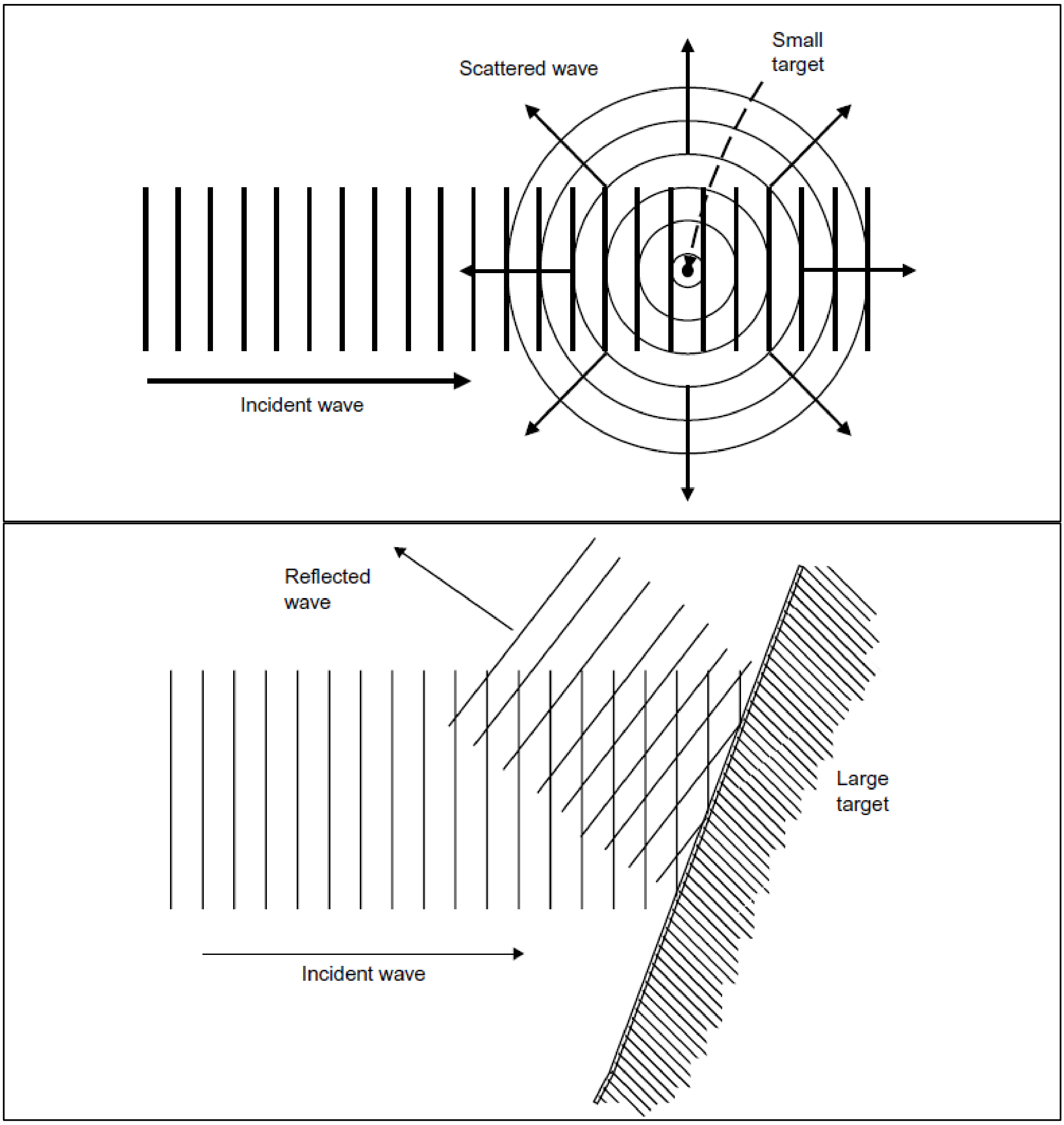

Figure 1.1.5 Scattering of sund by a small target on the top and scattering of sound by a large target below.
Source: Simmonds and MacLennan, 2005

The inhomogeneous structure of fish and their variable orientation yields different echo phases; when they interface with one another, they cause fluctuations in echo amplitude, introducing some incoherence otherwise, the echo amplitude is coherent (Demer et al., 2009). The ratio between the sum of coherent (e_c) and incoherent (e_d) components is expressed by $\gamma = \frac{e_c^2}{e_d^2}$.

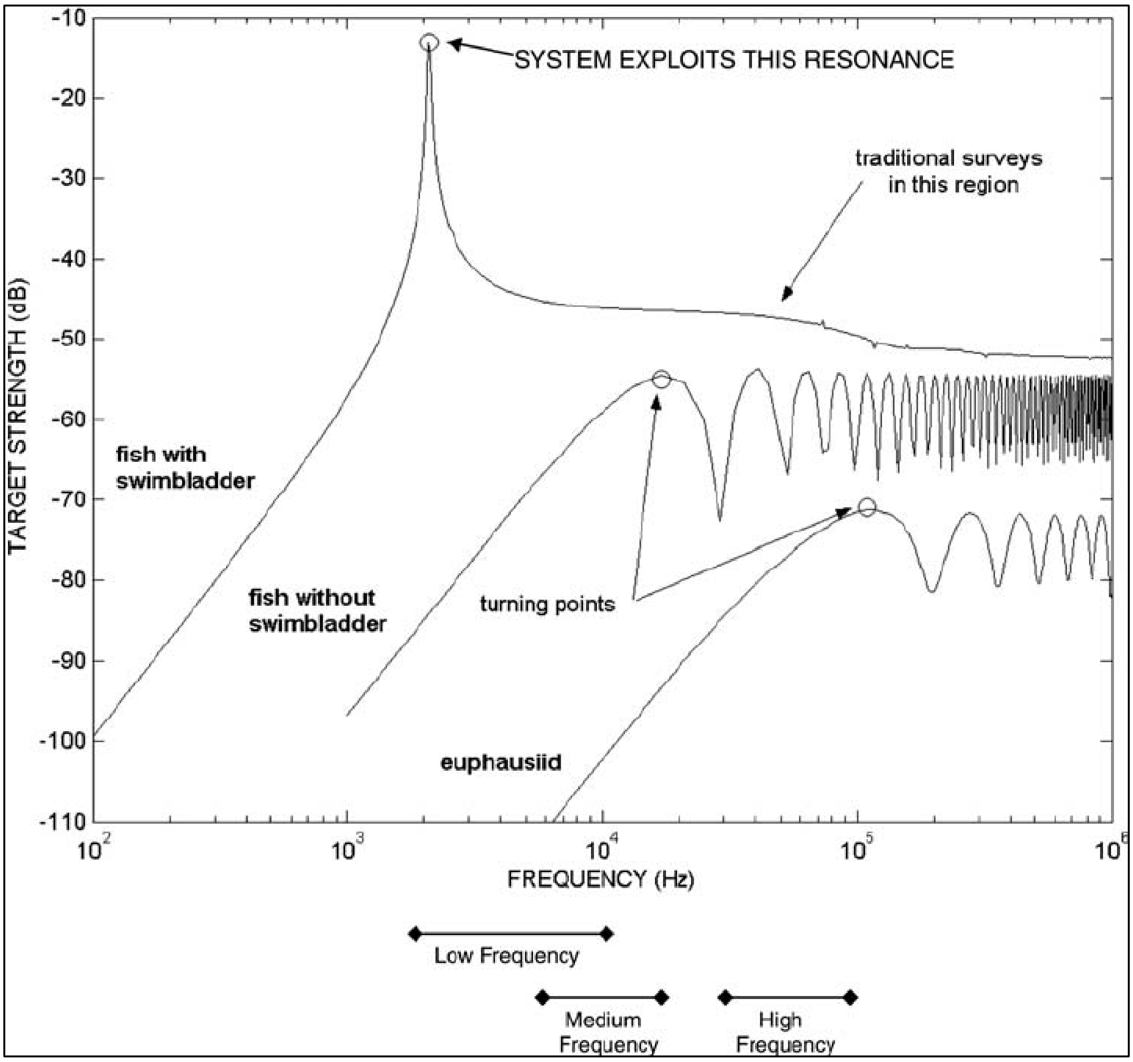

Figure 1.1.6. Target strength by organism categories along frequency domain.

For higher γ values, the PDF approaches a Gaussian distribution, whereas when γ is close to zero the PDF approaches a Rayleigh distribution (Clay and Heist, 1984). Consequently, the scatter distribution can change rapidly, leading to more complicated variations in relation to size and frequency than are suggested by simple geometrical models. Due to the above considerations, it is therefore generally accepted that the scatter of active fish shows a Rayleigh-like distribution (Stanton et al., 2004).

The backscatter of a single target in expressed in terms of mean backscattering cross section (σ_{bs}) that is the ratio of the incident intensity (*I1*) and the reflected intensity at 1 m from the target (*I2*): $I2/I1$ expressed in m^2/m^2. Nevertheless, the acoustic measurements are quoted in decibel (dB) units which is a logarithmic measure of the ratio of two intensities in Pascal (Pa). The decibel unit is used to describe the acoustic reflectivity of targets, namely the Target Strength (TS) which will be discussed in more detail in section 1.2.

1.1.3 Calibration

The calibration of acoustic instruments employed in the echo integration is based on the measurement of the backscattering cross-section of a known target. In order to avoid any bias, it is commonly used a sphere of known material made from tungsten carbide with 6% cobalt binder. Knowing the material proprieties and shape, the backscattering energy depends only on the dimension of the target which is also known. The calibration process is intended to check the distribution of the targets in the acoustic beam as well as the physical response of the instrument through the on-axis sensitivity, the Time-Varied Gain (TVG) and the beam pattern. The on-axis sensitivity is obtained by computing the direction in which the transmitted energy is greatest, where the backscatter from any targets is largest inside the acoustic beam at a constant range. The transmitted source level and the receiving voltage response is determined in this direction to compute the gain, which is defined by the intensity ratio at a certain distance keeping electrical power constant. The sensitivity changes with direction and the gain change with range. These differences in the backscatter energy are corrected through the TVG which takes into account the range dependence of echo in terms of delay between the echo and the transmitted pulse. The beam pattern is defined by the sound pressure propagation as a function of spatial angle of the transducer. If the transducer is a disc, the beam pattern is symmetrical and in any plane the angle from the axis is θ (Foote, 1987). It is represented in polar diagram and it is composed by a main lobe, where the sensitivity is high and side lobes, where the sensitivity is lower, interspersed by nulls where there is no sensitivity (Figure 1.1.5). The signal of a fish located between lobes in the nulls cannot be detected, it will be very weak if it is located in a side lobe while it will be at strong intensity if it is located in the main lobe.

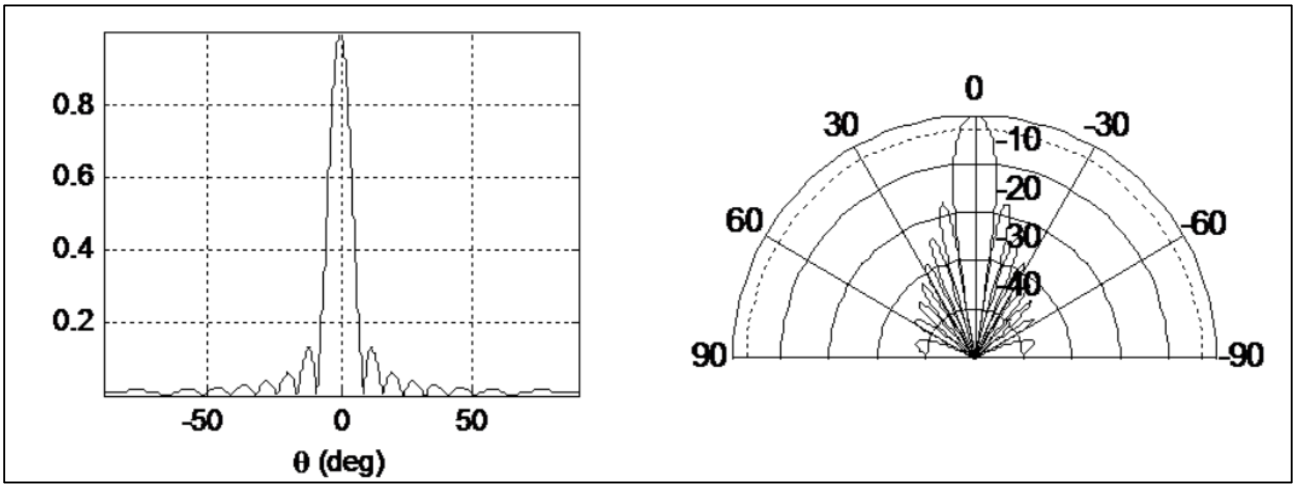

Figure 1.1 6. Examples of a beam pattern with a diameter equal to 50 wavelengths. The directivity pattern is shown in dB in the y axis dimensionless (left) and real (right) while the off axis angle of beam θ is shown in the x-axis. Source: Demer et al., 2015

The beam angle is usually defined as the measurement of the total angle where the sound pressure level of the main lobe has been reduced by 3 dB on both sides of the on-axis peak. It describes the beam width of a transducer. Often the beam pattern is measured as the equivalent beam angle, ψ, which is: "the solid angle at the apex of the ideal conical beam which would produce the same echo-integral as the real transducer when the targets are randomly distributed in space" (Simmonds and MacLennan, 2005). It describes the beam pattern in space with the spherical polar coordinates, θ (theta) and ϕ (phi) to solve the position of any point relative to the transducer. The equivalent beam angle is a fundamental measure for detecting the correct position of the single targets during the target strength measurements.

A sphere of known material, size and TS is usually moved throughout the acoustic beam to check the beam pattern characteristic, measure the variations in sensitivity due to the beam directivity calibration process and compute the gain. For the echo integration purpose, it would be enough to get the value from the centre of the main beam to adjust the gain, where the echo is strongest, but the calibration of the whole beam is needed for TS estimations (Demer et al., 2015). Figure 1.1.6 show a classical calibration procedure for TS estimates carried out with SIMRAD EK80 echosounder.

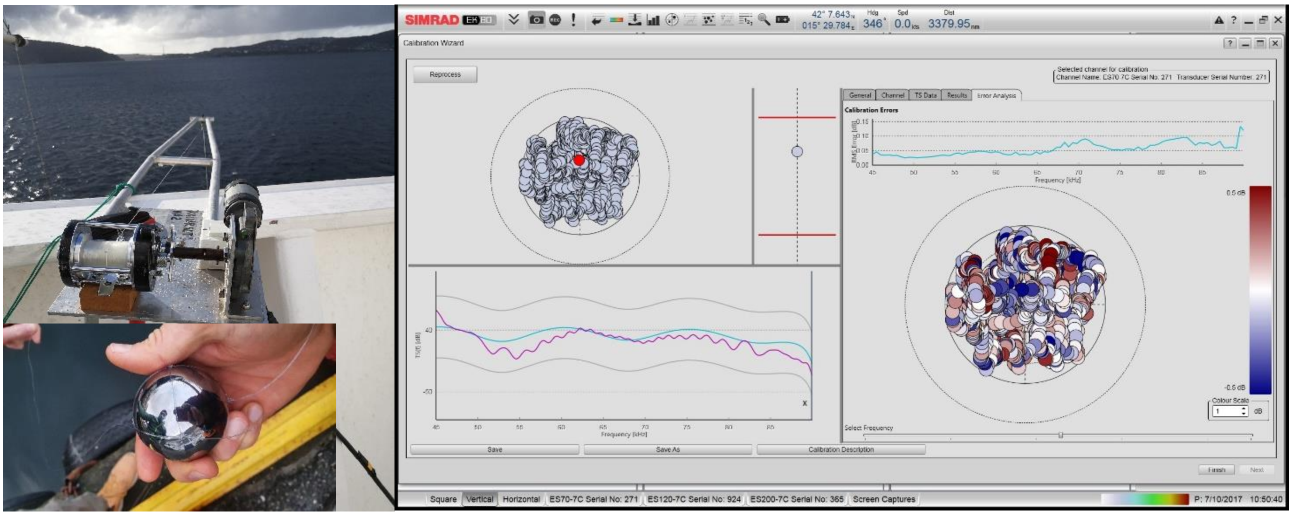

Figure 2.1.6. Calibration procedure. On the left the tools for calibration process: a 38.1 tungsten carbide calibration sphere and an automated road; on the right an example of calibration results of the whole beam obtained with EK80 in broadband (photo: A. Palermino 2021)

1.1.4 Acoustic measurements and echo integration

When there are many fish it is no longer possible to distinguish the single echoes by echo counting, but the echo intensity in the sample volume is still a measure of abundance in the water column. As a matter of fact, the basic acoustic measurement is expressed in terms of volume backscattering coefficient, s_v. It is obtained from the echo integral through the following formula:

$$s_v = \Sigma\sigma_{bs}/V_0 \ \ (\mathrm{m^2/m^2}) \qquad \text{(Eq. 1.1.3)}$$

Where V_0 is the sampled volume and σ_{bs}, is the backscattering cross-section, which describes all the single targets contributing to the echoes in that volume. s_v is more often displayed in terms of volume backscattering strength, which is the equivalent logarithmic measure,

$$S_v = 10log\ (s_v).\ \ (\mathrm{dB\ re\ 1\ m^2}) \qquad \text{(Eq. 1.1.4)}$$

The standard volume over several numbers of pings is called mean volume backscattering strength (MVBS). Similarly, the energy returned from a layer between two depths (*r*) is defined by the integral of S_v as the area scattering coefficient:

$$s_a = \int_{r_1}^{r_2} s_v \quad (\mathrm{m^2/m^2}) \qquad \text{(Eq. 1.1. 5)}$$

s_a is the product of s_v and distance which is dimensionless since its unit should be written as $\mathrm{m^2/m^2}$, meaning the integrated σ_{bs} per square meter of the layer surface. Notably, the fundamental parameter in fisheries acoustic for the measurements is the nautical area scattering coefficient (NASC):

$$S_a = 4\pi 1852^2 s_a\ (\mathrm{m^2/nmi^2}) \qquad \text{(Eq. 1.1.6)}$$

It is a measure of the abundance in terms of energy reflected and can be converted through the echo integration equation in the number of specimens per $\mathrm{nmi^2}$ (Ehrenberg and Lytle, 1972):

$$\rho_a = \frac{S_a}{4\pi < \sigma_{bs} >} \qquad \text{(Eq. 1.1.7)}$$

Where $< \sigma_{bs} >$ is the average backscattering cross-section.

The sum of the energy in echoes returned from selected part of the echograms can be converted into fish abundance through the echo integration. this is done by first squaring the digitized voltages sampled by the echosounder, then summing the results with respect to time or distance, over depth intervals corresponding to the depth channels or polygons (Simmonds and MacLennan, 2005):

$$E_i = \int_{t_1}^{t_2} |v(t)|^2\ dt \qquad \text{(Eq. 1.1.8)}$$

Where $v(t)$ is the voltage produced by the echosounder at time *t*. Therefore, the echo integral over many transmissions (N) is defined by:

$$E = \sum_{i=1}^{N} E_i / N \qquad \text{(Eq. 1.1.9)}$$

The echo integration assumes that the transducer is on a moving ship and the fish are also mobile in the acoustic beam. Thus many different fish will contribute to the total echo energy (E). When the number of detected fish is large, and when they are randomly distributed over the beam cross-section, then E is proportional to the number of fish per unit area. Hence, it is supposed that echo integration is a linear process, assuming that the output is proportional to the number of targets having similar proprieties. The linearity principle requires that the mean sampled value of the echo energy from the *n* targets will be the same as the sum of all the echo energies that would have been observed if each target were insonified separately. The two main assumptions required for linearity are that (i) the targets are randomly distributed in the insonified space, ensuring that the phases of the echoes from each target are randomly distributed relative to each other, and (ii) the targets move so that a new random set of phases is generated from each transmission. The echo energy from a single sample from the i'th ping is E_i. All the E_i will be different because of the changing echo phases and target locations within the beam and, at least for live targets free to behave naturally, the variability of the echoes from each target arising from the stochastic nature of the target strength (Simmonds and MacLennan, 2005).

The typical operating frequency used for small pelagic biomass estimate in echo integration is 38 kHz (O'Driscoll, 2004; Leonori et al., 2017; Leonori et al., 2021). This ultrasonic frequency is shown to be near the optimum for achieving the detection of commercially important fish in the presence of attenuation due to spherical spreading and absorption (Love, 1971). Additionally, 18, 70, 120, 200 and 333 kHz are employed during acoustic surveys to discriminate between target fishes and schools from other non-target marine organisms such as zooplankton. In fact, marine organisms without air inclusions such as swimbladderless fishes, liquid-like and hard-shelled zooplankton are characterized by a wake echo with stronger reflection at higher frequencies, due to the wavelength dimensions as shown in figure 1.1.6. E.g. the common mackerel (*Scomber scombrus*) doesn't have a swimbladder and at 38 kHz reflects around 20 dB less than swimbladder fish of the same size. This is a logarithmic value, therefore it means a difference of over 100 times (Scoulding et al., 2016). However, the echo integration method by itself cannot identify the species and length-frequency distribution (LFD) of target fish, therefore this is determined by trawl samples. A big effort was done during the past 30 years for the automatic identification of fish shoals and schools based on geometrical, locations and s_a descriptors (Fernandes, 2009; Iglesias et al., 2003; Scalabrin et al., 1995), but still some uncertainty remains. The multi-frequency technique improved the characterization of fish through the application of the relative frequency response formula published by Fernandes et al (2006) and by Pedersen and Korneliussen (2004). It is defined as the ratio between the s_v or σ_{bs} of a specific frequency and the reference frequency (38 kHz) (Pedersen et al., 2004; Fernandes et al., 2005). The resultant patterns sometimes are species-specific and are integrated in the classification process of post-processing software (i.e. Large Scale Survey System (LSSS) (Korneliussen et al., 2006). The developments of new echosounders able to send a broadband pulse started in 2010 (Stanton et al., 2010) can mark a turning point in the contribution of distinguishing marine organisms and sizes during echo integration, leading in a long perspective to overcome the necessity to carry out trawl samples. Modern echosounders can operate a complete range of frequencies from 10 to 260 kHz exploiting the principle of sending an chirp acoustic pulse which comprises a certain bandwidth, which can be successively converted in a single acoustic signal by exploiting the match filter and pulse compression equations (Reeder et al., 2004). In the future it could open the possibility to perform acoustic surveys by autonomous vehicles only, widely employed currently in parallel of research vessels all over the world (ICES 2021).

Acoustic methods have been routinely used worldwide since 1950 to evaluate population abundance, biomass, species temporal and spatial distribution (Leonori et al.,2021). The inclusion of acoustic indices in stock assessment models improves their accuracy and, consequently, the management of small pelagic fish stocks (Korneliussen, 2018). Fisheries-independent information is often free of many of the biases that characterize fisheries catch and/or landing statistics as well as the fisheries-derived indices of abundance, like the catch per unit effort (CPUE). This is often the case in small pelagic fishes due to how the fishery operates when an increasing trend in landings does not necessarily correspond to an increasing trend in the population and vice versa (Lucchetti and Bombace, 2011).

1.2 Target strength (TS)

The first attempts to measure the target strength of fish were done in the mid-1950 by some authors who tried to study the backscatter of the fish body and the swimbladder (Hashimoto and Maniwa, 1955; Harden Jones and Pearce, 1958). The beam pattern effect in each individual fish was the main challenge during the first measurements. To solve the issue due to the location of the fish in the beam and the loss of echo energy increasing the distance from the centre of the latter, indirect parametric and non-parametric methods have been developed (Ehrenberg, 1989). However, the TS measurement requires an accurate time-varied gain (TVG) compensation and accurate measurements of the overall system source level and receiving gain, which can be more easily done in the split-beam echosounder through the calibration process.

Equation 1.1.7 underlines the need for information on σ_{bs} of marine organisms. Numerical values associated with a particular echo signal for identified species of known size, biological and physical states, are especially valuable. As a matter of fact, the conversion of volume backscattering strength (S_v dB re 1 m^2), provided by the surveys, to an absolute biomass estimate requires knowing the species-specific acoustic backscattering cross-section. This parameter is often expressed in terms of target strength (TS) (Foote, 1987; Ona, 1990):

$$\boldsymbol{TS = 10\, log\, \sigma_{bs}/4\pi} \ \textbf{(dB re 1 m}^2\textbf{)} \qquad \text{(Eq. 1.2.1)}$$

in which sigma is the backscattering cross-section of fish or target. TS is the amount of incident wave reflected from a single target and includes the scattering proprieties of the species convolved with behaviour depending on the acoustic frequency used, fish body length, orientation (tilt angle) and swim-bladder features (Nakken and Olsen, 1977; Fässler et al., 2009). Notably, the swim-bladder is responsible for around 90% of the total energy reflected from a fish (Foote 1980). Therefore, the main distinction is between fish with or without a gas-filled swimbladder. The major contribution in acoustic backscatter of common mackerel, which is a swimbladdrless fish, is given by the bones and the presence of lipids (Gorska et al., 2005). Swimbladder fish are classified in physoclists species, characterized by closed swimbladder with gas exchange with circulatory system (such as the *Carangidae*), or physostomous species, with gas exchange effected by gulping air at the surface or by releasing a sphincter muscle on a duct leading to the exterior with swimbladder (such as the *Clupeidae*). The difference in physiology could determine an uncontrolled variation of the gas volume in physostomous species during day vertical migrations and escape behaviour from predators. Conversely, physoclists can adapt the amount of gas with high pressure keeping a well-inflated swimbladder also at high depth strongly affecting the target strength (Ona, 1990; Francis and Foote, 2003; Madirolas et al., 2017). Since TS is a stochastic variable (Simmonds and MacLennan, 2005), it can vary considerably within a species according to the behaviour and physiological state of each individual (Horne, 2003). Fish orientation is one of the parameters that most influence the TS especially when it is measured in unnatural conditions (Horne, 2003; Membiela and dell'Erba, 2018; Ona, 1990; Simmonds and MacLennan, 2005). It has been demonstrated that during *ex situ* experiments fishes display a steeper angle than in their natural state (Brooking and Rudstam, 2009; Wanzenböck et al., 2020). The choice of a correct frequency for specific studies is required because the TS is highly variable along the frequency range as shown in Figure 1.6.1 (Hazen and Horne, 2003; Gonzalez et al., 2020; Khodabandeloo et al., 2021). Additionally, the period during the acoustic survey may determine different TS values for the same species because of the influence of gonad-somatic and hepato-somatic index on the swimbladder volume and cross-sectional area (Ganias et al., 2015; Ok and Gucu, 2019). The stochastic nature of TS should be kept in mind when computing the TS of a species or group of specimens. Because of its nature, it could be one of the main sources of error in biomass estimates (Rose et al., 2000; Zwolinski et al., 2009). Geo-statistical approach is usually followed to determine the variance and statistical error in abundance estimates from acoustic surveys, but it does not take into account the variability of other intrinsic factors such as the calibration process, the sampling capacity related to the trawl net and the uncertainty in TS (Gimona and Fernandes, 2003), which alone could serve over the 20% of the variability (Scoulding et al., 2016).

In the 1987 Kenneth Foote published a milestone on the target strength use in echo integrators. He gathered the knowledge of the time giving reference TS values for physoclists and physotomous species mostly based on observations of gadoids and Atlantic herring, respectively (Foote, 1987). From the examined experiments, he assumed that the swimbladder grows proportionally to the length of the fish and there is a proportional relation between the mean backscattering cross-section (σ_{bs}) and the square of the fish length, providing the standard formulae for comparisons among species and geographical distributions which have been used until today as reference:

$$\boldsymbol{TS = m \log l + b} \ \textbf{(dB re 1 m}^{\mathbf{2}}\textbf{)} \qquad \text{(Eq. 1.2.2)}$$

and

$$\boldsymbol{TS = 20 \log l + b_2} \ \textbf{(dB re 1 m}^{\mathbf{2}}\textbf{)} \qquad \text{(Eq. 1.2.3)}$$

The single echo isolation or single target detection is the first step of the TS measurements. The theory says that in order to be able to separate two targets the distance between them should be at least half a wavelength (Soule et al., 1997), and are used the following criteria (1) two-way maximum beam compensation, (2) phase difference, (3) pulse length, and threshold level. If the received pulse width is within a certain criterion the echo is considered a single echo. This should be large enough to allow for the standard deviation of the axis angle, but by increasing the window, multiple targets could be retained. In order to overcome this problem, the parameter settings during the single target detection should be adjusted for each specific case (Handegard, 2007). The calibration process performed before each TS measurement allows to correct for the directivity of the beam. The maximum beam compensation is intended to reduce the sensitivity of smaller targets at the boundaries of the beam and increase the accuracy of the beam compensation function at high off-axis angles (Demer et al., 1999). Nevertheless, unwanted targets can still be retained due to the summation of amplitude, which determines a double energy signal coming from two single targets at the same range from the transducer (Sawada et al., 1993). For these reasons, the target strength measurements require well-separated targets and cannot be done in dense fish aggregations, limiting the application at fish shoals or along the boundaries of less dense fish schools. The night hours are particularly suitable because of the behaviour of most pelagic fish which spread near the surface feeding on plankton during darkness (Bonanno et al., 2021). To reduce the bias due to multiple target detection, Sawada and colleagues (1993) proposed a method based on the number of fish in effective reverberation volume providing two parameters, N_v to predict the probability of occurrence of multiple echoes and M, to compute the percentage of multiple echoes starting from s_v and TS values as follow:

$$N_v = c\tau\varphi r^2 n \qquad \text{(Eq. 1.2.4)}$$

where c is the sound speed, τ is the effective pulse width, φ is the equivalent beam angle, r is the range and n is the distribution density of fish: $n = Sv/TS$.

$$M = \frac{n-n_s}{n} * 100 \qquad \text{(Eq. 1.2.5)}$$

Successively, with the increasing employment of several frequencies in acoustic surveys, Demer (Demer, 1999) developed a method based on the detection of single targets at the same time at multiple frequencies to detect single targets and avoid the inclusion of unwanted targets. Specifically, targets were only accepted as individual scatters if the single-target detections at each frequency were matched to within ± 1 range cell at 38 kHz (0.1 m) and to an off-axis angular discrepancy of 1.5 degrees from each other.

The third step of TS measurements requires the exact paired of species and size of the target fish to solve the standard model equations 1.2.2 and 1.2.3 by applying a linear regression model, where m and b are respectively the slope and the intercept. Accordingly, once the TS distribution has been acquired it needs to be merged with the LFD of a specific species or the mean TS must be related to the mean total length (TL) of a single specimen (Dunford et al., 2015; Hannachi et al., 2004; Madirolas et al., 2017). In the last case, the mean-matching approach is simple but when many well-separated fish are insonified at the same time, it is not a smooth process. Another accepted approach has commonly been based on the on the attribution of the TS modal value to a given species and size based on modal values of LFD came from trawl haul samples (AcousMed, 2012). While the former method of matching mean TS and TL risks being too subjective, both approaches may fail to explain the stochastic nature of TS, which in repeated measurements of apparently similar targets yields a variety of values (Anonymous, 2012). However, TS-TL matching modes or means are usually acceptable only in particular circumstances, namely when analyzing numerous monospecific hauls, each characterized by a single year class of the same species (Simmonds and MacLennan, 2005). Unfortunately, data on ancillary species such as *T. mediterraneus, S. colias* and *S. sprattus* are scarce, therefore the TS-TL matching modes or means can seldom be applied. Even though, a statistical and objective method was proposed by MacLennan and Menz (1996), based on the Rayleigh assumption that the backscattering cross-section has an exponential distribution and gives greater importance to the modal than the mean values (MacLennan and Menz, 1996). Another interesting statistical approach based on TL-TS means matching has been developed by Kasatkina (2009), who devised an automatic method to reduce subjectivity and uncertainty when post-processing *in situ* TS estimates. Both methods potentially allow performing reliable assessments when few monospecific hauls are available.

TS measurement approaches can be grouped into three main categories:

In situ method

It consists of an acoustic measure of the fish in their natural environment using a split-beam echosounder and biological fish data from catch (i.e. pelagic trawl). The TS histogram data are then matched against the size-frequency distribution measured in the haul samples (MacLennan and Menz, 1996). In this case, it is derived essentially from the dorsal part of the directivity pattern in the dorsoventral plane of the scatter due to the negligible effect of roll (Nakken and Olsen, 1977). It is a summation or integral of σ_{bs} weighted by the probability density function (PDF) of the tilt angle. *In situ* measurements are currently considered the best available method, because, unlike *ex situ* approaches, it detects echoes as the fish swim (Henderson and Horne, 2007). Its main problem is the availability of monospecific hauls and gear selectivity. Kloser et al. (2011) and O'Driscoll et al. (2013) examined the possibility to employ optical instruments during *in situ* experiments which can contribute to estimating also the tilt angle of the specimens while they are insonified. It could be a very useful and less invasive method but it requires expensive instrumentation, because of the necessity to employ a stereo-camera and powerful flash. Moreover, it requires proximity to the fish which can affect their behaviour (O'Driscoll et al., 2013). Axelsen (1999) tried to collect TS data on *Trachurus capensis* in South Africa with the vessel in parallel with a submergible platform lowered near the seafloor because it allows collecting accurate measurements in high depth layer. Nevertheless, the author admits that the platform has the disadvantage of influencing fish behaviour. Different authors demonstrated the influence of the presence of a platform and especially the influence caused by the noise of the approaching vessel on fish behavior and in consequence the tilt angle (Barange et al., 1994; Hjellvik et al., 2008). Pelagic fishes usually perform a movement toward the bottom decreasing the tilt angle during swimming, which could lead to a significant bias during *in situ* measurements, especially when fish are aggregated in schools. Night measurements and collection of echoes near the bottom can help to reduce this phenomenon.

Ex situ method

This approach allows measuring the TS of live, anaesthetized or dead fish of known total length (TL) held in a cage or tethered at a predetermined depth (Thomas et al., 2002; Hazen and Horne, 2004). These experiments also afford direct computation of TS to TL regression, which is used to convert acoustic size to target size. *Ex situ* methods are practical and easy to use and are commonly adopted as a first approach to measure the TS of new species (Azzali et al., 2010). Complex and simple systems have been deployed during the last 20 years (Boswell and Wilson, 2008; Hazen and Horne, 2004; Henderson and Horne, 2007a; Horne et al., 2000; Nesse et al., 2009; Soule et al., 2010; Thomas et al., 2002). Horne et al. (2000) used a very simple experimental setup suspending fish with a dual-chamber swimbladder under the transducer vessel using a monofilament line and a hook. This method is seldom employed and the authors claim the hook can affect the fish behaviour and the fish echoes. More often the fish are measured when they are physically constrained in a small water space. Thomas et al. (2002) for example, assessed the TS of Pacific herring (*Clupea pallasii*) in a cage frame, monitoring the behaviour of the fish by camera. Hazen and Horne (2004) and Henderson and Horne (2007) employed a more sophisticated frame, composed by four monofilament lines connected to a sock between two PVC rectangles, which allowed manipulating the tilt angle of fish measured by a biaxial clinometer placed on the PVC frames. According to the authors, the only bias of the system can be due to the depth of the displacement which can lead to swimbladder compression after a previous period of acclimatization at surface pressure. Nasse et al. (2009) and Boswell et al. (2008) measured the TS of Bay anchovy (*Anchoa mitchilii*) and Atlantic mackerel (*Scomer scombrus*) in a tank and both claimed about the influence of the tank in the acoustic backscatter due to the increase in the Signal to Noise Ratio (SNR) (Boswell and Wilson, 2008; Nesse et al., 2009). Kubilius and Ona (2012) carried out an interesting experiment using a suspended cage equipped with camera and transducer to measure the target strength and tilt angle of sandeel (*Ammodytes marinus*). Many fish at the same time were placed in the cage and this could have affected the results, limiting the fish target tracking used to detect single targets (Kubilius and Ona, 2012). Moreover, the presence of other marine organisms (i.e. plankton) could influence the results of TS of this small and weak acoustic target fish. Studies conducted on dead fish as the one carried out by Azzali et al. (2010) could obviously encompass bias due to the physiological state of the fish.

Despite the ingenious attempts, it should be assumed a bias due to the artificial constraints on the natural behaviour of fish measured by *ex situ* experiments, which can be hardly removed.

Backscattering models

It is defined as “analytical and numerical expressions implemented using computer algorithms to predict acoustic backscatter” (Jech et al., 2015) and its amplitude. The acoustic wave equations are solved on real, approximate or geometrical fish body and swimbladder shape. The digital representations of target shape and properties, *e.g.* fish anatomy and morphometry (chiefly their swimbladder), material properties, and boundary conditions are required as input for each model (Jech et al, 2015). Samples of fish for backscattering model purposes are mainly collected during scientific surveys following a standard procedure. The fish are usually held in a tank for an acclimatization period at surface pressure, then they are anaesthetized and as soon as possible frozen. This limits the influence of external factors such as the depth pressure dependence of swimbladder volume (Fässler and Gorska, 2009; Henderson et al., 2008; Scoulding et al., 2015; Sobradillo et al., 2019; Yasuma et al., 2010). Often the backscattering model results agree with empirical experiments (Henderson and Horne, 2007a; Sobradillo et al., 2019; Sobradillo et al., 2021), but it is not a rule and sometimes the results diverge (Hazen and Horne, 2004; Peña and Foote, 2008; Sawada, 1999).

1.3 Backscattering models

A rich variety of models has been developed in the past to predict the acoustic backscatter as a function of depth, fish orientation, and frequency (Jech et al., 2015). *In situ* and *ex situ* methods are suitable for the measurement of the species-specific TS in the natural environment and under controlled conditions, respectively (Henderson and Horne, 2007b; Kubilius and Ona, 2012; O'Driscoll et al., 2018; Salvetat et al., 2020). But only the use of backscattering models allows to properly predict theoretical backscatter from accurate measurement and setting of organism anatomy, material proprieties, swimbladder morphology, tilt angles, and frequencies (Jech et al., 2015).

The overall TS of fish is a sum of the contribution of many organs of the fish body (Ona, 1990). However, the backscatter of a swimbladder fish, is attributable mostly on sound scattered from an air-filled or partly air-filled swimbladder, which is responsible for up to 95% of the TS at certain frequencies (Foote 1980). In physostomous fish species the volume of the swimbladder decreases with increasing water pressure when the fish descends to deeper depths. The effect of this depth-dependent phenomenon on the backscattered sound by fish has been modelled, for instance in Atlantic herring (Fässler and Gorska, 2009; Gorska and Ona, 2003), and it is known to affect the accuracy of acoustic surveys (Hjellvik et al., 2008). As the swimbladder is a gas-filled organ, it is conventionally modelled as a gaseous object, whereas the flesh of the fish has been traditionally represented by a unique liquid object (Furusawa, 1988) characterized by a very weak impedance due to the similar density with the surrounding waters. Therefore, the fish models require an accurate representation of this key organ. The simplest way to model a swimbladder is by a regular shape, for instance, a sphere (Andreeva, 1964), a finite cylinder (Clay, 1992) or a prolate-spheroid / ellipsoid (Furusawa, 1988). The first published models were based on the aforementioned geometrical shapes solved through analytical solutions (Anderson, 1950; Jech et al., 1995). The approximation of the swimbladder to a prolate-spheroid was pursued by a sectioning swimbladder into a compound sets of finite cylinders solved by Kirchhoff Approximation model (KA), deformed cylinder model (DCM) and Kirchhoff Ray approximation Mode (KRM) (Furusawa, 1988; Jech et al., 1995; Foote and Francis, 2002). They belong to the family of approximate analytical models. Successively, complex finite surface elements were solved using numerical models based on the solution of wave equations such as the Boundary Element Method (BEM), which use the integral of the Helmholtz equation, the Fourier matching model (FMM), which computes the transformed Helmholtz equation and the Finite Element Method (FEM) which involve the use of inhomogeneous Helmholtz equation (Francis and Foote, 2003; O'Driscoll et al., 2011). A summary of the main models applied in fisheries acoustic is shown in Table 1.3.1.

All the above-cited methods are three or two-dimensional geometrical approximations of the swimbladder, hence an accurate morphological study of this organ is required as a basis for modelling. Three-dimensional characteristics of the swimbladder were traditionally determined by dissections of samples (Kleckner and Gibbs, 1972; Foote, 1985), estimation of volume from the amount of gas contained in the swimbladder (Fine et al., 1995) and the measurements of solid slice obtained inflating the swimbladder with resin (E. Ona, personal comm.). Some authors are still using the dissection technique implemented by the use of camera directly on board, to acquire a snapshot of the fish swimbladder immediately after capture (Ganias et al., 2015; Ok and Gücü, 2019). But, most commonly, the same investigation is carried out at veterinary and medical facilities through imaging techniques such as magnetic resonance imaging (MRI), computed tomography (CT) or X-ray (an example is given in Figure 1.3.1) (Fassler et al., 2013; Gauthier and Horne, 2004; Peña and Foote, 2008; Scoulding et al., 2015).

Figure 1.3.1. Example of Computer Tomography scan carried out on specimens of *S. colias* at the veterinary JesiVet, Jesi, Italy

Table 1.3 1. Summary of the main models applied in fisheries acoustics

Model	Family	Accuracy	Range of validity	Limitations
Prolate Spheroid Model (PSM)	Analytical	Low	All angles	Weak accuracy at small L/λ. Not suitable for non-ellipsoid swimbladders
Boundary Element Method (BEM)	Numerical	High	All shape, all frequencies and all angles	Computing demands at high frequencies; inhomogeneous volumes; need an accurate 3D triangular mesh shape; reduced accuracy for weak scatters.
Finite Element Method (FEM)	Numerical	High	All shape, all frequencies and all angles	Computing demands at high frequencies; inhomogeneous volumes; need as accurate 3D triangular mesh shape reduced accuracy for weak scatters.
Fourier Matching Method (FMM)	Numerical	Exact	axisymmetric, all frequencies and all angles	Non-axisymmetric; convergence issues at high aspect ratios; 3D triangular mesh shape
Kirchhoff Approximation (KA)	Approximation	Approximate	High frequencies; near normal incidence; homogeneous material	Off-normal incidence; no circumferential waves; low frequencies
Kirchhoff Ray Mode (KRM)	Approximation	Approximate	All frequencies; high aspect ratio at low frequencies; near-normal incidence; homogeneous material	Off-normal incidence; no circumferential waves

All the models reported in Table 1.3.1 are still in use, each has advantages and constraints and the choice of the model depends on the target shape, the tilt angle range investigated, data availability and computing power availability (Gauthier and Horne, 2004; Jech et al., 2015b). Numerical models are computationally demanding and require detailed measurements of swimbladder and fish body characteristics. They account for the finer morphometric variation on the surface resulting in more precise and accurate measurements of acoustic reflectivity which can help to output broadband frequency patterns. Conversely, analytical models approximate complex swimbladder shapes by solving the backscatter from one or more simplified geometric figures but, they do not require extensive-computer power and high-resolution measurements (Reeder et al., 2004). Approximate analytical models are more accurate compared to the analytical ones but are less effective than the numerical model. However, they usually sum the fish body and the swimbladder backscatter. Body parts other than the swimbladder increase in relative importance at high frequencies, when the wavelength is much shorter than the scattering target (the swimbladder) and the swimbladder response becomes weaker. The study of the body backscatter could be particularly promising in species identification with broadband echosounder associated with spectral analysis, but this possibility has only been investigated in bladderless fish (Forland et al., 2014).

Generally, there is a good agreement between models and empirical experiments (Hazen and Horne, 2004; Horne et al., 2000; Reeder et al., 2004), but some authors found significant differences between models, *in situ* and *ex situ* experiments (Hazen and Horne, 2004; Peña and Foote, 2008; Sawada, 1999). Backscatter models are not intended to replace the empirical measurements, but they are useful to corroborate target strength experiments and to fill the gap in the knowledge of species that hardly fulfil the requirements of monospecific shoals of spread fish for the application of the *in situ* method.

The choice of the best models to be used on the species covered by this study was based on data and computational power availability along with the morphological characteristics of the swimbladder and fish body. The dual-chamber laterally-compressed swimbladder of sprat lends itself to more complex models i.e. FEM model which can be used despite the high computational power demanding due to the small size of the swimbladder which reduces the time and CPU needed for the computations. Conversely, the approximate prolate-spheroid shape of the swimbladder of Mediterranean horse mackerel and chub mackerel does not require an extensive effort to capture the morphological variability of the backscatter organ. Therefore, we decided to apply a KRM model as the best trade-off between model complexity and computational power demanding.

1.3.1 *Kirchhoff-Ray mode model (KRM)*

In Kirchhoff-Ray mode model (KRM) the swimbladder is sliced in a series of gas-filled cylinders, while the fish body is sliced in a series of fluid-filled cylinders (Figure 1.3.2). The backscatter of the fish is then obtained by the summation of the scattering energy from the two overlap cylinders (Gauthier and Horne, 2004) approximating the swimbladder as an object with a piecewise circular cross-section. Due to the approximation, the accuracy of KRM decreases at off-broadside angle over 45°. However, it is one of the most used backscattering models for fish TS estimation at high frequencies because it can take into account variations in the shape of the object computing the backscatter of stacked and potentially offset cylinders (Macaulay et al., 2013).

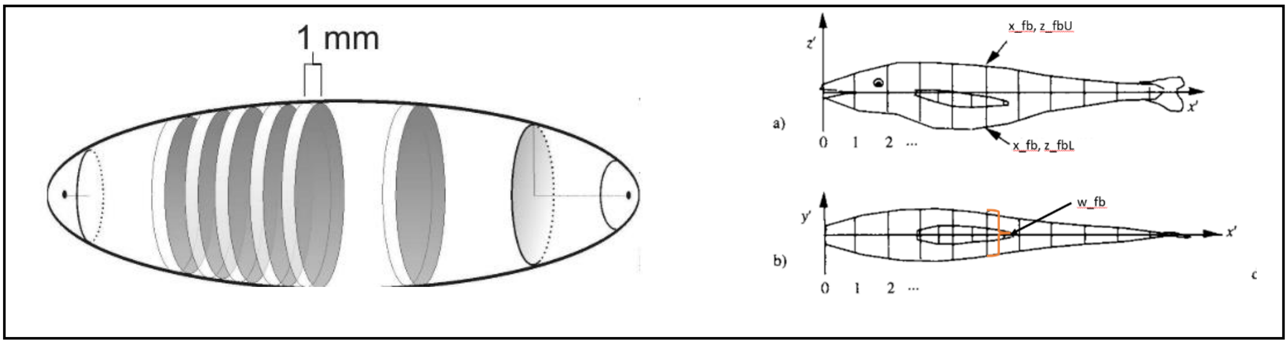

Figure 1.3.2: Swimbladder and fish body representation used in KRM model. Source: Clay and Horne 1994; Gauthier and Horne 2004

The scattered sound pressure P_{scat} at time t and range R from the object insonified by a plane incident wave of amplitude P_{inc} at the object is:

$$P_{scat}(t, R) = (P_{inc}/R)e^{i(kR-2\pi ft)}L(t) \qquad \text{(Eq. 1.3.1)}$$

where k is the wave number: $k = 2\pi/\lambda\ (m^{-1})$, f is the frequency (Hz) for the convolution of the sound wave, and $L(t)$ is the scattering length (m) of the object. The absolute square of the scattering length is equivalent to the backscattering cross-section of an insonified object (σ_{bs},m^2):

$$\sigma_{bs} = |L^2| \qquad \text{(Eq. 1.3.2)}$$

where L is a function of the insonifying frequency and length (L, m) of the object. Then the total scattering length of the fish is computed from the coherent addiction of the scattering amplitudes of the fish body and swimbladder:

$$L = gas\ cylinder\ L + fluid\ cylinder\ L \qquad \text{(Eq. 1.3.3)}$$

For more details about the mathematics formulae see Clay and Horne (1994).

The KRM required 2D dimensional representation of the fish body and the swimbladder. The measures shown in Figure 1.3.1 should be taken to compute the model: fish body coordinates along x-axis (*x_fb*), fish body width along x-axis (*w_fb*), lower height of the fish body along the x-axis (*z_fbl*), upper height of the fish body along the x-axis (*z_fbu*). The same measurements are collected for swimbladder. Several physical input parameters listed in Table 1 are required for the computations.

Table 1.3 2. Acoustic parameters required for Kirchhoff Ray mode model

Parameter	**Common values**
Ambient water sound speed (ms^{-1})	1490
Density ambient water (kgm^{-3})	1030
Sound speed inside fish body (ms^{-1})	1570
Density inside fish body (kgm^{-3})	1070
Sound speed inside swimbladder (ms^{-1})	345
Density inside swimbladder (kgm^{-3})	1.24

1.3.2 Finite Element Method (FEM)

It is more likely for a swimbladder to have an irregular shape rather than a spherical or ellipsoid one, because as already mentioned, many factors influence its volume and geometry. Therefore, numerical methods are required to solve irregular surface backscatter. The Finite Element Method (FEM) is one of the most powerful for backscattering estimation of an object with an arbitrary shape. It is computationally expensive but it assures accurate estimates (Jech et al., 2015). The total acoustic field p_t is given by the sum of the scattered and the background pressure field such that:

$$p_t = p_b + p \quad \text{(Eq. 1.3.4)}$$

$$p_b = p_{0e}{}^{-i(k*x)} \quad \text{(Eq. 1.3.5)}$$

The background pressure field is a plane wave of amplitude p_0 moving in the direction k with wave number $|\boldsymbol{k}| = 2\pi f0/c0$, where $f0$ is the frequency and $c0$ is the speed of sound. The governing equations are implemented as a scattered field formulation such that only the scattered field p is solved. The gas-filled object is located inside a computational domain of total radius R_i+ R_{pml}, where the layer of thickness R_{pml} is the absorbing Perfectly Matched Layer (PML) while R_i is the computational domain (Figure 1.3.3). The PML is used as a non-reflecting and absorbing boundary that mimics a domain stretching to infinity. After solving a pressure acoustics model, it is possible to determine the pressure outside the computational domain using the exterior field calculation.

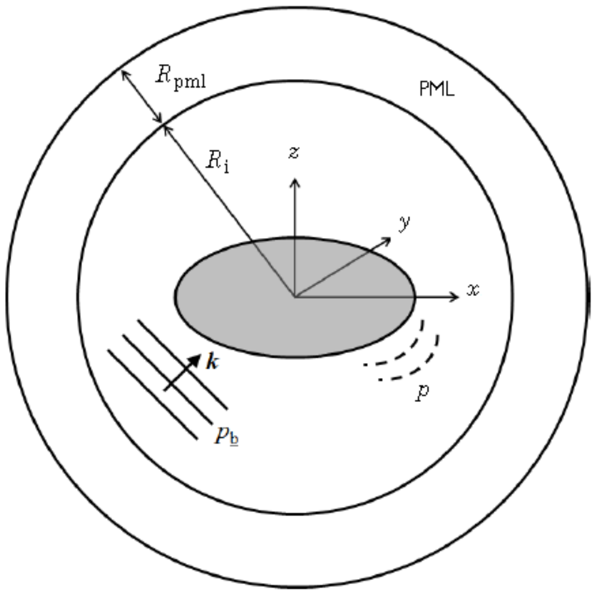

Figure 1.3.3. Sketch of the model system showing the incident background pressure field (*pb*), the scattered field (*p*), geometric scales, the radius of inner modelled water region (R_i), the Perfectly Match Layer *(PML)* and the thickness of absorbing PML (R_{pml}). Source: COMSOL Multiphisics 6

The exterior field calculation solves the Helmholtz-Kirchhoff (H-K) integral of the surface hit by the wave. It is needed to make a closed surface around all sources and scatter. The far-field backscattered pressure is estimated by computing the H-K integral between the homogeneous layer surrounding the object and PML. Through the use of the full H-K integral, it is possible to determine the exact exterior-field pressure (including phase) at any point and distance outside the computational domain. Note that in order to get a precise evaluation of the exterior-field variable, the evaluation of the H-K integral must be accurate. This requires having a good numerical estimate of the normal derivative of the pressure on the exterior-field calculation surface (adjacent to the PML layer at the boundaries of computational domain). Therefore, the thickness of this layer should be one-tenth of the element size in the domain.

The model solves the H-K integral for each union point of the meshes which makes the swimbladder shape. Higher the number of triangles more accurate the results. It is usually accepted to make a mesh shape with at least 10 mesh for each λ, hence $\lambda_{min}/10$ (Khodabandelo et al., 2021). The mathematical equation will be addressed in the following chapters. Nowadays, the FEM model is usually solved in the Acoustics Module of COMSOL Multiphysics software, where it is possible to find more details on the exterior field computations (COMSOL, 2021). An example of an ellipsoid with fine mesh surrounding by the water domain and the PML in COMSOL for the application of FEM model is given in Figure 1.3.4.

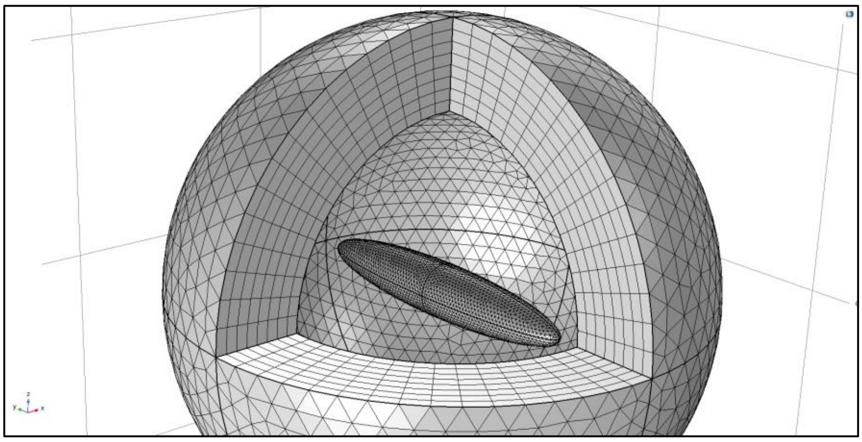

Figure 1.3.4. Example of the layers and boundaries made in COMSOL Multhyphisic Acoustic Module (v.5.6) for solving a finer mesh size shaped ellipsoid (mesh size= $\lambda_{min}/10$; frequency domain= 200 kHz)

1.4 Acoustic surveys in the Mediterranean Sea (MEDIAS Project)

The small pelagic fisheries in the Mediterranean Sea is responsible for the largest landings in terms of tons per year since the exponential developments of fisheries sector after the World War II (FAO, 2018). This motivated the scientists to the use of new technologies to monitor the status of the biological resources and in 70' was started the study of small pelagic fishes by acoustic methods. The earlier experiments were conducted in the Adriatic Sea (Kačić, 1972; Vučetić & Kačić, 1973; Azzali & Levi 1976; Kačić et al., 1976; Azzali & Burczynski, 1977) followed by South of Majorca (Oliver & Bravo de Laguna, 1976) and in the Gulf of Lion (Sacchi, 1975). Once acoustic method was recognized as a powerful tool to estimate the abundance and biomass of small pelagic fishes, other countries implemented this type of survey. It became quickly the common research method adopted for the study of species such as European sardine (*Sardina pilchardus*) and European anchovy (*Engraulis encrasicolus*) in the framework of national programs (Machias et al., 1997; Azzali et al., 2002; Tičina et al., 2006, Leonori et al., 2012a).

Thanks to the European Council Regulation (EC 199/2008), in 2009 the MEDiterranean International Acoustic Survey (MEDIAS) (http://www.medias-project.eu/medias/website/) was born as an extensive, regular, internationally coordinated acoustic surveys carried out in the framework of the EU Fisheries Data Collection Regulation (DCR) to assess the state of small pelagic stocks. The main objectives of these mandatory surveys are to provide fishery-independent data to support scientific advice within the FAO General Fisheries Commission for the Mediterranean Working Group on Stock Assessment of Small Pelagics (GFCM WGSASP, 2021) based on the use of a common protocol (MEDIAS, 2021). Abundance indices of anchovy and sardine obtained from acoustic data are used as tuning indices in analytical stock assessment models based on fisheries and catch allowing the evaluation of their spatial and temporal distribution (Giannoulaki et al., 2014; Carpi et al., 2015; Leonori et al., 2021). Conversely, acoustic data on non-target species has seldom merged in stock assessment in the Mediterranean Sea (Angelini et al., 2021). Currently, the MEDIAS project covers the Mediterranean waters of six countries (Italy, Spain, Croatia, France, Slovenia and Greece) (Figure 1.4.1). Additionally, Bulgaria and Romania participate annually to the MEDIAS steering committee meeting and four other non-EU countries have been involved, Morocco, Algeria, Tunisia and Turkey (Giannoulaki et al., 2021).

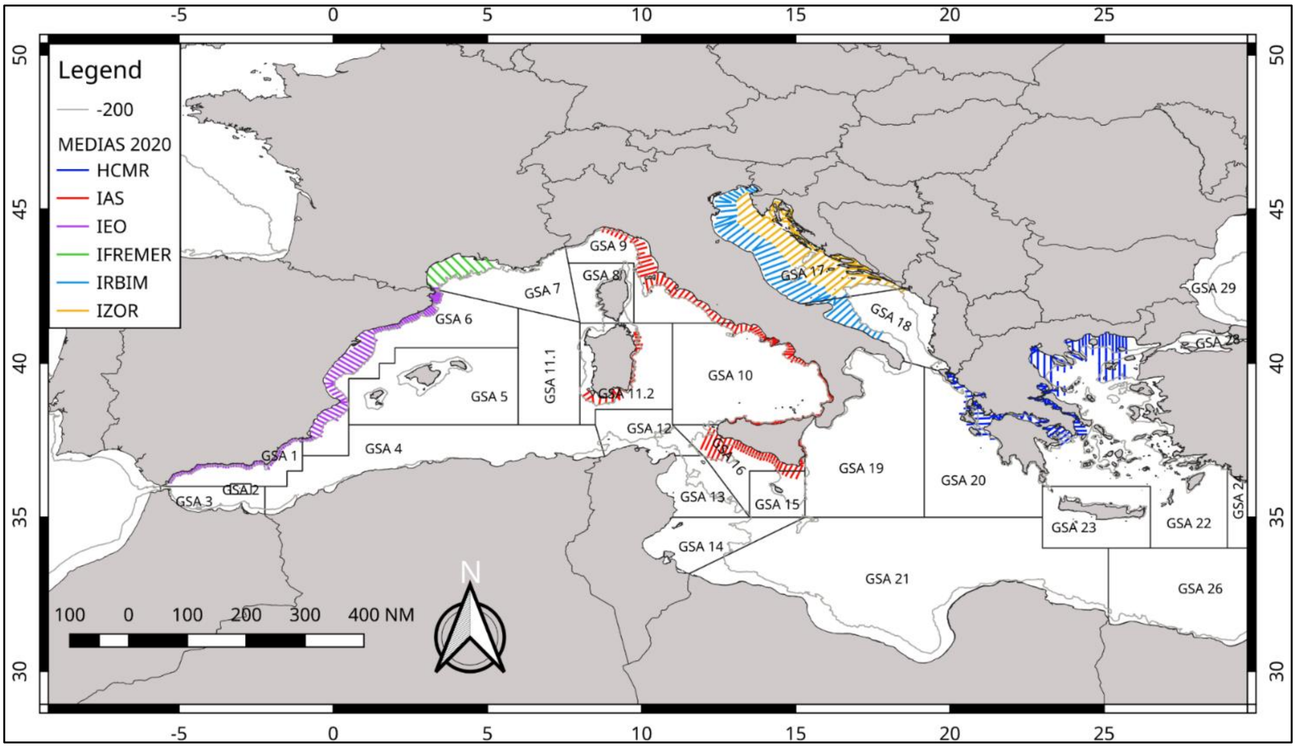

Figure 1.4.1. MEDIAS survey design. Map of transects covered by the MEDIAS surveys and GFCM Geographical Sub-Areas (GSAs) in the European Mediterranean Sea (Leonori et al., 2021). The discrete colors depicted the different research groups which perform the survey. Source: MEDIAS website (http://www.medias-project.eu)

The common protocol foresees the collection of acoustic data on board of research vessels equipped with split-beam scientific echosounders operating at least at 38 kHz, the principal frequency used for biomass assessment. Whether available, 18, 70, 120, 200, 333 kHz transducers are employed as complementary frequencies. The survey design consists of systematic parallel line transects perpendicular to the coastline, or adapted to the geomorphology of the survey area, carries out during the daytime in bathymetry between 10 and 200 m (Figure 1.4.1). Acoustic sampling should start at least at a bottom depth of 20 m, but it obviously depends on vessel draught. In order to avoid problems with the engine of hull noise or propeller cavitation the proper vessel speed during data acquisition should be between 8 and 10 knots. Fish sampling, indispensable for the identification of fish schools, should be carried out to obtain a sample of the echo traces that appear in the echogram and to obtain biological information and the LFD of each species. Therefore, the haul's location cannot be defined in advance and the sampling intensity depends on the occurrence of echo traces and the spatial characteristic of fish schools in each area. Indeed, there is no restriction on the trawl gear used as long as it is suitable to catch a representative sample of the target schools or layers. In any case, (1) the maximum cod-end mesh size should not exceed 24 mm, (2) the vessel speed during fishing operation should be 3.5-4 knots a (3) the duration of the haul should not be less than 30 minutes. Additionally, CTD and net plankton stations are routinely performed for the characterization of the pelagic ecosystem.

According to the MEDIAS common protocol, Echoview or Movies 3D software should be used for acoustic data analysis following a specific workflow detailed in MEDIAS handbook 2021 (MEDIAS, 2021). The Elementary Distance Sampling Unit (EDSU) for echo integration is fixed to 1 nautical mile (nmi) so as to provide the total fish NASC. The echo partitioning into species is based on echogram scrutinization either by direct allocation from acoustics and geometrical characteristic of the individual fish schools or, most commonly, by taking into account representative fishing samples. The TS equation for echo integration is currently applied depending on the area and "the application of common TS equations should derive from *in situ* estimations of TS, preferably based on acoustic data from the Mediterranean Sea" (MEDIAS, 2022). The only common TS equation is currently applied for sardine (MEDIAS, 2022):

$$TS = 20log(TL) - 72.6 \text{ dB} \quad (1.4.1)$$

While there is no agreement about the equation of anchovy which is still featured by some differences between areas. The other pelagic species are not mentioned and are gathered in a sub-group named OPS, with the exception of sprat, which is assessed using different TS values depending on the area of study (De Felice et al., 2021).

1.4.1 Acoustic surveys in the Adriatic Sea

The first acoustic survey performed in the whole Mediterranean Sea was conducted in the North-West Adriatic Sea, FAO Geographical Sub Areas (GSA) 17 in 1976 by the Acoustic Research Group of CNR IRPEM of Ancona on board of the research vessel R/V Salvatore Lo Bianco equipped with SIMRAD EK500 echosounder operating at 38 and 120 kHz (Azzali et al., 1980, 2002; Leonori et al., 2012a; Leonori et al., 2021). In 1987 and 1988 the surveyed area was extended to Middle and South-West Adriatic Sea involving the GSA 18 during the summer months. After four years of interruption, it was followed by further 7 years of acoustic surveys from 1992 to 1998 making the first long-time series for the western side of the Adriatic Sea (Azzali et al., 1997). Successively the vessel has been replaced with R/V Thetis in 1999 and 2000. In 2001 the building of the new R/V "G. Dallaporta" equipped with SIMRAD EK500 allowed the extension of the survey annually conducted in the Italian water to Slovenia, Montenegro and Albania in the framework of FAO AdriaMed project (Leonori et al., 2012b). Since the beginning of acoustic surveys, studies on target strength were conducted through *in situ* and *ex situ* experiments involving the use of cages specifically designed to increase the accuracy in abundance estimates. Its chief aim was to assess the biomass and spatial distribution of anchovy, sardine and sprat (*Sprattus sprattus*) on the Italian side of the Adriatic.

On the eastern side of the Adriatic, occasional small-scale acoustic surveys on a monthly seasonal basis aimed at collecting information on small pelagic fish for commercial fisheries, were undertaken in 1977, 1982 and 1986 on board of R/V BIOS. The latter was equipped with SIMRAD 540-4 echosounder operating at 30 kHz and EK38A operating at 38 kHz to assess the behaviour of small fish and estimate the relative abundance (Kačić, 1988). In 2002 Croatian scientists implemented a regular acoustic survey in the Eastern Adriatic Sea, which was merged in a national annual survey called PELMON which took place from 2003 to 2012 financed by the Croatian Ministry of Agriculture and Institute of Oceanography and Fishery (IOF) of Split (Tičina et al., 2006). The PELMON surveys of the pelagic ecosystem were carried out in September on board the R/V BIOS, which was equipped with the SIMRAD EK500 echosounder (38 kHz) extended in the Croatian territorial waters up to the midline of the Adriatic Sea.

Surveying at the same species and stocks, Croatia adopted TS equations for acoustic data conversion equivalent to the Italian side of the Adriatic Sea on anchovy, sardine and sprat (Tičina et al., 2006; Leonori et al., 2017). Due to the exploitation of the same stocks, an inter-calibration between the R/V BIOS used by Croatian scientists and the R/V Dallaporta used by Italian scientists was required. The exercise was performed in 2005 in the Neretva Channel, on the southern Croatian coast, to compare their acoustic and biological sampling measurements (Leonori et al., 2012a). In 2008 the R/V Dallaporta echosounder EK500 was replaced with the SIMRAD EK60 followed by the installation of 120 and 200 kHz in 2010. Accordingly, in 2009 a new ship named R/V BIOS DVA, was equipped with the same echosounder operating at 38 kHz before 2018 and at 120 and 38 kHz successively and with the same fish sampling equipment as the R/V Dallaporta. On board of the new vessel, two pilot surveys were performed before the harmonization with EU MEDIAS standard protocol in 2013. R/V BIOS DVA is currently working with the last version of SIMRAD echosounder, the EK80 operating at two frequencies: 38 and 120 kHz. Conversely, the R/V Dallaporta has undertaken a continuous development of acoustic equipment and it is currently equipped with the SIMRAD EK80 echosounder operating at four frequencies: 38, 70, 120 and 200 kHz. Two 120 kHz transducers are employed, one mounted vertically and one horizontally.

The main characteristics of each frequency are detailed in Table 1.4.1. The MEDIAS survey is carried out in parallel transects perpendicular to the coastline in the western part of the Adriatic Sea in GSA 17, GSA 18 and in the Albanian Coast in the framework of MARE-Albania project (De Felice et al., 2021) (Figure 1.4.2). In parallel to the acoustic data acquisition, a biological sampling is routinely carried out with a four seams pelagic net (upper panel is equal in mesh size to lower panel and so the two side panels between each other). This type of net is suitable for catching small pelagic fish schools composed by juveniles and adults of the target species. The selectivity is very low due to a cod-end of 18 mm stretched mesh length. The catchability is very high due to a vertical opening of 10 meters and horizontal opening of 18 meters at 4 knots of average trawling speed.

Table 1.4.1. Echosounder system details of R/V G. Dallaporta. GPT = General Purposes Transceiver. WBT = Wide Band Transceiver

Frequency	Transducer	Transceiver	Processor unit
38	SIMRAD ES38-7c	GPT	SIMRAD EK80
70	SIMRAD ES70-7c	WBT	SIMRAD EK80
120	SIMRAD ES120-7c	WBT	SIMRAD EK80
200	SIMRAD ES200-7c	WBT	SIMRAD EK80
120	SIMRAD ES120-7c	GPT	SIMRAD EK80

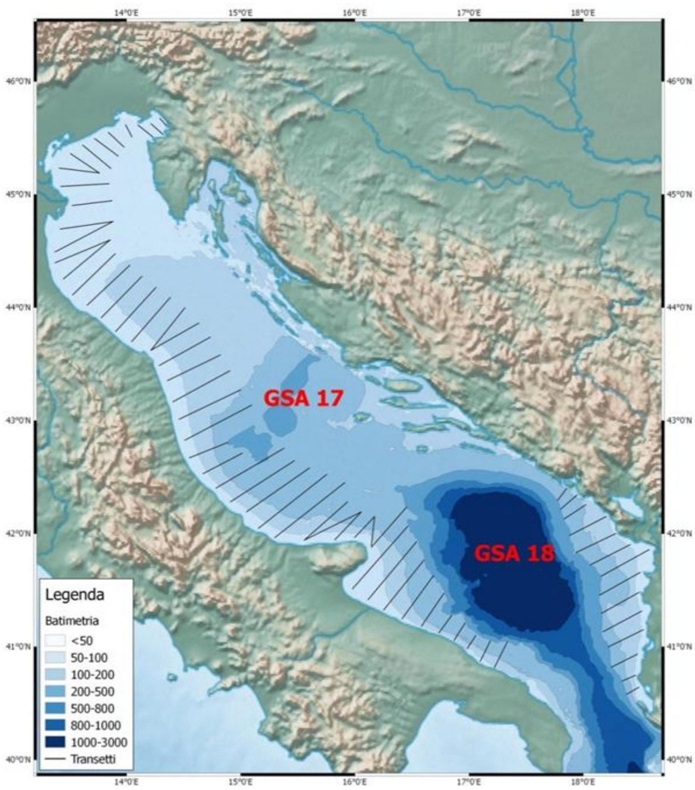

Figure 1.4.2. MEDIAS survey design carried out by the Ecosystem acoustic group of CNR-IRBIM of Ancona in the west side of the Adriatic Sea and Albanian waters and GFCM Geographical Sub-Areas (GSAs).

Net behaviour is monitored in real-time with the SIMRAD FX80 trawl sonar system. The system is equipped with a sonar head sampling the whole trawl mouth, an echosounder pointing downwards (i.e. trawl-eye) and a depth sensor that sends NMEA data to the EK80 to visualize the trawl depth on the sounder display as a continuous line. The skipper is able to manoeuvre the vessel and the trawl to secure the catch of the schools approaching the vessel having a real-time feedback if the schools of fish are entering in the net. Moreover, the SIMRAD FX 80 trawl sonar system, linked to SIMRAD PX Multisensor, is used to monitor the behaviour of the net and the capture of fish during fishing operations (Figure 1.4.3).

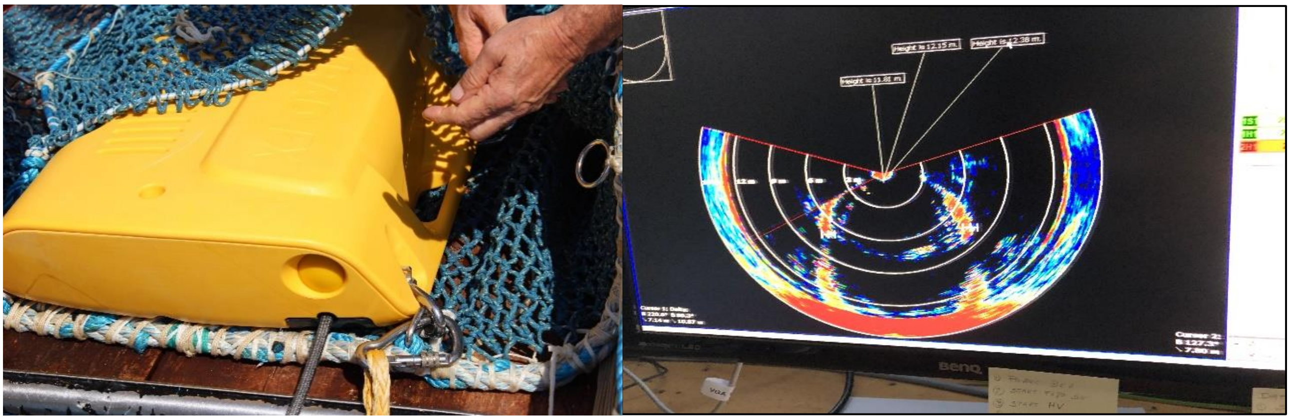

Figure 1.4.3. SIMRAD FX 80 trawl system. On the right an example of the output which shows the net mouth shape (photo: A. Palermino. 2020)

The acoustic data scrutinization is conducted in Echoview software (v. 10) following the MEDIAS common protocol. The schools are allocated mostly based on the catch sampling. The mixed-species is applied to convert the NASC into biomass estimates (Nakken and Dommasnes, 1975) entering the species-specific b_{20} values described in Table 1.4.2.:

$$E_i = {w_i(\sigma_i)E_m}/{(\Sigma w_j(\sigma_j)} \quad \text{(Eq. 1.4.2)}$$

Where E_m is the mean echo-integral of the mixture (NASC value nmi^2/m^2), $\langle\sigma_i\rangle$ is the mean backscattering cross-section (m^2) and must be computed from the target strength function and the size distribution of the species (see Eq. 1.2.2 and 1.2.3) and w_i is the proportion of a specific species in the mixture computed as a weighted average of the quantity of the i'th species caught at a haul k (q_{ik}) in kg over the total catch (q_k) in M trawl haul of an homogeneous region:

$$w_i = {(\sum_{k=1}^{M} q_{ik}/q_k)}/{M} \quad \text{(Eq. 1.4.3)}$$

The echo-integral contributed by each species is proportional to the product of w_i and $\langle\sigma_i\rangle$, hence E_i depends on the $\langle\sigma_i\rangle$, values of all the species in a mixture. The long series of historical data resulted in a biomass trend that is annually update in the North-Western Adriatic Sea from 1976 (Figure 1.4.4).

Table 1.4.2. b_{20} values in use in the Adriatic Sea in GSA 17 and 18

Species	b_{20} values (dB)	References
Engraulis encrasicolus	-74.6	(Anonymus, 2012)
Sardina pilchardus	-72.6	(MEDIAS, 2012)
Sprattus sprattus	-71.7	(Azzali, 1997)
Boops boops	-67	
Trachurus Spp.	-68.7	(Lillo et al., 1996)
Scomber colias	-68.7	(Lillo et al., 1996)
Sardinella aurita	-72.6	(MEDIAS, 2012)
Other pelagic	-71.9	

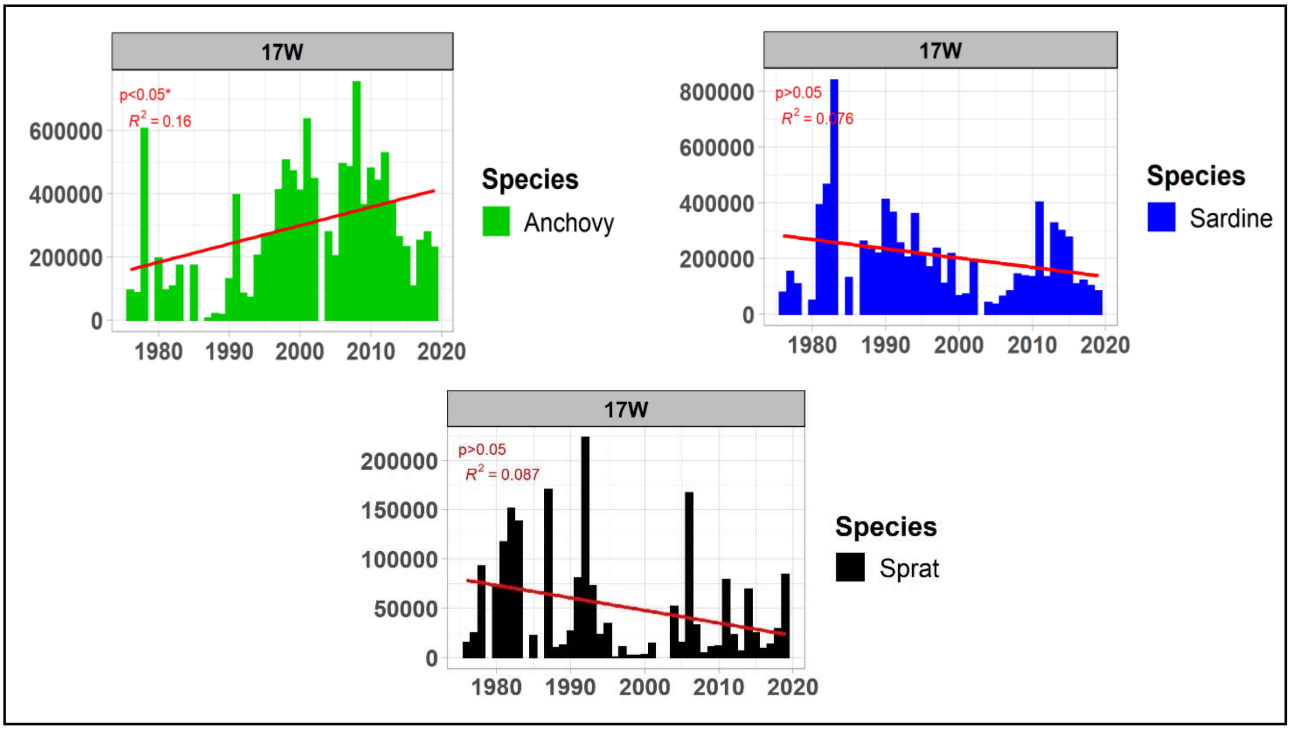

Figure 1.4.4. Historical biomass trend (tons) of anchovy, sardine and sprat in the western side of GSA 17 from 1976 to 2020, Source: Leonori et al., 2021

1.5 Target species

1.5.1 Trachurus mediterraneus

Figure 1.5 1. *Trachurus mediterraneus*. (photo: A. Palermino, 2020)

Trachurus mediterraneus (Steindachner, 1868) is a *Carangidae* species distributed along the temperate waters of the Eastern Atlantic Sea including the Mediterranean and Black Sea (Figure 1.5.2). It is found in the area with the other congeneric species *T. trachurus* and the rare *T. picturatus*. The Mediterranean horse mackerel inhabits shallow coastal areas and it can be considered a neritic species against the benthic habits of the common horse mackerel which has a wider and deeper distribution (Milisenda et al., 2018). It is commonly found between 20 and 200 m depth in schools. It is spread along all the GSA 17 in the Adriatic Sea, but MEDITS data shows just a few coastal persistency areas (Piccinetti et al., 2012). Nevertheless, the semi-pelagic behaviour of the species could determine likely inaccurate estimates with bottom trawler samples suggesting more accurate estimates of species biomass and distribution by the use of acoustic surveys. The spawning period extends from May to September with a slight difference from west to east Mediterranean (Demirel and Yüksek, 2013; Follesa and Carbonara 2019).

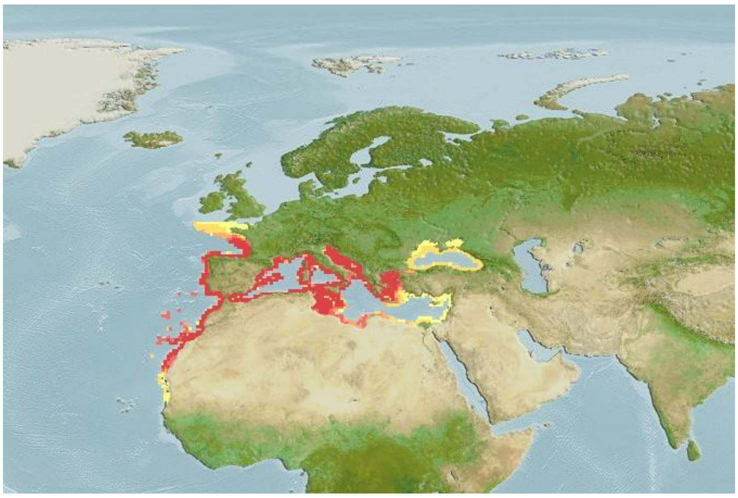

Figure 1.5.2 Geographical distribution of Mediterranean horse mackerel. Source: Forsee and Pauly, 2022

The Mediterranean horse mackerel is caught by different fishing gear, from bottom trawl to pelagic trawl and trammel nets. It accounts for 1.4% of the total landings in the Mediterranean and the Black Sea, with an annual mean of 15’692 tons (FAO, 2022). It composes a substantial portion of landings in the south-western Mediterranean (Carbonell et al., 2018) and the Adriatic Sea (Šantić et al., 2003) and is considered a commercial species in the entire basin and rarely discarded (FAO, 2022). However, since it is often pooled with *Trachurus trachurus*, data are not wholly reliable. The generalist behaviour and diet of *T. mediterraneus*, which prey mostly on zooplankton and fish, lead to a higher trophic level and different niches compared to *T. trachurus* (Albo-Puigserver et al., 2016; Bayhan et al., 2013; Georgieva et al., 2019). The first stock assessment carried out on the species in the Adriatic Sea suggested a good status of the resources which has resulted overexploited during the past years underlining the importance of a constant assessment of this important stock (Angelini et al., 2021). However, currently, *T. mediterraneus* is pooled with Other Pelagic Species (OPS) species in acoustic data scrutinization and together with the uncertainty on the TS does not allow to give a reliable specific assessment on its distribution and abundance from acoustic surveys.

1.5.2 *Scomber colias*

Figure 1.5 3. *Scomber colias*. Photographed by myself during MEDIAS survey 2022

Scomber colias (Gmelin, 1789) is one of the four epipelagic species belonging to the genus *Scomber* together with *Scomber scombrus*, *Scomber japonicus* and *Scomber australicus*. *S. colias* have been misidentified as *S. japonicas* until 2009 when genetic evidence proved his phylogenetic differentiation (Catanese et al., 2010; Infante et al., 2007). It differs from the congeneric species, for several morphological characteristics, but the main difference consists in the presence of the swimbladder, absent in *S. scombrus* (Scoles et al., 1998). Its distribution is shown in Figure 1.5.4. It is spread anti tropically in the warm and temperate waters of the Atlantic Ocean along the American, European and African coasts including the Mediterranean and the Black Sea. It is considered a panmictic species in the Mediterranean and Atlantic Seas (Zardoya et al., 2004) and the peak of the spawning period is registered during the summer months in the Mediterranean and the Adriatic Sea (FAO, 2019).

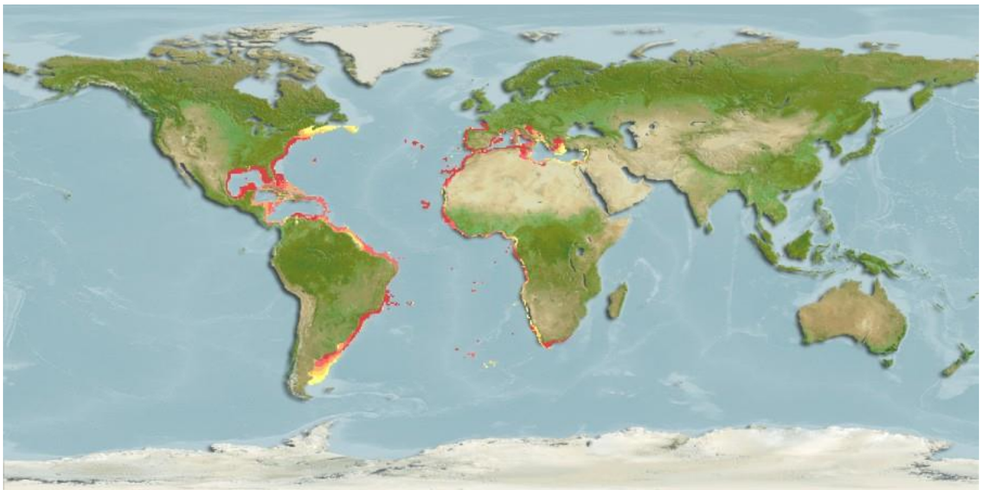

Figure 1.5.4. Geographical distribution of Atlantic chub mackerel. Source: Forsee and Pauly, 2022

S. colias composes a significant part of the landing in the Northwest African fisheries and European waters of the Atlantic Sea (ICES, 2020), while in the Mediterranean Sea, it accounts for a substantial proportion of landings only in the eastern Mediterranean fishing grounds (Bariche et al., 2006, 2007; Tsagarakis et al., 2012). Nevertheless, here the landing of the species is quite low with a total amount of landing in 2018 of about 12’313 tons (FAO, 2022). In Italy, the species does not have a high commercial value compared to the common mackerel and with annual landings of ca. 1200 tons (Eurostat, 2022). Conversely, in the Adriatic Sea, the landings of the species increased in the last two decades, from less than 200 tons fished by Slovenia, Croatia, Italy and Montenegro in 2005 up to 2100 tons in 2017. In this basin, it composes bycatch of the pelagic trawl and purse seine (Santojanni et al., 2005, STECF, 2016). However, since *S. colias* is often sold as *Scomber scombrus*, it is difficult to obtain reliable landings data (Zardoya et al., 2004). Despite its relatively low commercial value in the Mediterranean Sea, *S. colias* is planktivorous and piscivorous species that play an important ecological role in pelagic niches occupying an intermediate position in the trophic level (Albo-Puigserver et al., 2016; Šantić et al., 2003; Sever et al., 2006).

In the Atlantic Sea, the stock of this species is assessed yearly within a FAO-CECAS Working Group, where the catches have increased exponentially in the last 15 years compensating the decrease of sardine stock (ICES, 2020). Although the panmictic behaviour of the species leads to a unique stock between the two basins, in the Mediterranean Sea less is known and the stock has never been assessed. During the last ICES working group on the species, experts claimed there is no information available from acoustic surveys such as MEDIAS on chub mackerel (ICES, 2020). As a matter of fact, as already mentioned, here the data are collected as OPS together with the other non-target species. Moreover, the *S. colias* TS currently used for the conversion of acoustic data into biomass estimates rely on studies conducted on other species in the Pacific Ocean (Lillo et al., 1996).

1.5.3 *Sprattus sprattus*

Figure 1.5.5. *Sprattus sprattus* Photographed by myself during MEDIAS survey 2020

Sprattus sprattus (Linnaeus 1758) is one of the three clupeids species found in the Mediterranean Sea with *S. pilchardus* and *Sardinella aurita*. It inhabits the North Atlantic European waters, the Black Sea and the Mediterranean Sea (Figure 1.5.6). Since sprat prefers cold waters it is mainly distributed in the northern part of the basin in Northern Spain (GSA 6), in the Gulf of Lion (GSA 7) and in the Northern Adriatic Sea (GSA 17). In these regions, sprat is mainly found in shallow waters up to 50 m of depth. Its distribution in the Adriatic Sea depends on migration between the more productive area close to the Po River and the spawning ground in the Eastern Adriatic (Piccinetti et al., 2012; Tičina et al., 2000). Sprat is a multiple (batch) spawning species which reaches the peak of its spawning activities in the winter months in the Adriatic Sea (Tičina et al., 2000).

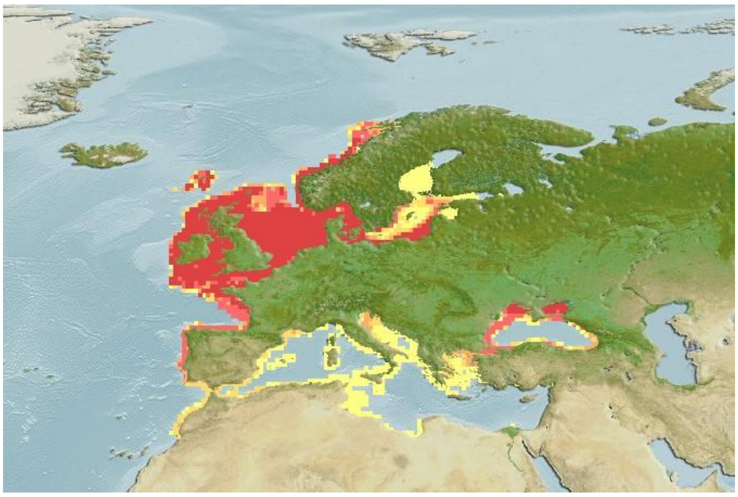

Figure 1.5.6. Geographical distribution of European sprat. Source: Forsee and Pauly, 2022

Sprat is the third fish species in terms of landing in GFCM area (Mediterranean and Black Sea) after anchovy and sardine with a mean of 49’985 tons landings annually from 2018 to 2020 (FAO, 2022). It is a target species of purse seine and pelagic trawl in the Black Sea, while in the Western Mediterranean Sea and the Adriatic Sea it is a low commercial value species which contributes with a very small percentage (less than 1%) to the landing of pelagic trawl because it is often discarded

(FAO, 2022). Also it is occasionally caught by the purse seine fisheries in the Ionian and Aegean Sea (Tsagarakis et al., 2012). Despite the low commercial value, in the Northern Adriatic Sea it is landing because is part of traditional food in several Italian regions, such as Emilia-Romagna and Veneto, with other ancillary species in the category of miscellaneous (Angelini et al., 2021). Here, it is generally considered an ancillary species for small pelagic fisheries, while in the Central and Eastern Mediterranean Sea the landing of this species is not documented (FAO, 2022).

In the pelagic ecosystem, sprat plays an important tropho-dynamic role by exerting both top-down control on zooplankton and being an abundant prey resource for piscivorous (Peck et al., 2012). But, as a cold favour species, it is threatened by the increasing sea temperature due to climate change which can lead in the future to a local extinction of the species in the entire Mediterranean Sea (Schickele et al., 2021). Moreover, the first stock assessment models carried out for sprat in the Mediterranean Sea have shown a pessimistic situation of the stock (Angelini et al., 2021). This confirms the decline in biomass registered from acoustic surveys in the Adriatic Sea shown in Figure 1.2.4. Therefore, it is fundamental to monitor the status of this stock through acoustic surveys. Long time series of sprat biomass is available in the Adriatic Sea thanks to the acoustic surveys carried out annually in both sides of the basin (Leonori et al., 2021). Nevertheless, only Azzali and colleagues in 1994 investigated the acoustic reflectivity of sprat in the Mediterranean Sea (Azzali et al., 1997), where the conversion factor b_{20} currently applied are heterogeneous and the values are mostly based on the TS of the other clupeids species (De Felice et al., 2021). The results published by Azzali et al (1997) are currently in use only in the Adriatic Sea. Conversely, several studies on TS aiming to give more accurate biomass estimates of sprat have been carried out in the Baltic Sea, North and Black Seas, (Didrikas and Hansson, 2004; Fässler and Gorska, 2009; Marinova and Panayotova, 2015; Panayotova et al., 2014).

1.6 Aim of the study

Despite the importance of the knowledge of the species-specific backscatter cross-section, most of the studies all over the world have been conducted on few target species, such as Atlantic herring (Nero et al., 2004), Argentine anchovy (Madirolas et al., 2017), common mackerel (Scoulding et al., 2016) and several gadoids species (Foote, 1987). Even the efforts, there are still many species of commercial importance which don't have a TS. In the Mediterranean Sea, the lack of data leads to a disagreement on the conversion factor values for most of the species (Table 1.6.1). The only one commonly employed by all the research groups relies on the target species *S. pilchardus* (-72.6 dB), while the values on the other most important commercial species, *E. encrasicolus*, resulted in heterogeneous as shown in Table 1.6.1 (MEDIAS 2022). For the remaining ancillary species, the b_{20} values are seldom investigated and the current conversion factors rely on studies conducted in other areas (see Table 1.4.2). This is the case of *S. colias* and *T. mediterraneus*, which are commonly gathered in the category of OPS during biomass estimates process. The values currently in use rely on studies conducted on other species of the same genus in the Atlantic and the Pacific Oceans (Barange and Hampton, 1994; Lillo et al., 1996; Axelsen, 1999; Svellingen and Ona, 1999; Axelsen et al., 2003; Robles et al., 2017). These values are not uniform in the Mediterranean Sea with a difference of up to -2.5 dB among countries (Table 1.6.1).

Table 1.6.1. Conversion parameter b_{20} in use for abundance estimates of pelagic fish species by areas and GSAs in the Mediterranean Sea during the MEDIAS project. Source: Table 1.4.2; MEDIAS, 2008; Doray et al., 2010

Species	**b_{20} values (dB re 1 m^2) by areas**				
	Adriatic Sea (GSAs 17 and 18)	Sicilian channel and Tirrenian Sea (GSAs 9,10,11,16,15)	Gulf of Lion (GSA 7)	Iberian coast (GSAs 1 and 6)	Aegean Sea (GSA 20)
Engraulis encrasicolus	-74.6	-75.3	-71.2	-72.6	-71.2
Sardina pilchardus	-72.6	-72.6	-72.6	-72.6	-72.6
Sprattus sprattus	-71.7		-71.2	-72.6	
Boops boops	-67				
Trachurus Spp.	-68.7	-71.2	-68.7	-68.7	
Scomber colias	-68.7		-70		
Sardinella aurita	-72.6	-72.6	-72.6	-72.6	-72.6

Moreover, to the best of the author's knowledge, no studies have been conducted on both species all over the world. Conversely, the TS of *S. sprattus* is well studied in the Black and Baltic Sea by *in situ* investigations, but not in the Mediterranean Sea, where only Azzali in 1995 investigated the TS of this species with a single beam transducer (Azzali, 1997) obtaining the value that is currently in use in the Adriatic Sea (see Table 1.4.2).

The importance of the study of non-target species increases in the Mediterranean Sea, where the pelagic ecosystem and the fisheries are multi-specific. In the Atlantic Ocean the stocks of *T. mediterraneus*, *S. colias* and *S. sprattus* are assessed yearly based on acoustic surveys carried out by the International Council for the Exploration of the Sea (ICES), whereas in the Mediterranean, despite their ecological and commercial importance, *S. colias'* stock has never been assessed, and only recently *S. sprattus* and *T. mediterraneus* stocks have been assessed, (Angelini et al., 2021). The multi-specificity of the Mediterranean Sea forces to use the mean nautical area scattering coefficient (NASC) allocated by haul composition through the mixed-species echo integrator conversion factor which converts the acoustic values into abundance in number (Nakken and Dommasnes, 1975). Consequently, the variability of sprat, Mediterranean horse mackerel and chub mackerel TS could also affect the biomass estimates obtained for the other two target species, anchovy and sardine. Additionally, a recent study demonstrates that sprat and Mediterranean horse mackerel, although the low commercial value compared to other species, are slightly overexploited in the Adriatic Sea (Angelini et al., 2021).

Nevertheless, fisheries acoustic technologies have advanced over time opening the possibility to employ a wide range of frequencies in broadband spectra as a key tool for qualitative analysis for species identification and TS measurement purposes (Korneliussen et al., 2018). The present study aimed to characterize the species-specific acoustic backscatter and increase the knowledge of species-specific TS for biomass assessment goal of *T. mediterraneus*, *S. colias* and *S. sprattus* through the application of *ex situ*, *in situ* experiments, analytical and numerical backscattering models. Data collected during four experiments on tethered fish conducted in 2013 were processed and analyzed. Additionally, the dataset of MEDIAS survey was scrutinized to find monospecific hauls useful for the application of the *in situ* method on these non-target species. The scrutinization resulted in only one sprat monospecific haul carried out in MEDIAS 2014 which was aggregated with new data specifically collected during MEDIAS 2020. Finally, backscattering models were implemented on the morphological and morphometric characteristics of the species in order to validate empirical data and characterize the species-specific acoustic fingerprint. The final goals were to provide reliable species-specific conversion parameter values, and acoustics diagnostic tools useful for the improvements of the assessment of the species dealt with this study in the Adriatic and the Mediterranean Sea.

To summarize:

- In Chapter 2, a pilot *ex situ* approach will be described, which involved tethering individual *T. mediterraneus* and *S. colias* specimens and placing them under the transducers to create a database encompassing a variety of body displacements and orientations.
- In Chapter 3, some issues will be addressed hampering *in situ* TS estimation as a result of the possibility of multiple target detection and the interpretation of single target TS values with respect to trawl catch LFD. Moreover, an assessment of the influence of TS on biomass estimates along the available time series will be presented.
- In Chapter 4, an analytical backscatter model (KRM) and a numerical model (FEM) will be employed on *T. mediterraneus*, *S. colias* and *S. sprattus* to compute the conversion factor as a function of tilt angle and frequency for comparisons purposes with the *ex situ* and *in situ* experiments results. The models allowed also the computation of broadband curve, relative frequency response and TS vs tilt angle curve to provide the species-specific characterization of acoustic backscatter as a function of frequency and tilt angle, beyond the estimates of conversion factor b_{20}.

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2. *Ex situ* experiment

First target strength measurement of *Trachurus mediterraneus* and *Scomber colias* in the Mediterranean Sea

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First target strength measurement of *Trachurus mediterraneus* and *Scomber colias* in the Mediterranean Sea

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Abstract

Knowing the species-specific target strength (TS) allows converting volume backscattering strength to numerical abundance. Since the acoustic surveys conducted for biomass assessment currently focus on the echoes of two or three target pelagic species in the Mediterranean Sea, the TS of non-target species has seldom been investigated in this basin. This is the first study of the TS of two pelagic species – Mediterranean horse mackerel (*Trachurus mediterraneus*) and Atlantic chub mackerel (*Scomber colias*) – in the Mediterranean Sea. A pilot approach using tethered live fish but not involving hooks and anesthetic was tested in experiments using a split-beam scientific echosounder operating at 38, 120, and 200 kHz. The mean TS was estimated for 29 live fish. The relationship between TS and fish length was determined with a standard linear regression model; the b_{20} conversion parameter was obtained with the slope forced to 20. b_{20} was computed at all frequencies for both species. The key values at 38 kHz were -71.4 dB for *T. mediterraneus* and -71.6 dB for *S. colias*. Although these results differ from those obtained with *in situ* and *ex situ* experiments using Pacific chub mackerel and other species of the genus *Trachurus*, they have the potential to provide new reference values for *T. mediterraneus* and *S. colias* biomass assessment in the Mediterranean

Sea. The proposed method removes some potential biases due to the unnatural behavior of anesthetized fish. Moreover, it provides an alternative to the use of hook, although the use of a piece of rope in place to the latter seems to rise the acoustic reflectivity of tethering apparatus.

Keywords: fisheries acoustics, target strength, *ex situ* experiments, Mediterranean horse mackerel, Atlantic chub mackerel.

Introduction

Scomber colias and *Trachurus mediterraneus* are two pelagic species found throughout the Mediterranean Sea (Scoles et al., 1998; Zardoya et al., 2004). Their lower commercial value compared to other pelagic species involves that they are often considered as minor species in this basin (Santojanni et al., 2005; FAO 2011; Carbonell et al., 2018). Yet, they are planktivorous and piscivores species that play an important ecological role in pelagic niches (Šantić et al., 2003; Sever et al., 2006). Their early life stages are secondary consumers like *Sardina pilchardus* and *Engraulis encrasicolus*, linking plankton to top predators in the trophic chain (Bănaru et al., 2019), whereas the mature stages are major clupeid predators (Šantić et al., 2003; Sever et al., 2006; Yankova et al., 2008) .

S. colias accounts for a substantial proportion of landings in the eastern Mediterranean fishing grounds (Bariche et al., 2006, 2007; Tsagarakis et al., 2012) whereas it is largely discarded in some Adriatic fisheries (Santojanni et al, 2005; STECF, 2016). In 2009 its total catch in the Mediterranean basin was about 12,000 tons (FAO, 2011). Since *S. colias* is often sold as *Scomber scombrus*, it is difficult to obtain reliable landings data (Zardoya et al., 2004). *T. mediterraneus* accounts for around 1.4% of all landings in the Mediterranean, with total catches of about 50,000 tons in 2014-2016 (FAO, 2018), and for a substantial portion of landings in the south-western Mediterranean (Carbonell et al., 2018) and the Adriatic Sea (Šantić et al., 2003). However, since it is often pooled with *Trachurus trachurus*, data are not wholly reliable.

In the Atlantic Ocean the stocks of *T. mediterraneus* and *S. colias* are assessed yearly based on acoustic surveys carried out by International Council for the Exploration of the Sea (ICES), whereas in the Mediterranean they have never been assessed, despite their ecological and commercial importance. The Scientific, Technical and Economic Committee for Fisheries (STECF) (2016) has highlighted the lack of species-specific landing and survey data for the genus *Trachurus* and *Scomber* and has stressed the importance of monitoring the status of *Scomber* in several geographical sub-areas

(GSAs). The collection of species-specific, fishery-independent data could help address the problem and provide sufficient information to assess their stocks.

Acoustic surveys are highly effective approaches to assess the distribution and abundance of pelagic and meso-pelagic species (Simmonds and MacLennan, 2005). Acoustic surveys have been conducted in the Mediterranean Sea since 2009 to monitor the status of these species (including Mediterranean horse mackerel and Atlantic chub mackerel) in the framework of the Mediterranean International Acoustic Surveys (MEDIAS), that involves several European countries joined by a standardized protocol (MEDIAS Handbook, 2020). However, the conversion of volume backscattering strength, provided by the surveys, to an absolute biomass estimate requires knowing the species-specific acoustic backscattering cross-section. This parameter is often expressed in terms of target strength (TS): TS = 10 log $\sigma/4\pi$ (Foote, 1987; Ona, 1990; Simmonds & MacLennan, 2005), in which sigma is the backscattering cross-section of fish. TS is the amount of incident wave reflected from a single target and it depends on the acoustic frequency used, fish body length, orientation (tilt angle) and swim-bladder features (Nakken and Olsen, 1977; Fässler et al., 2009). Notably, the swim-bladder is responsible for around 90% of the total energy reflected from a fish (Ona, 1990). Since TS is a stochastic variable (Simmonds and MacLennan, 2005), it can vary considerably within a species according to the behavior and physiological state of each individual (Horne, 2003). This should be kept in mind when computing the TS of a species or group of specimens.

TS measurement approaches can be grouped into three main categories:

- The *in situ* method consists of an acoustic survey of the fish in their natural environment using a split-beam echosounder and of their synoptic capture with a trawl net. The TS histogram data are then matched against the size frequency distribution measured in the haul samples (MacLennan and Menz, 1996). This is currently considered as the best available method, because, unlike *ex situ* approaches, it detects echoes as the fish swim (Henderson and Horne, 2007). Its main problems are the availability of monospecific hauls and gear selectivity.
- Backscattering models are "analytical and numerical expressions implemented using computer algorithms to predict acoustic backscatter" (Jech et al, 2015) and its amplitude. The digital representations of target shape and properties, *e.g.* fish anatomy and morphometry (chiefly their swim-bladder), material properties, and boundary conditions are considered as model inputs (Jech et al, 2015).
- *Ex situ* approaches allow measuring the TS of live, anesthetized or dead fish of known total length (TL) held in a cage or tethered at a predetermined depth (Thomas et al., 2002; Hazen and Horne, 2004). These experiments also afford direct computation of TS to TL regression, which is used to convert acoustic size to target size. *Ex situ* methods are practical and easy to

use and are commonly adopted as a first approach to measure the TS of new species (Azzali et al., 2010).

To the best of the authors' knowledge, there are no published studies investigating the TS of *T. mediterraneus* and *S. colias* in the Mediterranean Sea, whereas several studies have been conducted on species of the genus *Trachurus* in the Atlantic Ocean (Barange and Hampton, 1994; Lillo et al., 1996; Axelsen, 1999; Svellingen and Ona, 1999; Axelsen et al., 2003; Robles et al., 2017) and on the species *Scomber japonicas* in the Pacific Ocean (Gutiérrez and Maclennan, 1998; Lee and Shin, 2005; Kang et al., 2018).

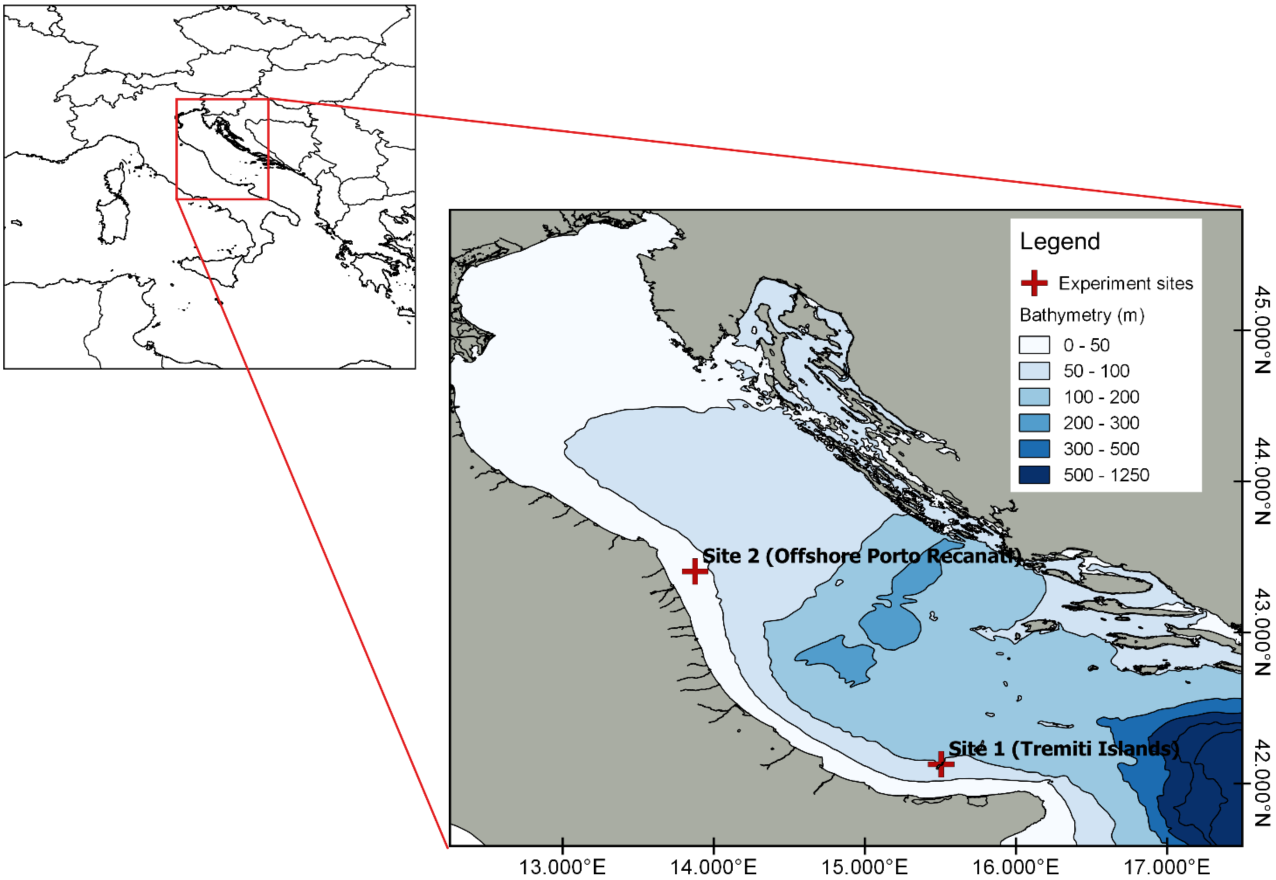

Figure 1. Sites (+) where the experiments were carried out at the Tremiti Islands (3 experiments, 12th, 14th, and 24th September 2013) and offshore Porto Recanati (one experiment, 26th September 2013).

We describe a pilot *ex situ* approach, which involved tethering individual *T. mediterraneus* and *S. colias* specimens and placing them under the transducers to create a database encompassing a variety of body displacements and orientations. By this method, we calculated their conversion factor which allows converting volume backscattering strength to species-specific biomass estimates.

Materials and methods

A total number of four experiments were conducted in the Adriatic Sea during the 2013 MEDIAS cruise. The experiments were conducted at the Tremiti Islands (3 experiments) and off Porto Recanati (one experiment) on board the R/V G. *Dallaporta*, which was equipped with a SIMRAD EK60 split-beam echosounder (Figure 1). The operating frequencies were 38, 120, and 200 kHz. The sound speed parameter was set by measuring water temperature, density, and salinity using a CTD (SEABIRD 911 PLUS) before each experiment. Routine calibrations (Demer et al., 2015) were performed using a 38.1 mm diameter tungsten carbide (with 6% cobalt binder) calibration sphere prior to TS measurements. The results of echosounder calibration and settings are shown in Table 1.

Table 2. Technical specifications and calibration parameters for the echosounder EK60 system used during the target strength measurements.

Specification	Tremiti Islands			Porto Recanati		
Frequency (kHz)	38	120	200	38	120	200
Absorption Coefficient (dB $\boldsymbol{m^{-1}}$)	0.0077	0.0501	0.0856	0.0083	0.05	0.0817
Sa Correction (dB)	-0.51	-0.34	-0.38	-0.61	-0.36	-0.31
Transducer Gain (dB)	25.54	25.32	25.41	25.77	26.14	25.15
Major Axis 3 dB Beam Angle (°)	7.03	6.35	6.41	7.07	6.26	6.36
Major Axis Angle Offset (°)	-0.07	-0.11	-0.05	-0.03	-0.01	-0.03
Major Axis Angle Sensitivity	21.9	23	23	21.9	23	23
Minor Axis3db Beam Angle (°)	6.97	6.54	6.32	7.07	6.27	6.3
Minor Axis Angle Offset (°)	-0.01	-0.04	-0.07	-0.09	0.02	-0.04
Minor Axis Angle Sensitivity	21.9	23	23	21.9	23	23
Sound Speed (m $\boldsymbol{s^{-1}}$)	1532.2	1532.2	1532.2	1525.3	1525.3	1525.3
Transmitted Power (W)	2000	250	150	2000	250	150
Transmitted Pulse Length (ms)	1.024	1.024	1.024	1.024	1.024	1.024
Two Way Beam Angle (dB re 1 Steradian)	-21	-20.4	-20.5	-21	-20.4	-20.5

Experimental design

The fish used in the experiments were caught in the area where the experiments would be conducted at night using hooks and lines as they were feeding near the surface (max depth, 60 m). After capture, they were allowed to acclimatize for 12 - 24 h in a tank (capacity, 200 liters) placed on deck and with running seawater. No more than 10 fish were held in the tank at the same time, to avoid further stress due to excessive density. Their handling was minimized and never exceeded 1 min. The experimental setup envisaged using the standard rig (rods, reels, and monofilament lines) for sphere calibration (Simmonds and MacLennan, 2005). A slight but crucial modification was that we suspended a tethered live fish, rather than the target sphere, under the transducers. Moreover, whereas the sphere is commonly tied at the end of the monofilament lines issuing from three rods, we used an 8 m monofilament line (0.60 mm in diameter) one end of which was tied to the three lines (two on the starboard side and one on the port side), whereas the other end was tied to a 1 kg lead weight. Two knots were tied by turning the main line on itself, to mark the place where the fish tether would be tied and to prevent the fish swimming too close to the weight (Figure 2).

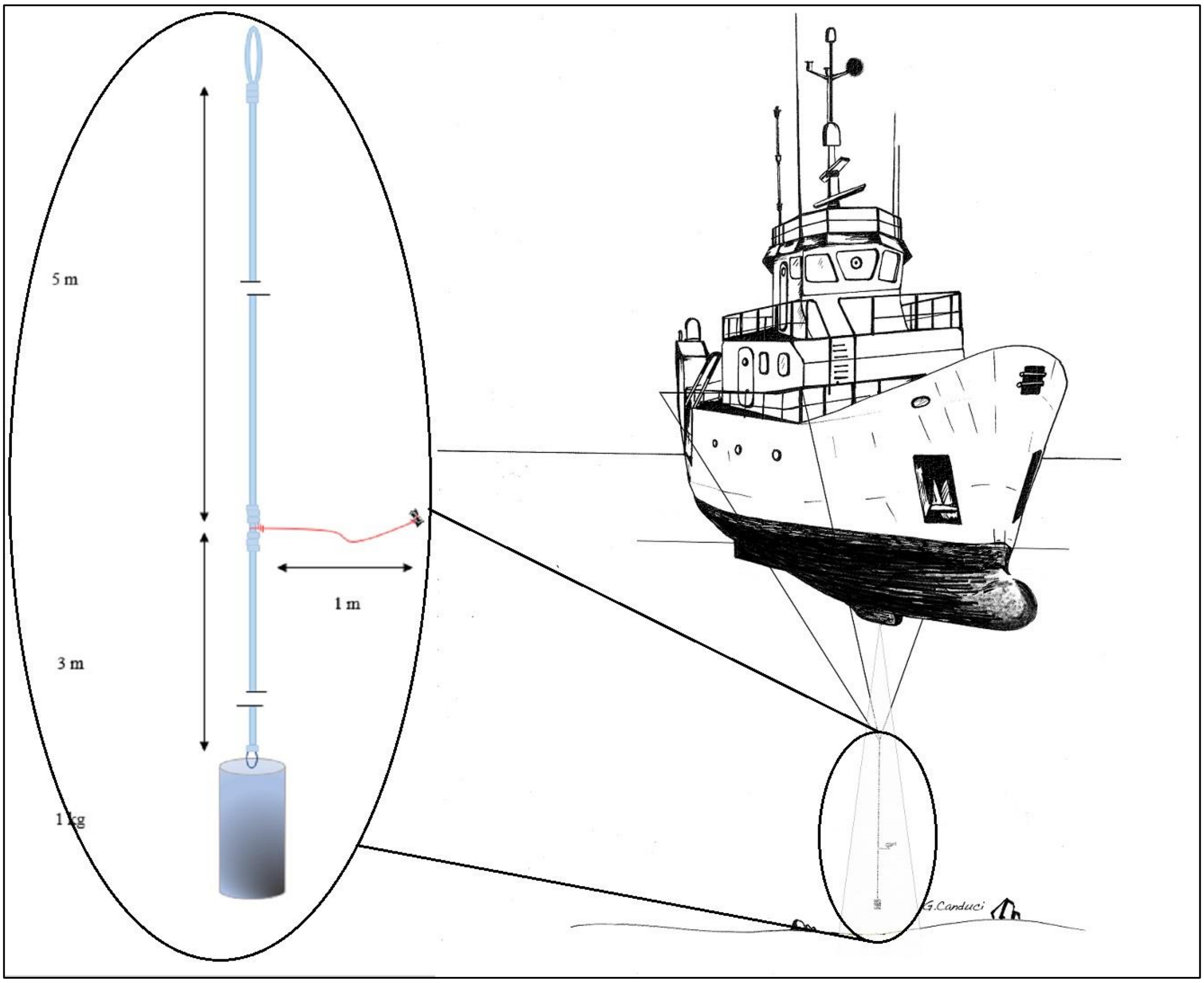

Figure 2. Experimental design.

The experiments involved using one fish at a time. Specimens were individually collected from the tank using a plastic fish basket that was immediately covered with a dark wet cloth, to reduce stress. To ensure that the fish remained still when it was placed on a table on deck, it was wrapped in and handled with the cloth. Then, a monofilament line (ca. 1 m in length and 0.40 mm in diameter) ending with a small fragment of synthetic rope (10 mm in length and 2 mm in diameter) was threaded through a thin needle, which was passed through the fish palate and made to exit through the maxillary and lacrimal bones. The monofilament was then pulled until the rope fragment stopped against the palate. With the fish still wrapped in the wet cloth, the free end of the line was tied to the main line. The fish was lowered into the sea and allowed to swim near the surface for around 10 min. All fish were highly vital. As soon as the specimen began to swim naturally around the rig, the lines of the three reels were lengthened further and adjusted, so as to place the fish into the acoustic beam at the desired depth, *i.e.* ≈21 m (*S. colias*) and ≈14 m (*T. mediterraneus*) at the Tremiti Islands and ≈18 m (*T. mediterraneus*) at Porto Recanati. Here the fish was again allowed to swim for 10 min before the TS measurements were performed. After the experiments the lines were gently reeled in and the fish were measured as soon as possible for the needed time in the lab for biometric parameters, gender and gonadal development in the shortest possible time. Thirty fish, 16 Atlantic chub mackerel (TL size range, 15.4 - 40.6 cm) and 14 Mediterranean horse mackerel (TL size range, 16.1 to 29.5 cm), were used in succession for the experiments. The study complies with the Italian animal research legislation (D. Lgs. N. 166 of 27/01/1992).

Table 3. List of the parameters used for the single-target detection split-beam Method 2 of Echoview software.

TS threshold	-62 dB
Pulse length determination level	6 dB
Minimum normalized pulse length	0.7 ms
Maximum normalized pulse length	1.5 ms
Two-way maximum beam compensation	4 dB
Maximum standard deviation minor-axis angle	0.6°
Maximum standard deviation major-axis angle	0.6°

Data analysis

The acoustic data were scrutinized using Echoview Software (v.10). The background noise removal and impulse noise removal functions were applied to the raw data according to the Echoview post-processing instructions (De Robertis and Higginbottom, 2007). Subsequently, the TS values were extracted through the single-target detection split-beam Method 2, whereby the application of an algorithm based on the standard phase deviation set on the split-beam angle data returns compensated TS values (Soule et al., 1997). The single-target detection parameters are listed in Table 2. To avoid including unwanted targets, only data from the depth layer where the fish was tethered were selected for single-target extraction. In all cases, the fish and the lead weight were clearly visible on the echogram, as shown in Figure 3. Any echogram section depicting considerable movement of the lead were discarded, since the current could push the fish outside the acoustic beam. In particular, the sections lacking evident lead echoes were marked as bad regions and cut, or else excluded using the Region Edit function. Careful examination of a control acoustic test, where the entire rig minus the tethered fish was placed under the acoustic beam (using a threshold of -70 dB and the same single-target parameters), allowed calculating the TS thresholds. A threshold of -62 dB was set at 38 kHz, -58 dB at 120 kHz and -57 dB at 200 kHz to provide a good compromise between the loss of fish echoes and the gain of echoes from the rope fragment or the monofilament line.

The TS to TL regression was calculated using the standard model: $TS = m \log L + b$ and the model proposed by Foote (1987): $TS = 20 \log L + b_{20}$. The latter model is based on the proportionality of the mean backscattering cross-section (σ) and the square of fish length. The standard model equation was solved by applying a linear regression model, where m and b were respectively the slope and the intercept and L was the TL of the specimens. The parameter b_{20} was estimated according to Simmonds & MacLennan (2005), to compute the equation proposed by Foote (1987) for each species and frequency.

Results

Data analyses were performed for all frequencies (38, 120, and 200 kHz), even though the frequency used most commonly for biomass estimations is 38 kHz. Only specimens presenting more than 50 single-target detections were included. A single individual did not reach this criterion and was excluded. Acoustic calibration with the standard sphere demonstrated little change between locations and no difference between days at the same site. The speed of sound was 1532 m/s at the Tremiti Islands and 1525 m/s off Porto Recanati. Analysis of the control test data (monofilament minus the fish) yielded a TS range of -69.4 to -61.4 dB (mean, -65.6 dB) at 38 kHz, which lent support to the threshold of -62 dB; a TS range from -67.3 dB to -57.7 dB at 120 kHz which lent support to the threshold of -58 dB and a TS range from -63 dB to -56.6 dB at 200 kHz which lent support to the threshold of -57 dB. As demonstrated by the TS distribution histograms shown in Figures 4 and 5, very few single targets reflected at -62 dB at 38 kHz. The histograms display a wide range of values (≥ 25 dB), from -62 dB to -35 dB for *T. mediterraneus* and from -61 dB to -30 dB for *S. colias*.

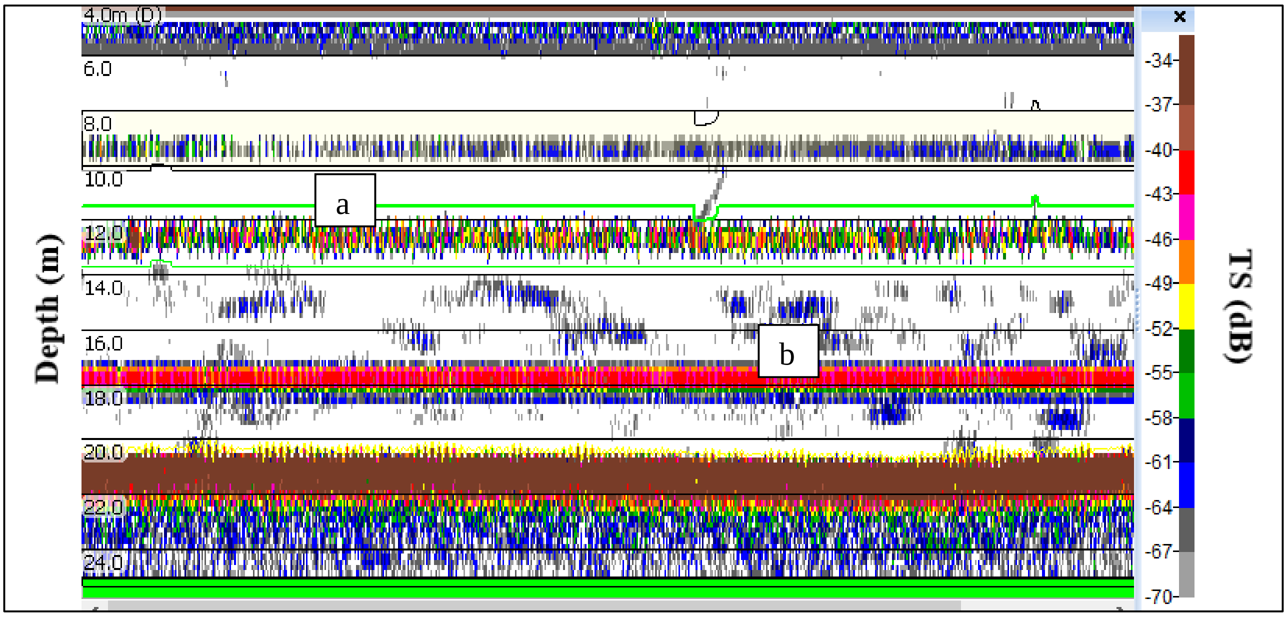

Figure 3. Representative echogram showing raw TS data of *T. mediterraneus* (frequency, 38 kHz). The color scale of TS (dB) values is on the right. The two layers are (a) the fish and (b) the lead weight. Pings horizontal number = 789.

The TS distribution can be often characterized by more than one mode and also mostly complies with the Gaussian distribution. The TS distribution of Atlantic chub mackerel specimens N8 (38.6 cm), N9 (39.7 cm), and N10 (15.4 cm) was characterized by a single mode to the right of the histogram, with a predominance of higher TS values (note the sign -).

The mean TS was computed on 16 Atlantic chub mackerel (TL size range, 15.4; 31.6 - 40.6 cm) and 13 Mediterranean horse mackerel (TL size range, 16.1 to 29.5 cm) based on the mean backscattering cross-section as $\overline{TS}$= 10log ($\bar{\sigma}_{bs}$). More details on biometric features of fish in Table S1 supplementary material. For *T. mediterraneus* TS mean ranged from -48.4 dB to -41.4 dB at 38 kHz, from -50.2 dB to -43. dB at 120 kHz, and from -49.14 dB to -41.9 dB at 200 kHz; for *S. colias* it ranged from -45.6 dB to -37.4 dB, from -47.6 dB to -41.1 dB, and from -46.8 dB to -40.5 dB, respectively. In Figures 6 and 7, the mean TS values are plotted against TL and the two regression lines are fitted in the same Cartesian plane, demonstrating that the mean TS values measured at 120 kHz and 200 kHz were lower and spread more widely around the slopes than those measured at 38 kHz. Mean TS increased significantly with TL for both species at 38 kHz (13 *T. mediterraneus* specimens: r^2=0.37; p= <0.05; 16 *S. colias* specimens: r^2=0.48; p= <0.01) and for Atlantic chub mackerel at 200 kHz (16 individuals; r^2=0.29; p= <0.05), whereas it showed a non-significant relationship with TL at 120 kHz, despite the fact that the trend was positive. The results of the fitted linear model are reported in Table 3.

Table 4. Results of the linear model regressions. The slope represents the parameter *m* in the standard equation. To compute the b_{20} values displayed below the slope was forced to 20.

Frequencies	***T. mediterraneus***		***S. colias***	
38 kHz	Slope= 15.1	b_{20}= -71.4	Slope= 15	b_{20}= -71.6
	Intercept *b*= -64.9		Intercept *b*= -63.8	
120 kHz	Slope= 11.6	b_{20}= -72.7	Slope= 10.9	b_{20}= -74.8
	Intercept *b*= -61.4		Intercept *b*= -60.7	
200 kHz	Slope= 11.8	b_{20}= -72.2	Slope= 12.4	b_{20}= -74.5
	Intercept *b*= -61		Intercept *b*= -62.7	

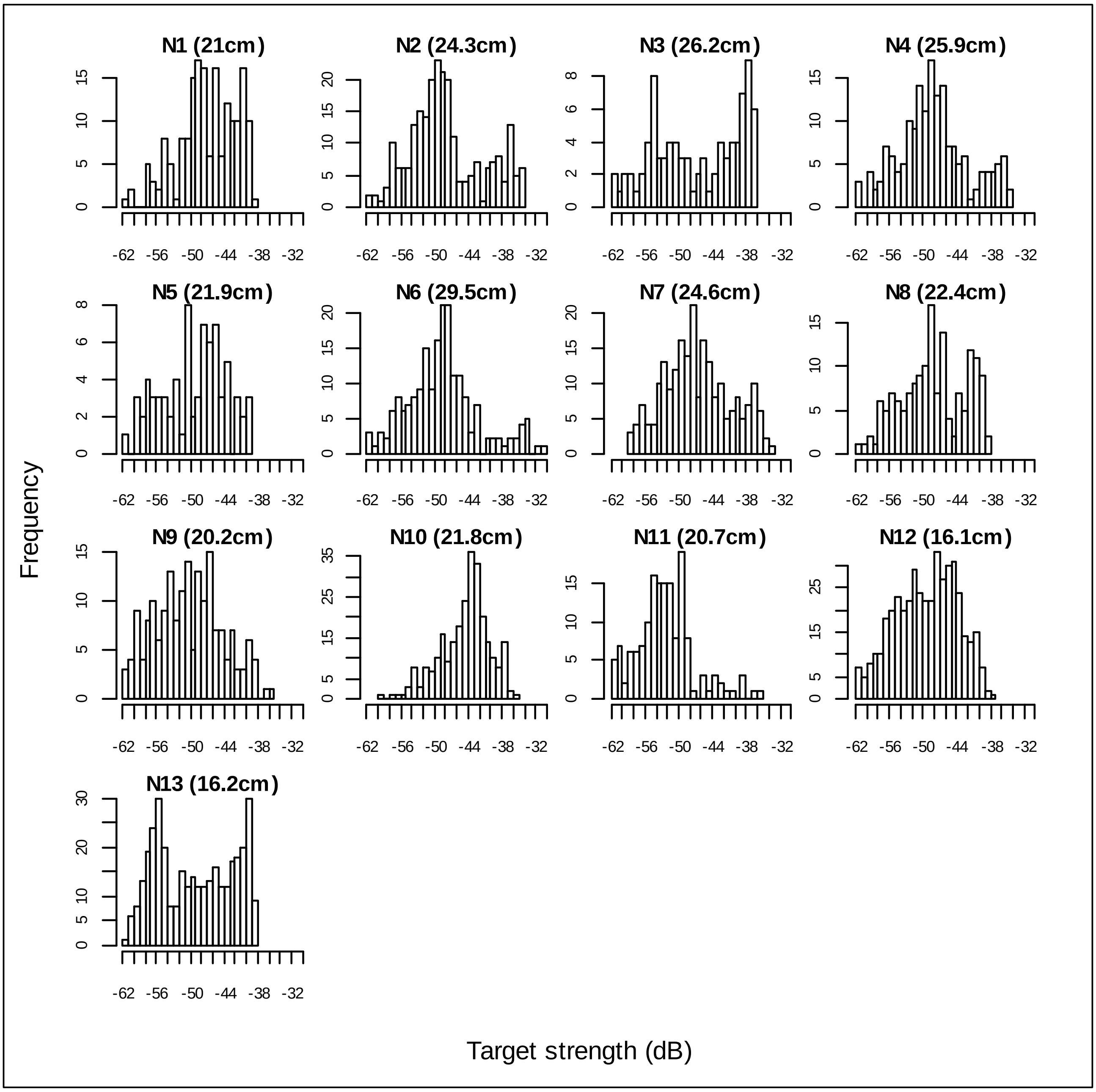

Figure 4. TS distribution of *T. mediterraneus* at 38 kHz. The ID and total length of each specimen are in parentheses.

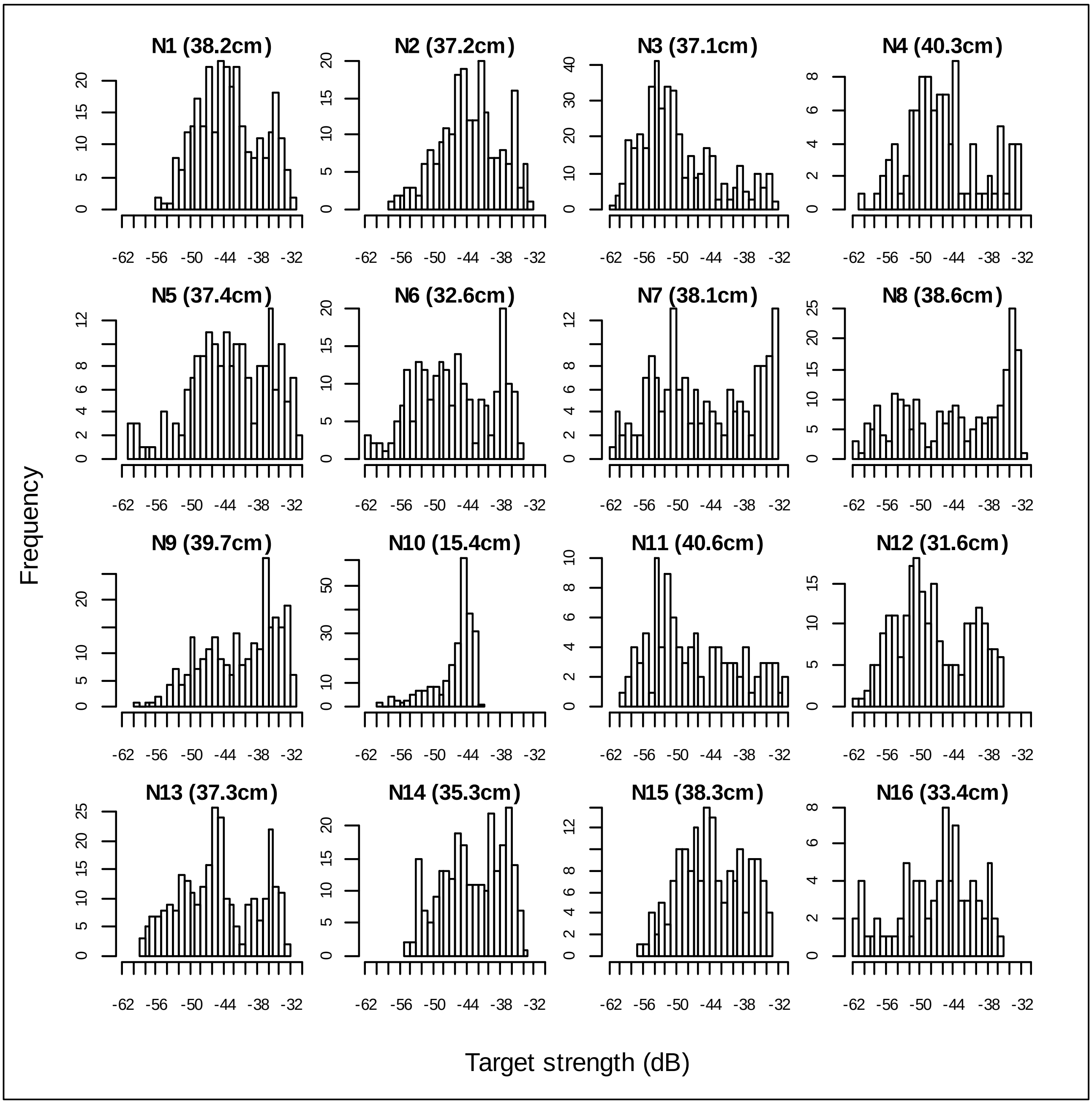

Figure 5. TS histograms of *S. colias* at 38 kHz. The total length and ID of each specimen are in parentheses.

Discussion

This is the first *ex situ* experiment performed on the genus *Trachurus* and on species *S. colias* (Lee and Shin, 2005; Robles et al., 2018). Use of the correct echosounder and echo detection settings and scrutinization of the raw data enabled accurate single-target detection and direct comparison of the TS and TL of each specimen. Notably, the tethered individuals used in *ex situ* experiments are often hooked and anesthetized, despite the fact that the anesthetic and the hook affect both fish behavior and echo reflection (O'Driscoll et al., 2018). According to Simmonds and MacLennan (2005), experiments carried out on immobile or not healthy fish are insufficiently accurate. The present experiments were devised to overcome these problems, obviating the use of the hook, hence of the anesthetic, allowing the fish free to move around the tethering apparatus.

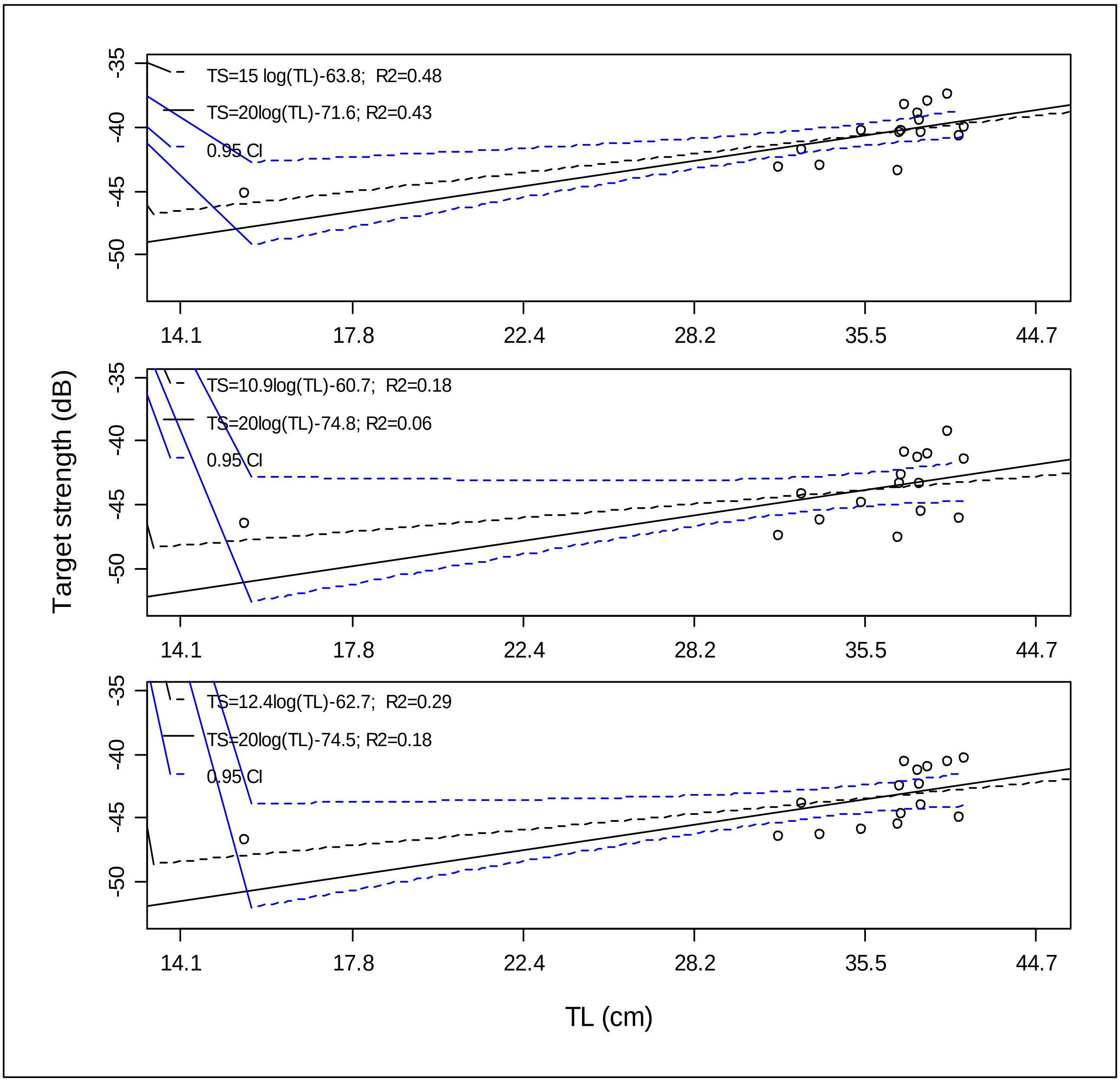

Figure 6. Target strength length (in logarithmic scale) relationship for *S. colias* calculated at 38 kHz (upper panel), 120 kHz (middle panel), and 200 kHz (lower panel). The results of the standard linear regression model (continuous line) and those obtained with the slope forced to 20 (dashed line) are also shown. Dots represent the mean TS of individual fish, while dashed blue lines report the 95% Confidence Interval.

The scrutinization of control test data during which no fish were tide to the line, resulted in great values of maximum TS especially for the higher frequencies. Hence, our attempt to use a small piece of rope in place of hook brought to worse results on the acustic gain of echoes coming from the tethered apparatus compared to other *ex situ* experiments that involved hooks (Thomas et al., 2002; Henderson and Horne, 2007; Boswell and Wilson, 2008). On the other end, all fish displayed a good vitality and data reported in Figures 4 and 5 clearly show the stochastic displacements of the fish, since TS variability largely depends on fish tilt angle and roll (McClatchie et al., 1996; Horne et al., 2000).

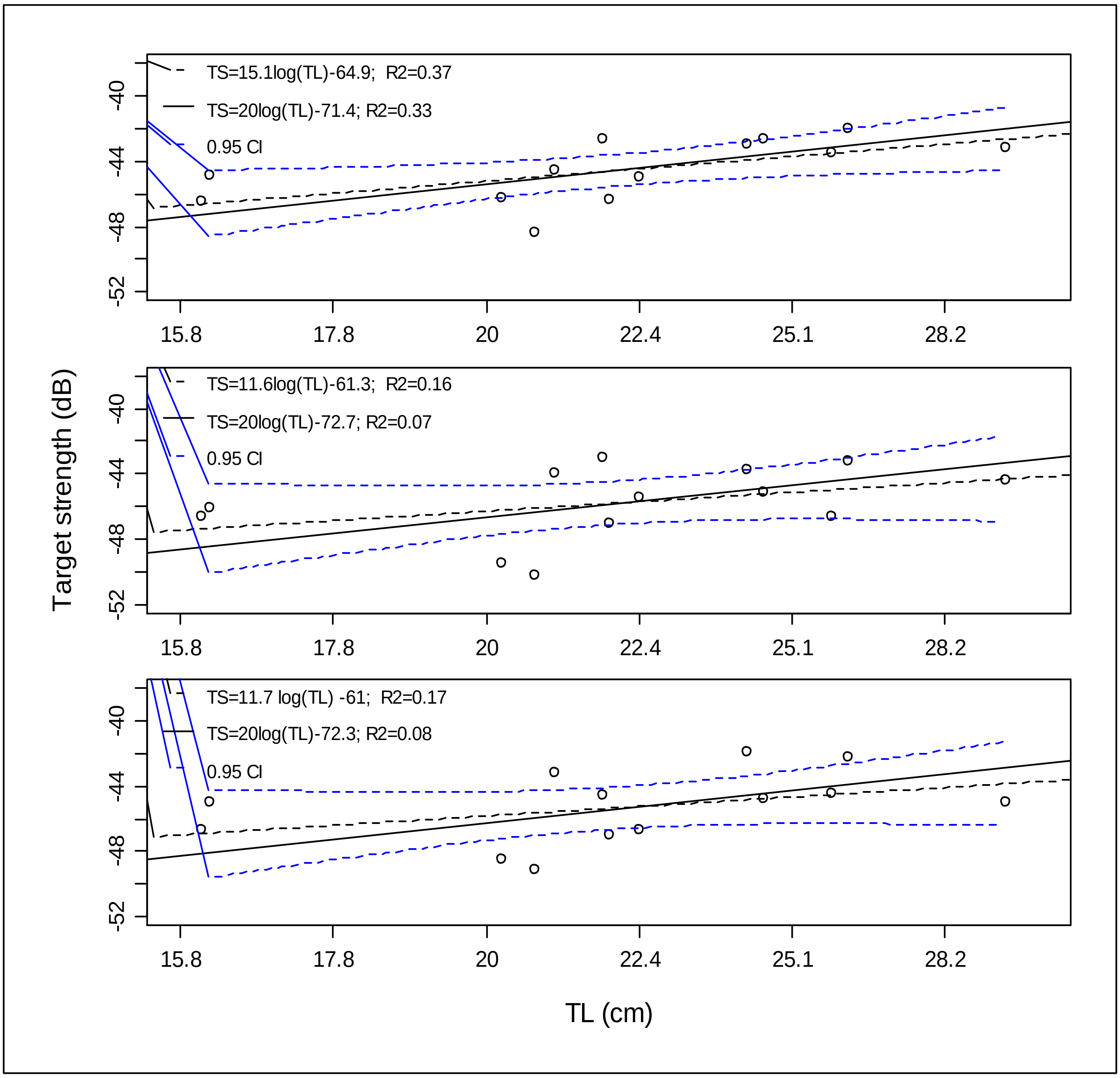

Figure 7. Target strength-TL relationship for *T. mediterraneus* at 38 kHz (upper panel), 120 kHz (middle panel), and 200 kHz (lower panel). The results of the standard linear regression model (continuous line) and those obtained with the slope forced to 20 (dashed line) are also reported. Dots represent the mean TS of individual fish, while dashed blue lines report the 95% Confidence Interval.

Generally, data indicated that we had acquired a wide range of tilt angles and fish movements, which however could not be measured. Results of specimens N8, N9 and N10 of Atlantic chub mackerel are likely due to the predominance of a narrow range of negative tilt angles during TS measurements. As a matter of fact, fish orientation influences the numbers of modes and echo intensity. Moreover, while swimming, the mean tilt angle of most fish with a swim-bladder is close to 0° (Henderson and Horne, 2007; Kubilius and Ona, 2012). Peña and Foote (2008) have described a mean angle between +1° and

-6° with a standard deviation of 8° to 18° for the genus *Trachurus*. Our data – showing multiple modes and a Gaussian distribution included in a 30 dB range as well as low values of the slopes (Table 3) – as a matter of fact, the Figures 4 and 5 suggest that the tethered fish displayed a good range of tilt angles during the experiments. Indeed, several *ex situ* experiments involving medium-large fish have described a slope exceeding 20, probably because the fish were forced in a near-horizontal position exposing the swimbladder to a tiny positive or negative tilt angle due to the general offset of this organ to the body axis (Simmonds and MacLennan, 2005). All our values are lower than 15.1. Furthermore, in all cases r^2 is greater in the standard equation (Figures 6 and 7), indicating a more limited adaptation of the TS to TL regression with the slope forced to 20. The great variability in the mean TS shown in Figures 6 and 7 at all frequencies underline how for these species, fish characterized by 10 cm different size can bring to around the same mean TS. This may be due to an incongruent growth of the swim-bladder compared to the square of fish TL, abnormal fish behavior, high variability in tilt angle or the narrow size range considered (*T. mediterraneus*, 15.4; 31.6- 40.6 cm, S. colias, 16.1 - 29.5 cm). Therefore, the conversion parameter of the standard model could be more accurate for *S. colias* and *T. mediterraneus*. Nevertheless, the limited size range and number of specimens used in our experiments involve that we must accept as the best function the 20 log L dependence (Simmonds and MacLennan, 2005; McClatichie et al., 1996). The use of target strength–length regressions forced through a slope of 20 appears to be useful for the comparison within the same species and between species of the same group as suggested by McClatchie et al. (1996). Since there are no published acoustic data on *T. mediterraneus*, and *S. colias* (Table 4), the most rational approach seems to use a model fit to 20 to compare our data to those of the three most widely studied species, *Trachurus capensis* (Cape horse mackerel), *Trachurus symmetricus murphy* and *Scomber japonicus* (listed in Table 4). Which are characterized by similar physiological and morphological features to the species deal with this study, in fact they present the same swim-bladder anatomy (Fisher et al., 1981).

Table 5. Overview of published b_{20} values for the genus *Trachurus* and the species *S. japonicus*. The mean TS ($\overline{TS}$) or the TS range (/) are reported where available.

References	Species	Method	Freq (kHz)	TL (cm)	TS (dB)	b_{20} (dB)
Svellingen & Ona, 1999	*T. capensis*	*In situ*	38	$\overline{17.2}$ $\overline{15.8}$	$\overline{-42.1}$ $\overline{-41.4}$	**-66.8** **-65.2**
Axelsen, 1999	*T. capensis*	*In situ*	38	$\overline{17.5}$	-55/-28 -55/-36	**-75** **-76.2**
Axelsen, 2003	*T. capensis*	*In situ*	38	$\overline{17.2}$ $\overline{18}$ $\overline{26.7}$ $\overline{25.1}$ $\overline{25.1}$	$\overline{-45.9}$ $\overline{-49.5}$ $\overline{-48.8}$ $\overline{-46.9}$ $\overline{-48.4}$	**-70.9** **-74.9** **-77.5** **-75.1** **-76.6**
Barange & Hampton, 1994	*T. capensis*	*In situ*	38	$\overline{40.2}$	$\overline{-38.5}$	**-66.8**
Lillo et al., 1996	*T. symmetricus murphyi*	*In situ*	38	22-40		**-68.91**
Peña & Foote, 2008	*T. symmetricus murphyi*	*Model*	38	20-47	-28/-47	**-72.1**
Robles et al., 2018	*T. symmetricus murphyi*	*In situ*	38 120	27-38	$\overline{-38.11}$ $\overline{-38.79}$	**-68.9** **-69.6**
Gutierrez & MacLennan, 1998	*T. capensis*	*In situ*	38	33-48		**-68.15**
Gutierrez & MacLennan, 1998	*S. japonicus*	*In situ*	38 120	26-30		**-70.95** **-70.8**
Lee & Shin, 2005	*S. japonicus*	*Ex situ*	120 200	26.2-38.3		**-66.9** **-71.1**
Svellingen & Charouki, 2008	*S. japonicus*	*In situ*	38 120	$\overline{21.8}$	$\overline{-50.9}$ $\overline{-53.1}$	**-77.6** **-79.8**

Axelsen and co-workers (Axelsen, 1999; Axelsen et al., 2003) obtained very low b_{20} values for *T. capensis* in an experiment involving a submersible transducer that was suspended just above the fish school. Barange and Hampton (1994) documented that the escape behavior of Cape horse mackerel can influence the backscattering cross-section results. As a matter of fact, the TS distribution shifted downward during trawling operations, due to an increased tilt angle. This suggests that the results reported by Axelsen and co-workers may have been influenced by fish escape behavior. In our study this bias was removed by performing all TS measurements with the vessel stationary. If the results of Axelsen and co-workers are excluded, the published b_{20} values of *Trachurus* range from -65.2 dB to -72.1 dB. At -71.4 dB, notably, our b_{20} is similar to the one obtained by Peña and Foote (2008) by applying the Kirchoff Ray Mode model to a 3D swimbladder shape, but it is lower than those reported by other researchers. This can be explained by the smaller size of the *T. mediterraneus* specimens used in our experiments (mean TL, 22.4 cm; range, 16.2 - 29.5 cm) and by the test conditions, which may influence fish behavior. The comparison of our data to the reference values used by the MEDIAS research groups for biomass assessment, which come from Lillo et al. (1996), yields a 2.5 dB lower value.

TS experiments have seldom been performed at 120 kHz and 200 kHz, due to the sporadic use of the latter frequencies in fish biomass estimations. Indeed, frequency selection should minimize the $L(length)/\lambda(wavelength)$ ratio (Demer et al., 1999; Hazen and Horne, 2003; McKelvey and Wilson, 2006). The TS to TL relationships found in the present study demonstrate that measurements at 38 kHz provide the best-fit linear models (*T. mediterraneus*, $r^2 = 0.37$; *S. colias* , $r^2 = 0.48$). However, the other two frequencies were also used to gather data to improve the multifrequency approach (Korneliussen, 2018). The difference between $b_{20} = -72.7$ dB at 120 kHz, reported in Table 3, and $b_{20} = -69.6$ dB reported by Robles et al. (2018) for *T. symmetricus murphyi* is probably to be attributed to the application by the authors of a -55 dB threshold besides the different fish size range.

Since Mediterranean horse mackerel is a physoclist species whereas Atlantic chub mackerel is a physostomous species (Park et al., 2015), we expected a greater difference between them, as reported in the literature for other fish families (Foote et al., 1986; Foote, 1987). The difference in their acoustic reflectivity is usually due to depth, since Atlantic chub mackerel is unable to compensate for the volume compression caused by pressure (Thomas et al., 2002; Gorska and Ona, 2003; Fässler et al., 2009b; Ganias et al., 2015). However, all the specimens used in the experiments were first acclimatized in a tank and insonified at about the same depth, thus removing the variability related to this parameter. Moreover, the comparison between physoclist and physostomous species is often performed against clupeids, which are physostomous but show significant morphological differences from *Scombridae* (Somarakis et al., 2000; Fisher et al., 1981). Therefore, the comparison between *Carangidae* and *Scombridae* does not necessarily reflect the classic distinction. The similarity may be attributed to a similar shape of the swim-bladder rather than to similar meristic and morphological features. Further modeling experiments based on fish anatomy and swim-bladder dimension and shape will likely clarify this issue.

As shown in Figures 4 and 5 and in Table 3, TS (and consequently b_{20}) is highly variable within the same species and genus. This variability can be associated to a number of environmental factors, resulting in a different morphological adaptation and reflection (Horne, 2003; Hannachi et al., 2004; Fässler and Gorska, 2009). Since the physical environment influences fish physiology and morphology (Scoles et al., 1998), as demonstrated for *S. japonicus* and *T. mediterraneus* in the Black and Mediterranean Seas (Turan, 2004; Erguden et al., 2009), TS may vary among areas as reported for herring (Ona, 1990), among other species. Therefore, the $b_{20} = -68.7$ dB used by some MEDIAS researchers to estimate *T. mediterraneus* and *S. colias* biomass (Lillo et al., 1996) and the $b_{20} = -70.9$ dB obtained in the Pacific Ocean and used by IFREMER for *S. colias* (Gutierrez and MacLennan, 1998), may be insufficiently accurate.

Conclusion

In the framework of the MEDIAS, the data of the two species, collected for biomass assessment purposes, are pooled under the category "other species". The b_{20} values obtained in the present study, -71.4 dB for *T. mediterraneus* and -71.6 dB for *S. colias*, can be used for single-species assessment and can be considered as a starting point to overcome the regional knowledge gap in the acoustic backscattering coefficients of non-target species. The main limitation of our approach is related to its possible influence on fish swimming behavior, although it can be less invasive than the other methods used in *ex situ* experiments. The specimen size range considered in the study may also be too narrow. Further work is clearly needed to validate our approach. A backscatter model would be able to support our conclusions by enabling processing factors, such as the tilt angle, which are not measured in this study. In contrast, *in situ* experiments are difficult to perform to assess these species, since they require monospecific hauls but have to be conducted at night, when several other fish species come to the surface to feed, thus forming multispecies schools. Altogether, our findings have the potential to be used for future biomass estimations of Atlantic chub mackerel and Mediterranean horse mackerel, provided that they are validated by further investigations of their backscattering cross-section.

CREdiT authorship contribution statement

Antonio Palermino: Formal analysis, Data curation, Writing-Original Draft, Visualization. **Andrea De Felice:** Conceptualization, Methodology, Validation, Investigation, Writing- Review & Editing, Supervision. **Giovanni Canduci:** Conceptualization, Methodology, Investigation, Writing- Review & Editing. **Ilaria Biagiotti:** Writing- Review & Editing. **Ilaria Costantini:** Invesigation,Writing-Review & Editing. **Sara Malavolti:** Writing- Review & Editing. **Iole Leonori:** Supervision, Conceptualization, Project administration, Writing- Review & Editing, Resources.

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Supplementary material

Tabele S1. Biometric measures of *Scomber colias* and *Trachurus mediterraneus* individuals used during *ex situ* experiments in September 2013. TL: Total length, FL: Fork length, W: weight. Sex and maturity stage have been determined following the ICES scale (ICES, 2008)

ID	Date	Species	TL (cm)	FL (cm)	W (g)	Sex and maturity stage
N1	24/09/2013	*Scomber colias*	38.2	-	493	F6
N2	24/09/2013	*Scomber colias*	37.2	-	486	F6
N3	24/09/2013	*Scomber colias*	37.1	-	424	M6
N4	24/09/2013	*Scomber colias*	40.3	-	512	F5
N5	24/09/2013	*Scomber colias*	37.4	-	467	M6
N6	24/09/2013	*Scomber colias*	32.6	-	260	F5
N7	24/09/2013	*Scomber colias*	38.1	-	478	F5
N8	24/09/2013	*Scomber colias*	38.6	-	493	F3
N9	24/09/2013	*Scomber colias*	39.7	-	498	F5
N10	24/09/2013	*Scomber colias*	15.4	-	25	IND
N11	12/09/2013	*Scomber colias*	40.6	33.7	554.0	M3
N12	12/09/2013	*Scomber colias*	31.6	26.7	264.0	F5
N13	12/09/2013	*Scomber colias*	37.3	31.5	417.0	F5
N14	12/09/2013	*Scomber colias*	35.3	29.4	342.0	M3
N15	12/09/2013	*Scomber colias*	38.3	31.7	502.0	F5
N16	12/09/2013	*Scomber colias*	33.4	28.4	310.0	F5
N1	24/09/2013	*Trachurus mediterraneus*	21	-	71.8	M2
N2	24/09/2013	*Trachurus mediterraneus*	24.3	-	108	F1R
N3	24/09/2013	*Trachurus mediterraneus*	26.2	-	120.6	F5
N4	24/09/2013	*Trachurus mediterraneus*	25.9	-	119.9	F1R
N5	24/09/2013	*Trachurus mediterraneus*	21.9	-	73.8	F5
N6	14/09/2013	*Trachurus mediterraneus*	29.5	23.8	170.0	M1
N7	14/09/2013	*Trachurus mediterraneus*	24.6	20.2	110.0	M2
N8	14/09/2013	*Trachurus mediterraneus*	22.4	18.1	93.0	F6
N9	14/09/2013	*Trachurus mediterraneus*	20.2	16.4	66.0	F5
N10	14/09/2013	*Trachurus mediterraneus*	21.8	19.7	80.0	M5
N11	14/09/2013	*Trachurus mediterraneus*	20.7	17.1	66.0	F2
N12	14/09/2013	*Trachurus mediterraneus*	16.1	13.2	30.0	M2
N13	14/09/2013	*Trachurus mediterraneus*	16.2	13.5	34.0	F1

3. *In - situ* experiment

Preliminary target strength measurement of *Sprattus sprattus* and its influence on biomass estimates in the Adriatic Sea (Mediterranean Sea)

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Preliminary target strength measurement of *Sprattus sprattus* and its influence on biomass estimates in the Adriatic Sea (Mediterranean Sea)

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Abstract.

Post-processing data analysis has the ability to affect target strength (TS) measurement, which in turn influence the biomass estimates of acoustic surveys. *In situ* experiments are considered as the best method to measure species-specific acoustic reflectivity since fish are measured in their natural environment without constrains. Nevertheless, this approach poses some problems concerning the methods to be applied to analyze acoustic data, especially when only a small number of monospecific hauls are available. In this study we tested several alternative ways to compute the b_{20} of sprat (*Sprattus sprattus*) in a poor data state in the Mediterranean Sea where the TS of the species is seldom studied. The method proposed by MacLennan and Menz (1996) and a pilot approach were used in parallel with the implementation of three single target detection algorithms to analyze two monospecific sprat hauls performed in the Adriatic Sea in 2014 and 2020. Next, we applied a Monte Carlo simulation to investigate the influence of TS variability on biomass estimates. We obtained six b_{20} values (range, -68.8 dB to -65.5 dB), all higher than the current known value of -71.7 dB. The resulting coefficient of variation in the overall uncertainty on absolute biomass estimates overcame

the 17%. The different models provided different total biomass estimates, however, the method proposed herein applied on high-density filter single target detection data suggest a value of b_{20} between -67.5 dB and -68.8 dB for sprat in the Mediterranean Sea, which significantly reduces absolute sprat biomass values between 2014 and 2021.

Keywords: fisheries acoustics, target strength, *in situ* measurement, sprat, Mediterranean Sea

Introduction

Sprattus sprattus (Linnaeus 1758) inhabits the North Atlantic European waters, the Black Sea and the Mediterranean Sea. Since sprat prefers cold waters, its spatial distribution is limited to the northern part of the basin in Northern Spain, Geographical Sub Area (GSA) 6, in the Gulf of Lion (GSA 7), and in the Northern Adriatic Sea (GSA 17), see Figure 1. Where sprat have been mainly found in shallow waters up to 50 m of depth. Its distribution in the Adriatic Sea depends on migration between the Po River and the spawning ground in the Eastern Adriatic (Piccinetti et al., 2012; Tičina et al., 2000). As a matter of fact, sprat is a multiple (batch) spawning species which reaches the peak of its spawning activities in the winter months in the Adriatic Sea (Tičina et al., 2000). Sprat is the third species in terms of landing in GFCM area (Mediterranean and Black Sea) after anchovy and sardine with a mean of 57’427 tons’ landings annually from 2016 to 2018 (FAO, 2020). The bulk of captures in the Mediterranean Sea focuses in the Adriatic Sea, where in the same period the average annual landings was ~200 tons (Angelini et al., 2021). In the pelagic ecosystem sprat plays an important tropho-dynamic role by exerting both top-down control on zooplankton and being an abundant prey resource for piscivorous (Peck et al., 2012). But, as a cold favouring species, it is threatened by the increasing sea temperature due to climate change which can lead in the future to a local extinction of the species in the entire Mediterranean Sea (Schickele et al., 2021). The abundance of sprat is annually estimated by means of acoustic surveys, but despite its importance, it is less studied in the Mediterranean Sea compared to the other clupeids, *Engraulis encrasicolus* and *Sardina pilchardus*.

Acoustic surveys are highly effective methods to assess the distribution and abundance of pelagic species (Simmonds and MacLennan, 2005). However, the conversion of volume backscattering strength, provided by the surveys, into absolute biomass estimate requires knowing the species-specific acoustic backscattering cross-section, which in turn depends on species and size composition. This parameter is expressed in terms of target strength (TS), i.e. the amount of incident wave reflected from a single target, which depends on the acoustic frequency used and on fish body length, orientation (tilt angle), and swimbladder features (Nakken and Olsen, 1977; Fässler et al., 2009). The swimbladder is responsible for ~ 90% of the total energy reflected from a fish (Ona, 1990). TS is expressed as follows: TS = 10 $\log_{10} \sigma/4\pi$ (dB re 1 m^2; Foote, 1987; Ona, 1990; Simmonds and MacLennan, 2005), where σ_{bs} is the acoustic backscattering cross-section. Since TS is a stochastic variable (Simmonds and MacLennan, 2005), it can vary considerably within a species according to the behaviour and physiological state of each individual (Horne, 2003). This should be kept in mind when computing the TS of a species.

TS measurement techniques can be grouped into three main categories: *ex situ,* where fish TS is measured in controlled experiments (Nasse et al., 2009; Azzali et al., 2010; Kubilius et al., 2012; Palermino et al., 2021); backscattering modelling, where analytical and numerical algorithms are implemented to predict acoustic backscatter (Jech et al., 2015); and *in situ*, where fish are measured in their natural environment (MacLennan and Menz, 1996; O'Driscoll et al., 2017; 2018; Salvetat et al., 2020). The latter is currently considered the best available method, because it detects echoes as the fish swim naturally and without constraints (Henderson and Horne, 2007), even though the scarcity of monospecific hauls, instrument availability and gear selectivity still involve limitations. The introduction of the split-beam echosounder has overcome the physical and technical (equipment) limitations of direct *in situ* TS measurement methods, which are its chief disadvantages (Ona, 1999). Notably, it potentially allows locating individual fish in the three-dimensional space via the electrical phase deviation between the signal received from each of the four quadrants.

Nevertheless, TS uncertainty is still a major source of variance of the abundance indices computed with acoustic methods (Rose et al., 2000; Scoulding et al., 2016). The possibility that multiple targets may be detected as a single target, advises against measuring the schools usually encountered in daylight and effectively confines *in situ* measurements to the night hours, when fish are scattered (Simmonds and MacLennan, 2005). In addition, although the single target detection algorithm split-beam method 2 implemented in Echoview software reduces the sampled volume by setting a cut-off on pulse length, beam compensation, and phase deviation, to reduce multiple target retention, a proportion of overlapping echoes may still be falsely detected as single targets (Soule et al., 1997). A high- density filter is usually applied to implement multiple target rejection; this involves a good knowledge of the species-specific TS, based on the mean length of the trawl catch which however pose some bias (Madirolas et al., 2017; Sawada et al., 1993). Therefore, some problems persist. However, multiple target rejection has been further improved by Demer et al. (1999) using a multi-frequency algorithm on spatial matching criteria between transducer faces.

Another issue affecting the *in situ* measurement of TS is the interpretation of the relationships among fish size, acoustic frequency, and TS (Love, 1971). In fact, the acoustic equations, to convert the

Nautical Area Scattering Coefficient (NASC) into abundance, require TS as a function of fish Total Length (TL) according to the standard model (Simmonds and MacLennan, 2005):

$$TS = m \log_{10} L + b \qquad (1)$$

and the model proposed by Foote (1987):

$$TS = 20 \log_{10} L + b_{20} \qquad (2)$$

The conversion factor b_{20} under study is obtained from this second model, where L is the TL of a single fish or mean TL of a fish school. The general starting point for a correct *in situ* measurement is a good match between the modes or means of the fish-length frequency distribution (LFD) derived from the trawl catch and those derived from the TS acoustic measurement frequency distribution on the same population (Dunford et al., 2015; Hannachi et al., 2004; Madirolas et al., 2017). However, the aforementioned approach risks being too subjective and may fail to explain the stochastic nature of TS, which in repeated measurements of apparently similar targets yields a variety of values (Anonymous, 2012). TS-TL matching modes or means are usually acceptable only in particular circumstances, namely when analyzing numerous monospecific hauls, each characterized by a single year class of the same species (Simmonds and MacLennan, 2005). Notably, the variance in TS values could be explained by the theoretical approach that takes into account the echo amplitude probability density function (PDF) of fish scattering (MacLennan and Menz, 1996). As a general proposition, the choice of model depends on sonar transmission wavelength relative to target size (scattering size). For physoclistous and physostomous fishes, the scattering size roughly corresponds to the swimbladder gas volume (Ona, 1990). If L < wavelength (λ), scattering is Rayleigh type, whereas if λ < L, it is said to be geometrical. However, the wavelengths commonly used in fisheries acoustics are related to the size of discrete scatters such as swimbladder dimensions (Love, 1971), but the inhomogeneous structure of fish and their variable orientation yields different echo phases. Consequently, the scatter distribution can change rapidly, leading to more complicated variations in relation to size and frequency than are suggested by simple geometrical models. Due to the above considerations, it is therefore generally accepted that the scatter of active fish shows a Rayleigh-like distribution (Stanton et al., 2004). A statistical, objective method proposed by MacLennan and Menz (1996) is based on the Rayleigh assumption that the backscattering cross-section has an exponential distribution and gives greater importance to the modal than the mean values (MacLennan and Menz, 1996). Another interesting statistical approach based on TL-TS mean matching principle has been developed by Kasatkina (2009), who devised an automatic method to reduce subjectivity and uncertainty when post-processing *in situ* TS estimates.

This work was designed to gain insight into the acoustic reflectivity of sprat (*Sprattus sprattus*) in the Adriatic Sea. Data on this physostomous species in the Mediterranean Sea are more scarce than in other seas (ICES, 1983; Didrikas and Hansson, 2004; Marinova and Panayotova, 2015); indeed, only one study has been conducted in the Adriatic Sea (Azzali et al., 1997) with single beam transducers. Therefore, since monospecific sprat hauls are rare in this area, we tested different methods to establish the differences in the results on b_{20} if several methods are applied in a poor data state. In this study, we address some issues that hamper *in situ* TS estimation as a result of the possibility of multiple target detection and the interpretation of single target TS values with respect to trawl catch LFD. We compare two post-processing statistical approaches based on the Rayleigh scattering assumption according to MacLennan and Menz (1996) and giving more weight to the mode values through an improvement of Kasatkina's theoretical approach (2009). We then apply the two models to data processed with a high-density filter algorithm and a multi-frequency single target detection algorithm, described below. Moreover, we provide estimations of sprat biomass and uncertainty using the b_{20} value currently in use for the Adriatic Sea and those computed in this work. Finally, we stress how post-processing techniques could affect *in situ* TS measurement results which in turns increase the biomass estimation uncertainty.

Materials and methods

The acoustic measurements were performed onboard the R/V *G. Dallaporta* equipped with SIMRAD EK60 in 2014 and SIMRAD EK80 in 2020 split-beam scientific echosounders at operating frequencies of 38, 120, and 200 kHz and 38, 120, and 70 kHz, respectively. The sound speed parameter was set by measuring water temperature, density, and salinity using a CTD probe (SEABIRD 911 PLUS) before each experiment. Routine calibrations (Demer et al., 2015) were performed before each surveys close to the location of the experiment using a tungsten carbide calibration sphere with 6% cobalt binder measuring 38.1 mm in diameter. The transmitted pulse length was set to 1.024 ms for all frequencies in 2020, while in 2014 the 38 kHz's pulse duration was set to 0.512 ms. The transmitted power was fixed to 2000 W for 38 kHz and 250 W for 120 kHz. Further details on calibration results can be found in Table S1. We excluded the 200 kHz and 70 kHz frequencies, because they were not employed in both surveys. Fishing operations were conducted using a mid-water trawl net with an 18 mm cod-end mesh size. The SIMRAD FX80 trawl sonar instruments, mounted on the trawl, allow monitoring net depth, height, and width as well as the fish biomass entering the net. In both hauls, the net was cast at a seabed depth of 30 m at a vessel speed of ~ 4 knots for 30 min at the sites shown in Figure 1. After each fishing operation, a subset of at least 100 individuals per target species was subjected to biometric measurement onboard (precision, 0.1 g for weight and 1 mm for TL) and then frozen for further analysis in the laboratory. Further procedure details are reported in the MEDIAS Handbook (2021).

Only monospecific night hauls with sprat biomass > 90% are usually considered reliable for *in-situ* measurement. Nevertheless, after the scrutinization of a total of 353 hauls carried out between 2013 and 2021 during MEDIAS survey conducted in the western Adriatic Sea and Slovenian waters, due to the limited number of sprat monospecific hauls, we tried to lower the threshold up to > 75%, without any results. Only two hauls carried out in 30 August 2014 and 4 July 2020, during MEDiterranean International Acoustic Surveys (MEDIAS) cruises in the Adriatic Sea (Leonori et al., 2021) resulted suitable for *in-situ* TS measurement. The hauls were composed by sprat for the 96.2% in biomass and 96.7% in number in 2014, 98.7% in biomass and 98.9% in number in 2020 (see Table S2 for more details on hauls composition).

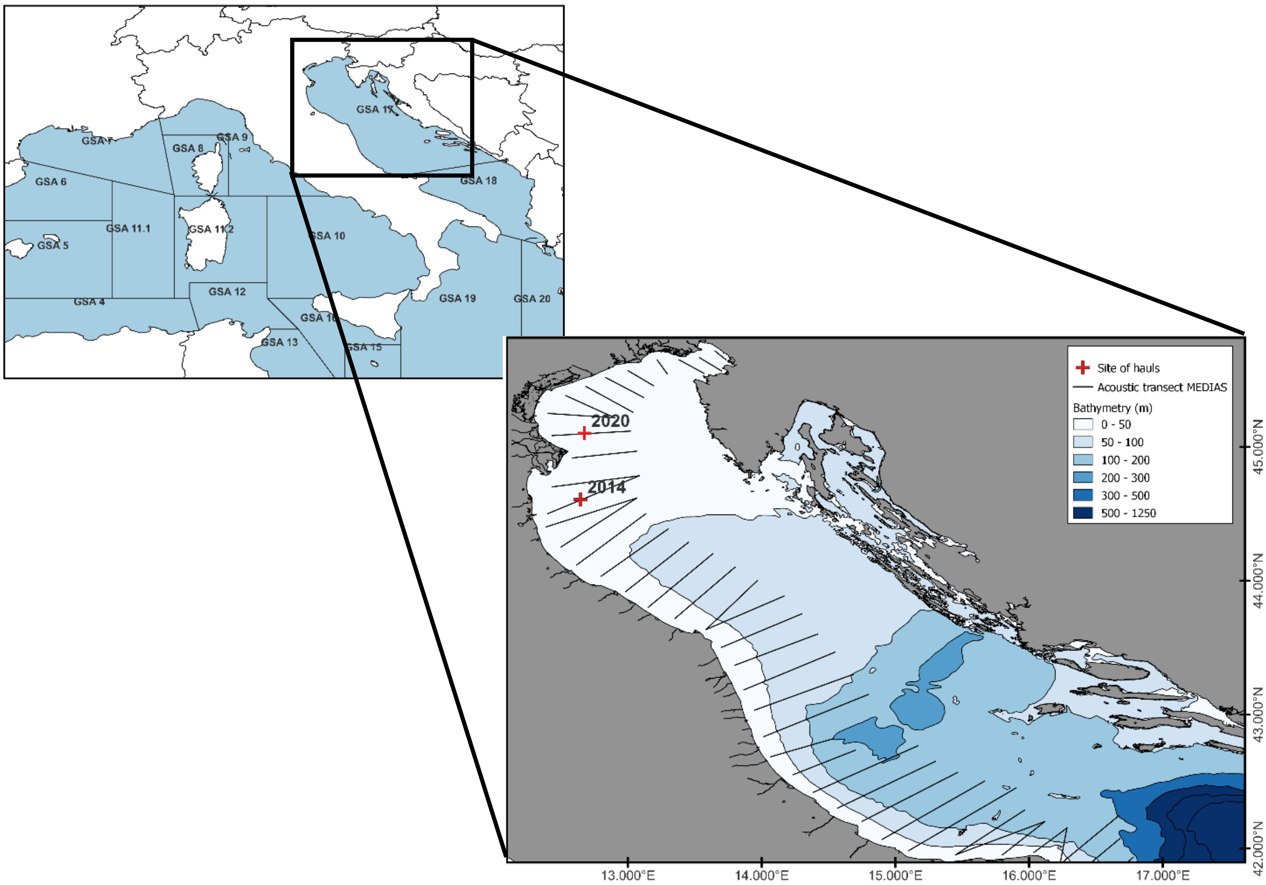

Figure 1. GFCM Geographical Sub Areas (GSAs) division of the Western Mediterranean Sea on the top. Below the map of the GSA 17 with the acoustic transects annually carry out in the Western Adriatic Sea and the locations of the two sprat monospecific hauls performed in the Northern Adriatic Sea.

Single target detection algorithms

The acoustic data were scrutinized using Echoview Software (v.10). Only the layer defined by the headrope and footrope was considered for further analysis, by applying a mask over the two lines as shown in Figure 3. The background noise and impulse noise removal functions were applied to the raw data according to the Echoview post-processing instructions (De Robertis and Higginbottom, 2007). Subsequently, a multi-frequency method based on the difference in mean volume backscattering strength (S_v dB re 1 m^2/m^3) between 38 and 120 kHz, was applied to separate fish with a swimbladder from other organisms such as macro-zooplankton following the workflow detailed in MEDIAS report (2018). The latter was settled employing some results on multi-frequency backscatter reported in SIMFAMI Project (2005, chapter 3) for the species of the area of study (Fernandes et al., 2015; MEDIAS, 2018). For more details on the workflow described above see Figure S1. TS values were extracted from the selected echoes through the single target detection split-beam Method 2, whereby application of an algorithm based on the standard phase deviation set on the split-beam angle data returns compensated TS values (Soule et al., 1997). This process was implemented on both hauls before further analysis. The single target detection parameters are listed in Table 1.

Table 1. List of the parameters used for the single target detection split-beam Method 2 of Echoview software.

TS threshold	**-62 dB**
Pulse length determination level	6 dB
Minimum normalised pulse length	0.7
Maximum normalised pulse length	1.5
Two-way maximum beam compensation	4 dB
Maximum standard deviation minor-axis angle	0.6°
Maximum standard deviation major-axis angle	0.6°

In this work, we applied a high-density filter algorithm (Ona, 1999; Sawada et al., 1993), where the probability of the detection of multiple target echoes is determined from the estimated density of fish. After gridding the echograms of each haul in cells with a horizontal size of 5 pings and a vertical size of 1 m, we applied the high-density filter (Ona, 1999). We set the threshold of the total number of fish in the reverberation volume Nv at 0.04. To account for the probability of detecting multiple targets, we set a threshold of 0.7 on the ratio for multiple echoes M. We extracted the single target TS values obtained by this procedure to compute also a multi-frequency algorithm on spatial matching criteria following the principle published by Demer et al. (1999) without taking into account the angular position of the target. Combining the synchronized signals of two adjacent split-beam transducers, the algorithm drops the chance to detect unresolvable and constructively interfering target. We applied the match ping time tool to all single targets, to synchronize them before applying the method to the 38 and 120 kHz frequencies. Then, based on the target range detected at the two frequencies, we applied the single target intersection operator algorithm in Echoview, which returns only single targets from one frequency (38 kHz) within a specified range of any single target detected at the other frequency (120 kHz). The tool computes the range in real world coordinates giving transducer location (x, y, z) and orientation (angle offset). For more details on the second part of the workflow see Figure S2. The results were visually inspected to check process outcome.

Models and analysis of data uncertainty

After computing the single target detection, considering the Rayleigh-like distributed echo amplitude and the exponential PDF of the acoustic cross-section discussed above, we applied the model proposed by MacLennan and Menz (1996). Through this, we compared the set of TS values to the LFD of the specimens caught by the trawl to estimate the constant b_{20} in equation (2). TS at step intervals of 0.5 dB and fish length in 0.5 cm class were set in the equations. A number of values suitable to encompass the intervals close to the modes were included in the analysis, to account for the stochastic variation of TS. Then, an initial value for b (b_0) was guessed and the theoretical TS distribution computed. Subsequently, the theoretical distribution was compared to the observed distribution. The sum of squares (S), using normalized residuals in each TS interval, was applied to select the best b that minimized S following an iteration of b values in that direction. Then, a normal distribution was fit around the modal values of LFD and plotted against the TS mode of observed TS dataset under the theoretical PDF (from -42 dB re 1 m^2 to – 49 dB re 1 m^2) to obtain the mean and standard deviation of the best b_{20} fitting the TS distribution assuming a $20\log_{10}$ TL.

At the same time, the method proposed by Kasatkina (2009) was employed to provide a set of TS-TL values for each TL class and TS distribution, with a bin range of 0.1 dB as suggested in AcousMed project (2012). The first point of the TL distribution was mapped at the first point of the TS distribution. Any other point of the TL distribution (with an ordinal number NL_i and length L_i) was mapped at point NTS_i of the TS distribution according to the following rule:

$$NTS_i = NTS_1 + r * (NTS_i - NTS_1) \qquad (3)$$

$$r = \frac{NL_i - NL_1}{NL_2 - NL_1} \qquad (4)$$

where NL_2 and NL_1 are the ordinal numbers of fish in the sample distribution (i.e. the last and the first), NTS_2 and NTS_1 are the ordinal numbers of TS values in the sample distribution (the last and the first), and TS_i (with the ordinal number NTS_i in the TS sample) is the TS value corresponding to the NL_i^{th} fish with length L_i. The index *i* is used to show that there can be several fish with the same length in the sample. As suggested by Kasatkina, the result is suitable for estimating b_{20} with the bootstrap technique. Therefore, following the theoretical principle of an exponential PDF, we applied a five hundred weighted bootstrap regression model in the R package “Boot” on TS-TL dataset, to give more weight to the mode values. Each TS-TL was scaled by the frequency of TL values in a simple way:

$$Weight = TL_f / TL_t \quad (5)$$

where TL_f is the repeated times of that TL value (0.5 cm classes) and TL_t is the amount of TL samples. The weighted bootstrap method gives high odds that can be randomly extracted for values with a higher weight (Efron and Tibshirani, 1993).

Both methods were developed separately by application of single targets extracted with the high-density filter algorithm and the multi-frequency algorithm. For the subsequent discussion we will use the following terms to indicate the different methods employed: modified Kasatkina high-density filter (KH); modified Kasatkina multi-frequency filter (KM); MacLennan and Menz high-density filter (MMH); MacLennan and Menz multi-frequency filter (MMM). Finally, we tested how the choice of the *in situ* post-processing model could affect species abundance estimates. The indices were computed using 2014 and 2020 data for the northern Adriatic Sea area, including Slovenian waters (Figure 1), where sprat is more abundant (Leonori et al., 2012, 2017, 2021). The mean nautical area scattering coefficient (NASC) allocated by haul composition through the mixed-species echo integrator conversion factor was converted to abundance in number according to the mixed-species formula (Nakken and Dommasnes, 1975), which in turn was converted to biomass in weight (kg) using the length-weight relationship parameters of each year reported in Table 2. Next, 10,000 Monte Carlo simulations were performed to estimate the uncertainty due to TS values. A normal distribution was assumed; the input variables were the TS functions with estimates of mean b_{20} and standard deviations. The b_{20} value in the formula was repeatedly changed, starting with the original -71.7 dB and then the values obtained with the two methods described above. The confidence limits of the original b_{20} value were computed from the raw data, resulting in a standard deviation of 0.1 dB. The biomass variation is the ratio between -71.7 dB re 1 m^2 and the values obtained with the new b_{20}. Afterwards, we extended the analysis described above to the time series from 2014 to 2020. Sprat abundance obtained throughout this process was converted into biomass in weight using the length-weight relationship presented in Table 2. Successively, the mean biomass and the overall uncertainty was estimated using the upper and lower limit of b_{20} values (-65.5 to -71.7 dB re 1 m^2) as input variables for each year. The steps of the methods applied in this paper are summarized in Figure 2.

Table 2. Total length-weight parameters for the sprat biomass equation. L is mean sprat length in the study area, a is the intercept, b is the slope of the relationship, and R^2 is the coefficient of determination.

Year	TL (cm)	a	b	R^2
2014	11.3	0.0192	2.5459	0.914
2015	9.3	0.0058	3.0476	
2016	8.8	0.0057	3.0578	
2017	9.8	0.0044	3.1653	
2018	8.6	0.0031	3.3515	
2019	9.1	0.0034	3.3077	
2020	9.5	0.0031	3.3018	0.965
2021	10.1	0.0067	3.0348	

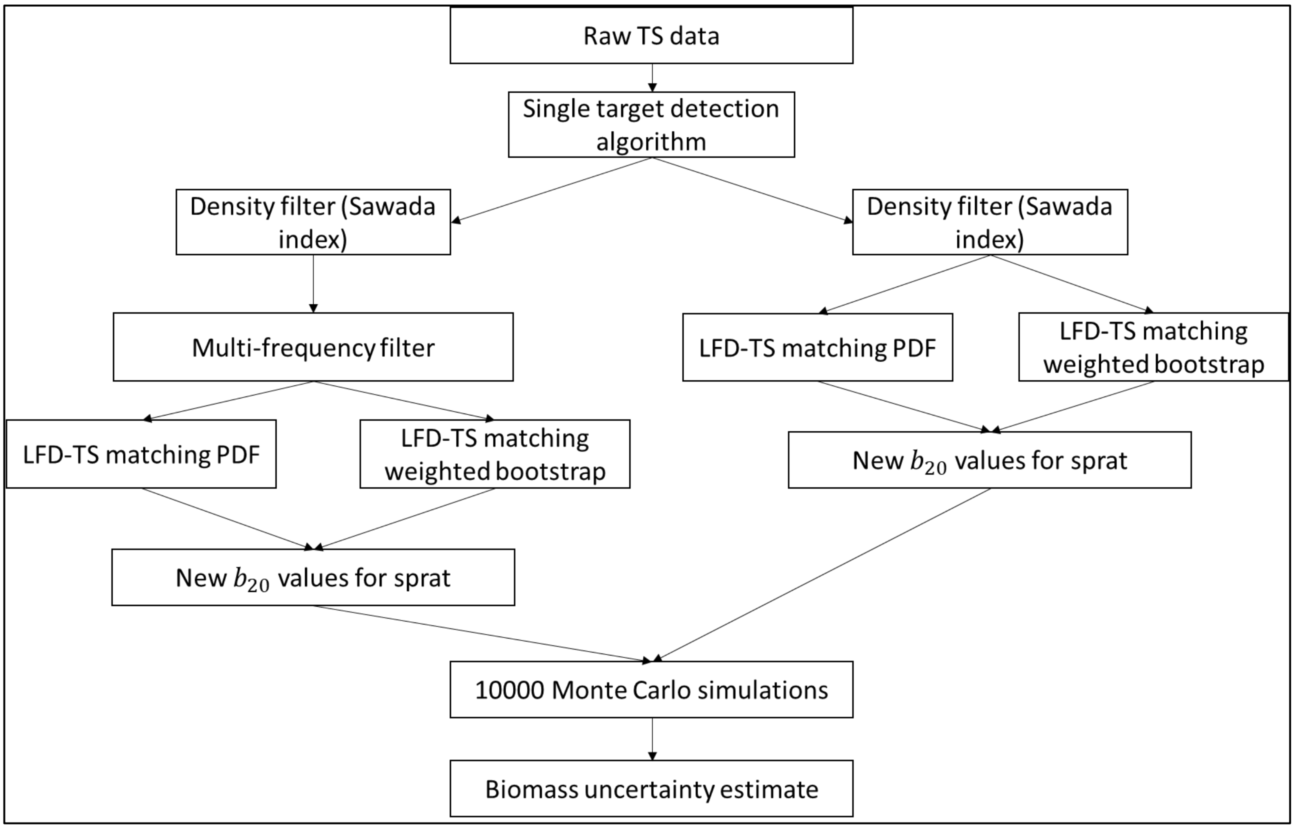

Figure 2. Workflow that describe the series of step applied for the computation of the b_{20} values of sprat and biomass uncertainty estimates. The "LFD-TS matching PDF" represents the application of the method proposed by MacLennan and Menz (1996), while the "LFD-TS matching weighted bootstrap" point out the new method propose herein.

Results

The raw TS data collected during the two hauls, shown in Figure 3, clearly depict a layer close to the bottom where sprat is found at low density and specimens swim at a distance from one another. The single target detection algorithm yielded 1606 single targets in 2014 and 915 in 2020 at 38 kHz; 833 and 1043 single targets at 120 kHz respectively in 2014 and 2020. Application of the high-density filter rejected approximately 10% and 13% of single targets, leaving 1394 and 823 single targets at 38 kHz, and 1% and 8% of single targets at 120 kHz, leaving 820 and 960 single targets in 2014 and 2020 respectively. Considering a pulse length of 1.024 ms in 2020, we applied a range of acceptance of 0.7 m for both frequencies obtaining an exclusion of 67% of targets in 2020. The same threshold, applied for 2014, lead to a reduction of 71% single target retained, being consistent with the results gained for the other year although the different pulse length (0.512 ms) as shown in Table 3. The TS frequency distributions clearly show two modes, one at -59 dB re 1 m^2 and the other at -44 dB re 1 in 2014 and -46 dB re 1 m^2 in 2020. In both years, they matched the LFD of the specimens collected with the pelagic trawl, which in 2014 consisted predominantly of sprat measuring 11.5 cm, with a small portion of specimens measuring 9.5 cm, and in 2020 consisted of specimens measuring 11 cm and 8.5 cm (Figure 4). The TS and TL statistics of the two experiments are reported in Table 3. The TS statistics were computed in the linear domain. The theoretical TS distribution was obtained by superimposing a Rayleigh distribution on the sprat mode (10.5-12 cm) and adjusting b to give the best fit over the TS mode -49 to -42 dB re 1 m^2 (Figure 5). The theoretical and the observed TS distributions on the best mode are comparable at the minimum sum of square. The MMH yielded a b_{20} of -65.6 ± 0.57 dB re 1 m^2 in 2014 and of -66.9 ± 1.17 dB re 1 m^2 in 2020, while the improved multi-frequency detection MMM resulted in a lower mean backscattering cross-section of f -66.2 dB re 1 m^2 in 2014 and higher in 2020 (65.6 ± 0.5 dB re 1 m^2). Comparison of the LFD and the TS distributions according to KH, by adjusting the weight of mode values, yielded the bootstrap distributions shown in Figure 6, where b_{20} in 2014 was -67.5 ± 0.2 dB re 1 m^2, with a difference of 1 dB re 1 m^2 compared to the non-weighted bootstrap, and -68.8 ± 0.25 dB re 1 m^2 with a difference of 0.5 dB re 1 m^2, in 2020.

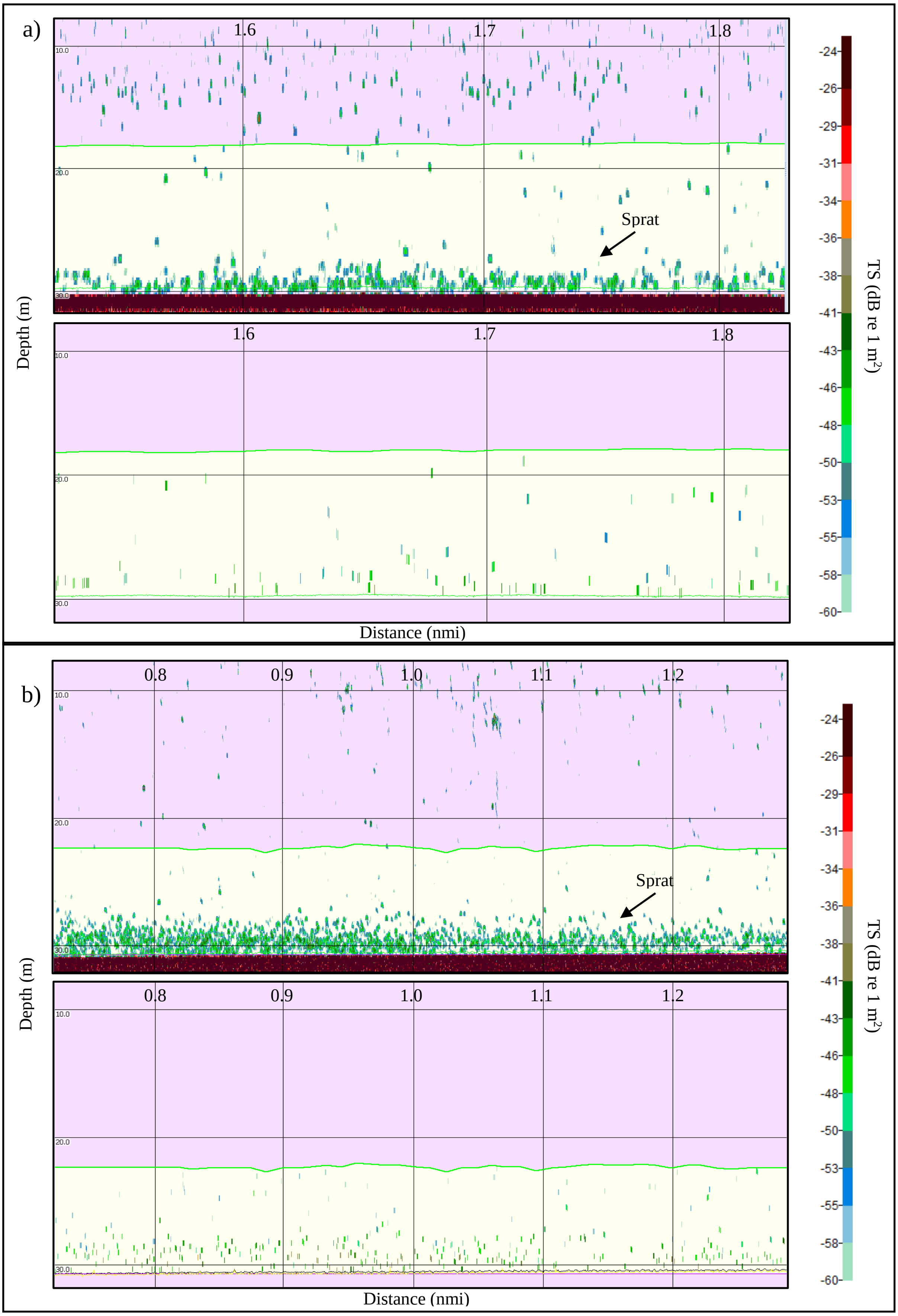

Figure 3. Snapshots of raw TS echograms on the top and point scattering 40 log R TVG detected after acoustic data processing from 2014 (a) and 2020 (b). Green line: depth of the headrope detected by the trawl sonar. The purple regions are the exclusion region cut off for the TS single target detection analysis. The Kaijo colour scale on the right shows the TS values. The differences between the two echograms are due to different pulse durations.

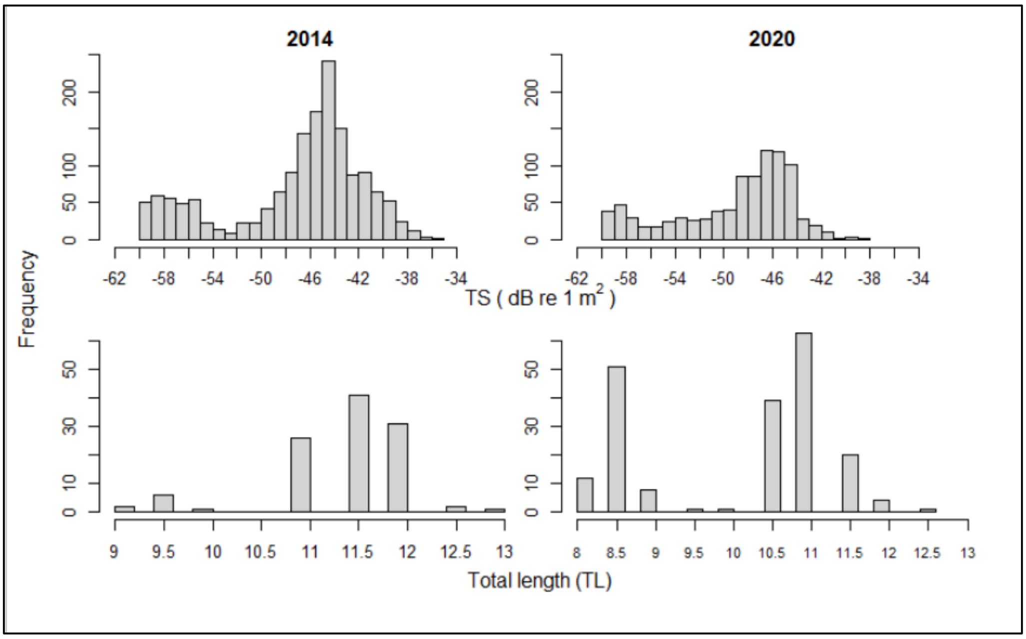

Figure 4. Upper panel: TS distributions of the single targets detected in *in situ* measurements. Lower panel: length-frequency distribution of sprat caught after the TS measurements.

The analysis of single target data, extracted after application of the multi-frequency algorithm (KM), yielded higher b_{20} of -65.5 ± 0.09 dB re 1 m^2 in 2014 and -66.1 ± 0.1 dB re 1 m^2 in 2020. The wide b_{20} differences between MMH and MMM yielded a biomass variation of 9% in 2014 and 16% in 2020 while the change from KH to KM resulted in a biomass decrease of 22% in 2014 and 27% in 2020. In contrast, compared to KH, the application of MMH involved a mean biomass reduction of 22% in 2014 and of 19% in 2020 (Table 4).

Table 3. Fish total length and TS statistics after the single target detection algorithms on raw TS data collected at 38 kHz.

Year	**TL mean (cm)**	**TL mode (cm)**	**TL range (cm)**	**TS mean (dB re 1 m^2)**	**TS mode (dB re 1 m^2)**	**TS range (dB re 1 m^2)**	**TS SD**	**No. of targets**
				High-density filter				
2014	11.5	11.5	9-13	-44.5	-44.7	-60/-35.4	5.77	1394
2020	10	11	8-12.5	-47	-46.6	-60/-38.6	4.81	823
				Multi-frequency filter				
2014				-44.9	-45.2	-59.6/-35.5	3.77	397
2020				-46	-45	-58.8/-38.8	2.26	272

The mean biomass reduction is considerably lower after application of the multi-frequency filter algorithm with the exception of MMM in 2014. Moreover, we detected divergent results characterized by an increase of 9% of biomass in 2014 and a decrease of 7% in 2020 with the application of MMM rather than KM. Comparison of 2020 and 2014 data highlighted a general biomass reduction in the study area with the new b_{20} values. Application of the new conversion factors yielded estimates almost two times lower than the current ones. The uncertainty in TS varies considerably between methods, as shown in Table 4. However, the overall uncertainty depicted in Figure 7, stresses the large influence of variable TS values on biomass estimates. An important divergence was detected at high sprat biomass level in 2014 and 2019, when the reduction of biomass estimated by the use of the new b_{20} value (-68.2 dB re 1 m^2), reached the 25%. Conversely, at low sprat biomass levels we detected negligible differences such as in 2016 (Figure 7).

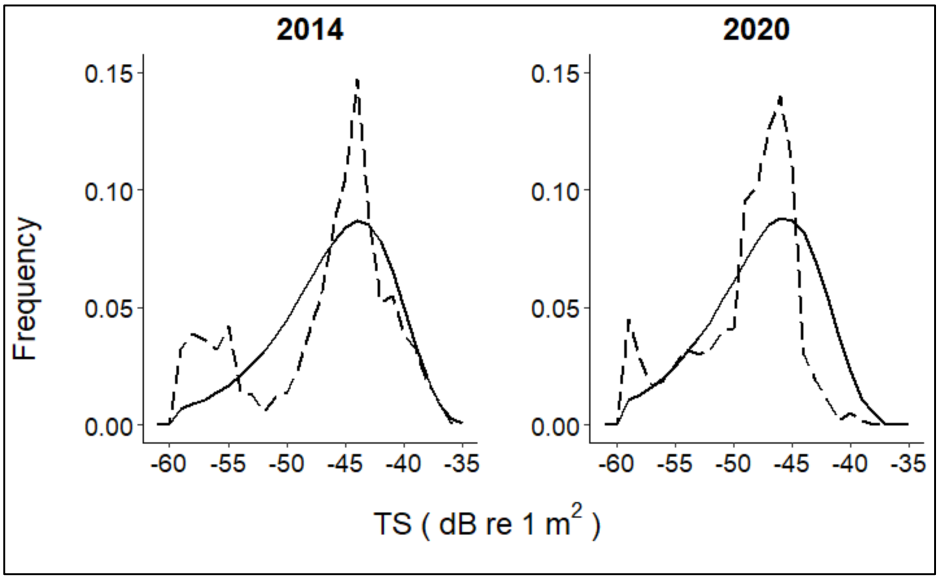

Figure 5. Observed TS relative frequency (dashed lines) and best fit theoretical relative frequency (continuous lines) at 1 dB intervals.

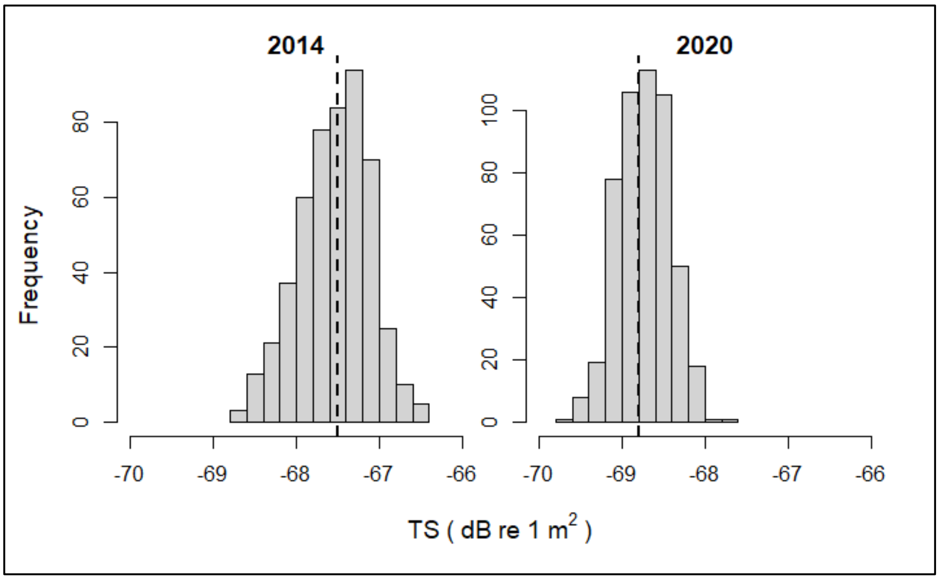

Figure 7. Bootstrap distribution derived from the weighted bootstrap results (TS data after application of the high-density filter algorithm). Dashed lines: bootstrap statistical mean (t) values.

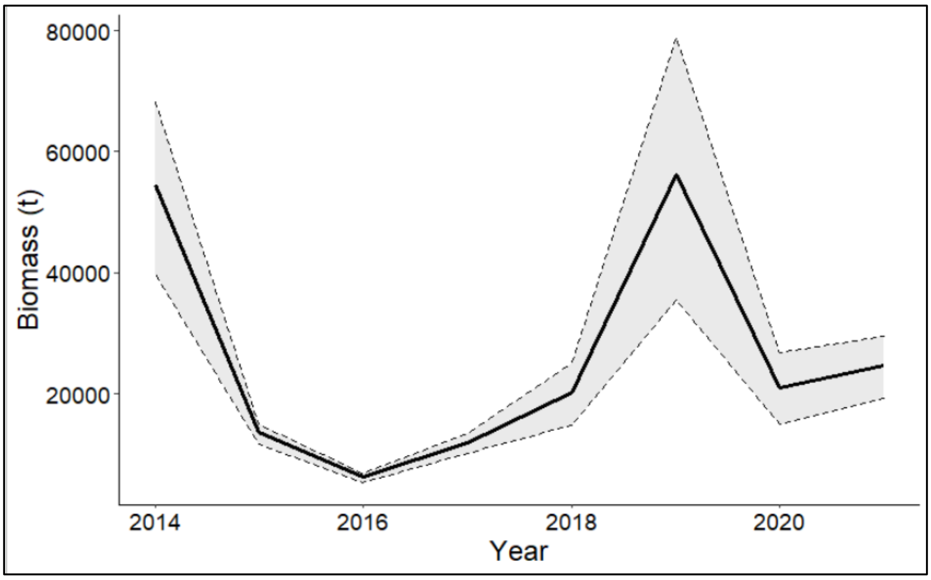

Figure 6. Mean biomass trend of Sprat in the North-Adriatic Sea (solid line) and 95% confidence interval (shadow area) which express variability due to the b_{20} uncertainty. The upper and lower dashed line depicted the biomass estimated using the current b_{20} (-71.7 dB re 1m^2) and b_{20} = - 65.5 dB re 1m^2 respectively.

Table 4. Fish biomass estimates statistics obtained with different b_{20} values: modified Kasatkina high-density filter (KH); modified Kasatkina multi-frequency filter (KM); MacLennan and Menz high-density filter (MMH); MacLennan and Menz multi-frequency filter (MMM). s.d., standard deviation; CV, coefficient of variation: 95% CI, 95% confidence interval.

	2014					**2020**				
Biomass statistics	**Current -71.7 dB**	**KH -67.5 dB**	**KM -65.5 dB**	**MMH -65.6 dB**	**MMM -66.2 dB**	**Current -71.7 dB**	**KH -68.8 dB**	**KM -66.1 dB**	**MMH -66.9 dB**	**MMM -65.6 dB**
Mean (t)	69,245.3	48,813.8	38,175.2	38,710.2	41,856.9	26,758.6	21,109.4	15,348.7	17,062.2	14,332
s.d. (t)	407.8	1,077.4	466.7	2,930.4	427.4	872.2	530	208.4	2,453.1	1,152.8
CV	0.006	0.02	0.012	0.08	0.01	0.03	0.03	0.01	0.14	0.08
Upper limit (95% CI t)	69,923.9	50,571.8	38,175.2	43,578.6	42,557.4	28,155	21,993.1	15,694.4	21,190	16,269.8
Lower limit (95% CI t)	68,572.7	47,040.9	37395.2	33,958.6	41,157.41	25,286.3	20,240.7	15,010.2	13,101.6	12,487.5
Ratio		1.42	1.81	1.78	1.65		1.27	1.74	1.57	1.86
Overall uncertainty					CV= 0.17 s.d. (t)=9,166.1				CV= 0.18 s.d. (t)=3,682.1	

Discussion

The slight reduction (10% - 13%) in the number of single targets detected after application of the high-density filter showed that the fish were widely scattered during the night measurements of TS, a situation that made the specimens suitable for the *in situ* experiment. The echogram showed that fish were dispersed and distributed in a layer during the night hours both in 2014 and in 2020. Sprat appeared to show an opposite behaviour to anchovy and sardine, which during the night usually perform a diel vertical migration to feed on plankton near the surface (Bonanno et al., 2021). The workflow employed for the selection of single targets ensured the nearly exclusive retention of the echoes from single specimens. A suitable amount of single targets was also retained after implementation of the multi-frequency filter as shown in Table 3. This second stage of the procedure provided the largest contribution to rejection of the weaker targets which affected the lower tail of the TS distribution but did not compromise the frequency around the TS modes. We tested a method based on the tool of Echoview software that can be easily applied to every dataset in addiction or in alternative to the density filter algorithm. Nevertheless, the aforementioned function could suffer from the exclusion of the angular position and high distance threshold of the target that may result in an overestimation or underestimation of suitable single targets retained and can justify the rejection of weaker targets.

In this work we applied two methods useful to correctly match TS and LFD distribution. The method proposed by MacLennan and Menz (1996) is entirely based on the Rayleigh scattering theory. In some circumstances, the echo amplitude of fish does not follow the theory (Stanton et al., 2004), and turns into a geometric scattering or a Rayleigh scattering due to frequency, incidence angle, and target size. This is attributable mainly to target orientation, which can vary significantly between species and within the same species (Korneliussen et al., 2018; Palermino et al., 2021). Conversely, in swimming fish the echo amplitude is mostly incoherent and the PDF conforms to the Rayleigh PDF (Korneliussen et al., 2018). Sprat has a small and laterally compressed swimbladder (Palermino et al., unpublished data) which generally, at 38 kHz, does not show backscatter directivity characterized by geometric scatter. Therefore, in our case the Rayleigh PDF may be considered as a good theoretical approximation to the actual trend of TS distribution, although some limitations persist (Demer et al., 1999). The second method proposed herein is a mathematical improvement of the formula developed by Kasatkina (2009) according to the principle of exponential distribution of backscattering TS (Simmonds and MacLennan, 2005). The matching criteria proposed by Kasatkina are useful to link a single TS to the corresponding TL from the whole dataset. Nevertheless, the results give the same importance to all TS-TL combinations. Conversely, Figure 4 clearly demonstrated that not all values cover the same influence on the trend of single target backscattering strength and the LFD. However, the implementation of a weighted bootstrap showed that the variability of the magnitude could be taken into account, resulting in different b_{20} and TS values useful for subsequent analysis.

The two methods KH and MMH yielded a difference of 1.3 dB re 1 m^2 between 2014 and 2020 and a constant 1.9 dB re 1 m^2 difference between them in each year. Conversely, the gap was narrower after the application of the multi-frequency algorithm between KM and MMM and years. We observed an opposite trend characterized by a maximum difference of 0.7 dB re 1 m^2. Therefore, the variability of b_{20} between 2014 and 2020 could be mainly attributed to the choice of the model. However, other factors may influence TS changes. The different pulse lengths used in 2014 and 2020 during TS measurements can affect the results (0.512 ms in 2014 and 1 ms in 2020). A shorter pulse length involves a greater bandwidth (Khodabandeloo et al., 2021), but no significant differences have been found among the mean TS values measured at different pulse durations in other studies (Sobradillo et al., 2021). Moreover, we got a similar rejection percentage of single targets after the application of the multi-frequency algorithm using the same distance threshold (0.7m), which indicate a negligible difference due to the pulse length in our measurements. The differences in swimbladder fullness and orientation can also influence TS, limiting the use of the new values during daytime acoustic surveys as demonstrated for other species (Bonanno et al., 2021). Moreover, an important element that increases uncertainty is the possible avoidance behaviour of clupeids, when the vessel approaches the schools in daytime (Hjellvik et al., 2008). Nevertheless, the spreading near the bottom which characterized the sprat behavior during nighttime, showed in Figure 3, suggests that sprat might not be affected by the vessel. When avoidance to the vessel is detected, it is expected that fish swim towards the bottom (Barange and Hampton, 1994; Hjellvik et al., 2008), but in our case the sprat is mainly found close to the bottom, therefore no evidences of escape behavior were detected for the species either during day and night.

The TS fluctuations was included and modelled in the biomass estimates using Monte Carlo simulation, a common, strong approach to calculate bias and ensure precision of individual and multiple sources of uncertainty in acoustic surveys (O'Driscoll, 2004). This technique allowed highlighting the important role of post processing techniques on TS uncertainty, since the inadequate knowledge of sprat acoustic reflectivity alone could result in a coefficient of variation (CV) of sprat biomass estimates between 17% and 18%. Figure 7 clearly displays an overestimation of sprat biomass during the past years using the current conversion parameter instead of the b_{20} values obtained here. If our TS measurement are correct, then the sprat biomass in the Adriatic Sea would be about one and a half times lower than previously estimated. It is therefore important to gain deeper insight into TS variability, which is one of the most important sources of bias in acoustic surveys

(Rose et al., 2000; Scoulding et al., 2016). A greater knowledge of overall uncertainty would help improve survey design and the choice of post processing data analysis, particularly due to the multi-species pelagic stock that characterizes the Adriatic Sea (FAO, 2020). The variability of sprat TS could also affect the biomass estimates obtained for the other two clupeid target species in the Adriatic Sea, anchovy and sardine, through application of the mixed-species formula (Nakken and Dommansens, 1975). However, it does not necessary affect the stock assessment of these species because the acoustic surveys are generally used as a relative time series index in the stock assessment process (Carpi et al., 2015).

TS is expected to increase with increasing TL following the assumption stated by Foote (1987) on the proportional growing of swimbladder and the square of fish length, nevertheless for some species this has been questioned (McClatchie at al., 2003). In our case, the mean TS of a sprat individual measuring 11.5 cm was higher than the one of an individual measuring 10.5 cm (see Table 3). Nevertheless, the few data and size range available do not justify the use of b_{20} in equation 2 rather than b in equation 1. The b_{20} conversion factor was employed in this study mainly based on literature and for comparison purposes with values reported by other authors. Although our work is limited to two samples and a narrow sprat size range, to the best of our knowledge this is the first *in situ* experiments carried out on *S. sprattus* in the Mediterranean Sea with a split-beam transducer (Azzali et al., 1997). The b_{20} values presented herein are significantly higher than the -71.05 dB re 1 m^2 obtained in the neighboring Black Sea (Marinova and Panayotova, 2015; Panayotova et al., 2014) and higher than those currently in use in the Mediterranean Sea (De Felice et al., 2021), whereas they are similar to those found in the Baltic Sea (Didrikas and Hansson, 2004; Fässler and Gorska, 2009). The difference from the values of -71.7 dB re 1 m^2 currently in use in the Adriatic Sea (Azzali et al., 1997; De Felice at al., 2021) may be due to several reasons. This value was measured in May 1994 in the mid-Adriatic Sea near Ancona, using a single-beam echosounder on spread fish with a mean TL of 13.2 cm. A single beam transducer provides less accurate detection of single targets, due to the difficulty of resolving target position inside the beam (Simmonds and MacLennan, 2005). Our values are also higher than the historical ones for physostomous clupeids given by Foote in 1987 and by ICES in 1983. Those experiments could now be re-evaluated in the light of the recent findings, which benefit from technological advances and algorithms development (Adrian Madirolas et al., 2017; Ok and Gücü, 2019; Sobradillo et al., 2021). In fact, the current use of split-beam transducer enhances the detection of the precise position of fish in the 3D space being particularly suitable for TS measurements (Simmonds and MacLennan, 2005). Therefore, a difference between 2.4 and 5.6 dB, found in this work, might be correct. It is difficult to make assumptions about the best model and filter algorithm based on the analysis of a small dataset such as ours. Some authors consider the multi-frequency algorithm the best approach to avoid target overlap (Scoulding et al., 2016); however, we found an unexpected rejection of weaker targets within the multi-frequency filter method which could affect the results. Moreover, both hauls were conducted at night, when fish are scattered and found at low density. For these reasons, we consider the density thresholds more reliable. We demonstrated that different post-processing steps and models can yield a difference exceeding 2 dB. However, the KH method for the TS-LFD relationship proposed herein yields more conservative values that are closer to the current one and to results reported by other authors compared to MMH which is based on MacLennan and Menz (1996) method. We thus suggest for sprat in the Mediterranean Sea a b_{20} value between -67.5 and -68.8 dB re 1 m^2, even if these results could be hopefully updated for sprat in case of future availability of more monospecific hauls and wider length frequency distribution in the sample. Despite the poor dataset and LFD available do not allow to use these as general values for the species in the Mediterranean Sea at the moment, the b_{20} value of -68.2 proposed in Figure 7 as the new conversion factor for sprat may be considered as a starting point to reevaluate the sprat-specific TSs now in use in the Mediterranean Sea.

CREdiT authorship contribution statement

Antonio Palermino: Conceptualization, Methodology, Formal analysis, Data curation, Writing- Original Draft, Visualization. **Andrea De Felice:** Validation, Writing- Review & Editing, Supervision. **Giovanni Canduci:** Investigation, Writing- Review & Editing. **Ilaria Biagiotti:** Investigation, Writing- Review & Editing. **Ilaria Costantini:** Writing- Review & Editing. **Michele Centurelli:** Writing- Review & Editing. **Iole Leonori:** Supervision, Validation, Project administration, Writing- Review & Editing, Resources.

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Supplementary materials

Table S2. Technical specifications and calibration parameters of the EK80 echosounder system used for the target strength measurements.

Specification	2014		2020	
Frequency (kHz)	38	120	38	120
Absorption Coefficient (dB m-1)	0.00784	0.0498	0.0099	0.0498
Sa Correction (dB re 1 m2)	-0.67	-0.34	-0.57	-0.76
Transducer Gain (dB re 1 m2)	25.22	25.32	25.27	26.87
Major Axis 3 dB Beam Angle (°)	7.05	6.35	6.98	6.61
Major Axis Angle Offset (°)	0.05	-0.11	0	0.09
Major Axis Angle Sensitivity	21.9	23	21.9	23
Minor Axis3dB Beam Angle (°)	7	6.54	6.94	6.52
Minor Axis Angle Offset (°)	0.07	-0.04	-0.02	-0.014
Minor Axis Angle Sensitivity	21.9	23	21.9	23
Sound Speed (ms)	1513.9	1513.9	1509.4	1509.4
Transmitted Power (W)	2000	250	2000	250
Transmitted Pulse Length (ms)	0.512	1.024	1.024	1.024
Two Way Beam Angle (dB re 1 Steradian)	-21	-20.4	-20.7	-20.7

Table S2. Sprat monospecific haul species composition

2014					2020				
Species	**Weight (g)**	**%**	**number**	**%**	**Species**	**Weight (g)**	**%**	**number**	**%**
Engraulis encrasicolus	672	3.0	75	3.2	*Sardina pilchardus*	31.5	0.2	2	0.1
Sprattus sprattus	21200	96.2	2292	96.7	*Engraulis encrasicolus*	28.7	0.1	9	0.5
Scomber scombrus	168	0.8	3	0.1	*Sprattus sprattus*	20200	98.7	1975	98.9
					Merlangius merlangus	198.4	1.0	10	0.5
Total weigth	22040		2370		Total	20458.6		1996	

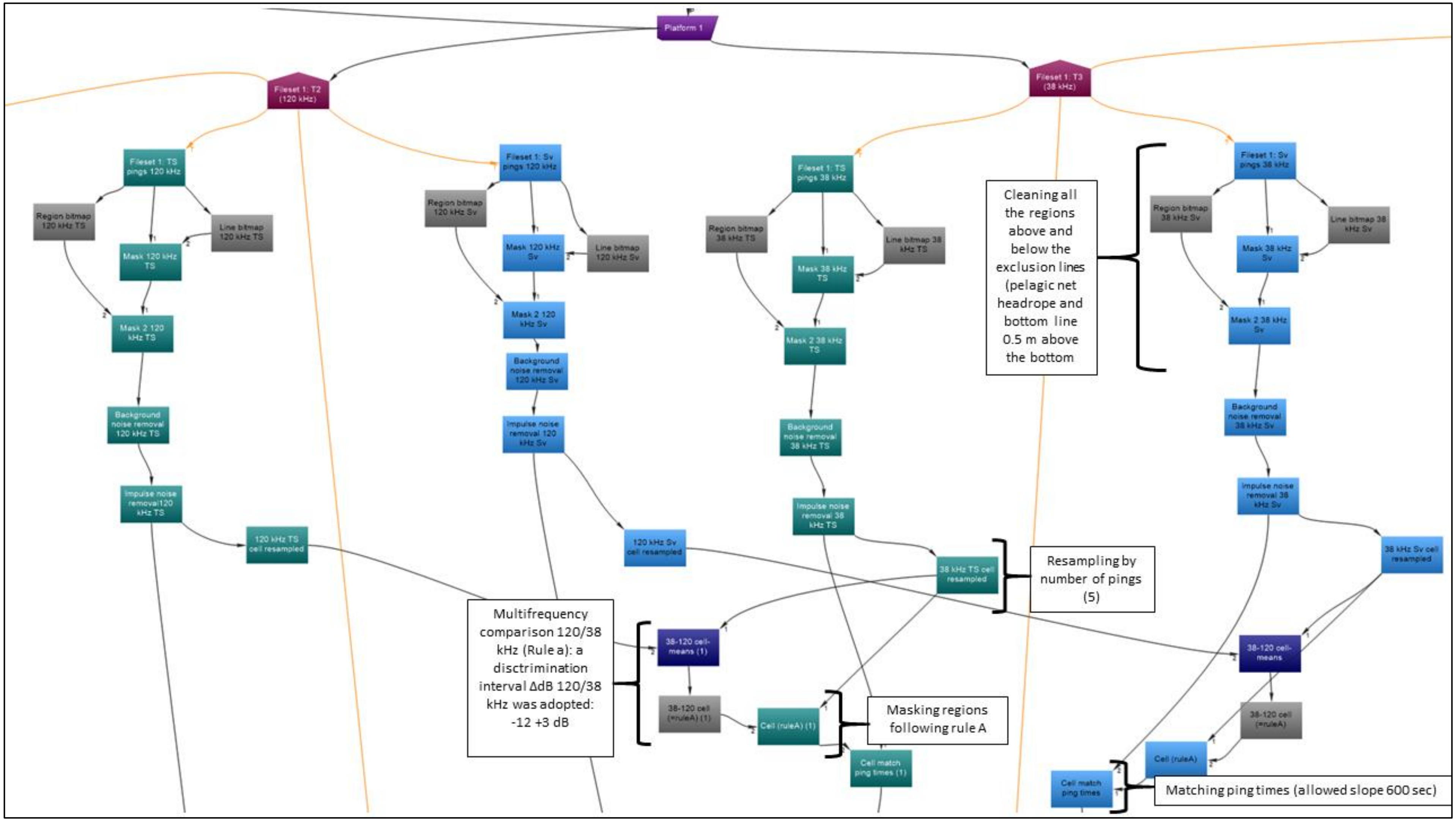

Figure S1. Workflow employed in Echoview software v 10.0 for the scrutinization of echogram

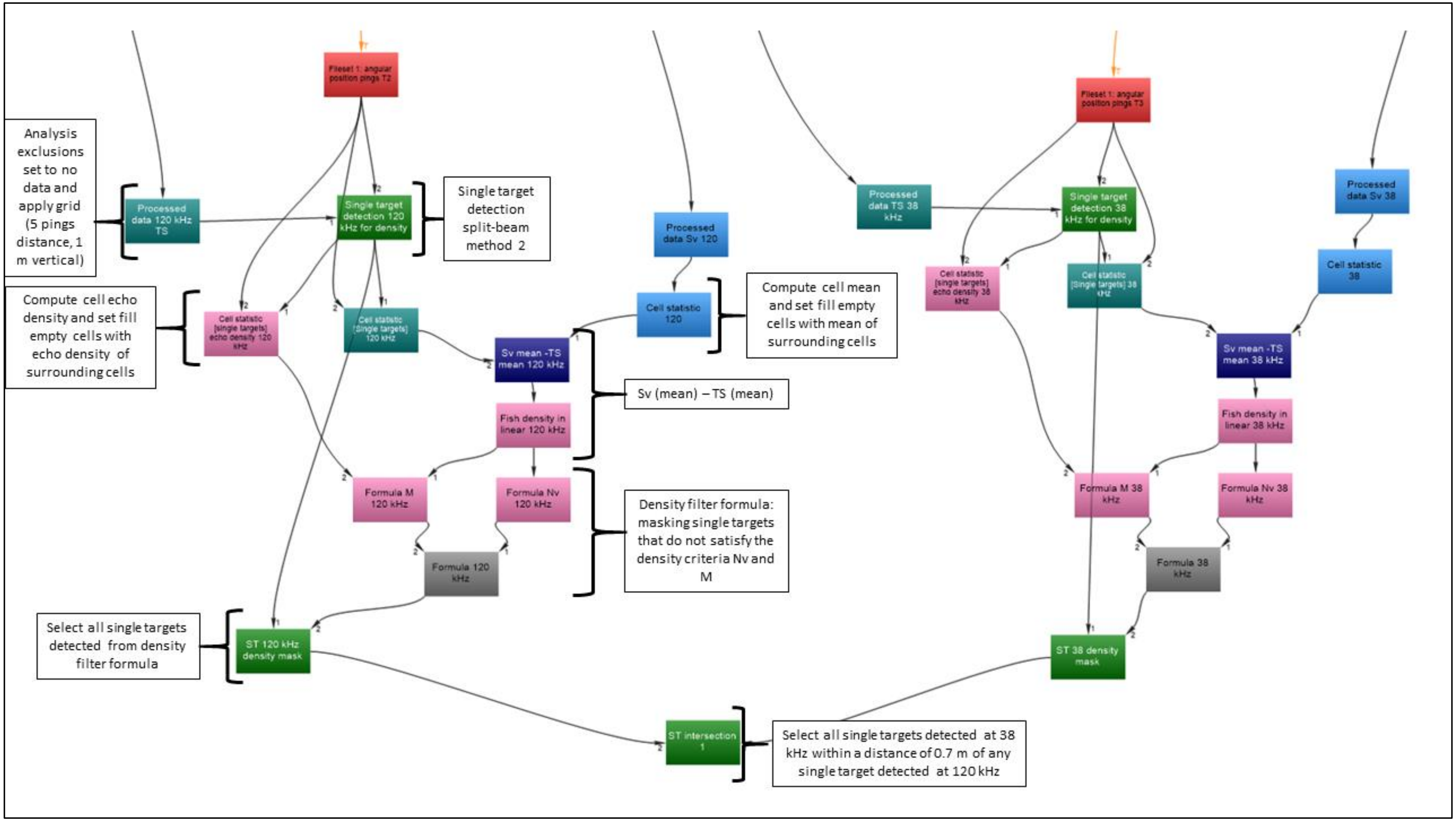

Figure S2. Workflow employed in Echoview software v 10.0 for the TS analysis

4. Backscattering models

Application of backscattering models for target strength measurements of *Trachurus mediterraneus* and *Scomber colias* in the Mediterranean Sea

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Application of backscattering models for target strength measurements of Trachurus mediterraneus and Scomber colias in the Mediterranean Sea

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Extended abstract

Acoustic surveys are highly effective to assess the distribution and abundance of pelagic and mesopelagic species [1]. However, the conversion of volume backscatter to biomass estimate requires knowing the target strength (TS) relation for each species. The TS relation depends on the acoustic frequencies, fish body length, orientation (tilt angle), and swim-bladder features [2], [3]. TS measurements can be grouped into *ex situ*, where the fish are measured in controlled experiments [4], [5], backscatter models, in which algorithms are implemented to predict acoustic backscatter [6], and *in situ*, where the fish are measured in their natural environment [7]. The latter is considered the best method for target strength measurements in nature, but the use of backscattering models allows to properly predict theoretical backscatter from accurate measurement and setting of organism anatomy, material proprieties, swimbladder morphology, tilt angles, and frequencies [6]. With a continuous increase in interest in the use of broadband in acoustic surveys, backscattering models give insight into the feasibility of implementing broadband techniques for species identification purposes. Moreover, these are useful to corroborate empirical measurements and to fill the gap in the knowledge

of species that hardly fulfil the requirements of monospecific shoals of spread fish for the application of the *in situ* method. This is the case of *Trachurus mediterraneus* and *Scomber colias* in the Mediterranean Sea.

In this study, X-Ray and Computer Tomography scans were performed on 82 individual fish collected during the Mediterranean International Acoustic Survey (MEDIAS) 2020 and 2021 carried out in the Adriatic Sea (Mediterranean) [8], to generate 2D digital images and 3D swimbladder models for the measurements of precise morphometric characteristic using as input for Kirchhoff ray mode model (KRM) and Finite Element Method (FEM). The image processing was carried out on Image J, 3D slicer and 3D Doctor software. KRM model accounts for resonant and geometric backscatter using a low mode solution and Kirchhoff-Ray approximation to sum the energy of consecutive gas-filled and fluid-filled cylinders of 1 mm thick. KRM is an analytical model that approximates the shape of the body and swimbladder, hence does not require extensive computation [9]. While numerical models such as Finite Element Method (FEM) can solve the backscattering pressures of arbitrary fluid or gas-filled shapes, but it is computationally demanding [10].

We employed the KRM on *T. mediterraneus* and *S. colias* to estimate the backscatter as a function of frequency and tilt angle. The acoustics parameters settled for the model are reported in Table 1. A broadside angle of 90° was considered for broadband backscatter computations. Successively, the mean TS was computed as a function of tilt angle ranging between 65° and 115° due to the loss of accuracy of the model increasing the off-broadside angle [9]. The KRM package in R was performed for the KRM calculations, while FEM model was run in the Acoustics Module of COMSOL software. So far, the FEM model was implemented to assess the accuracy of KRM results only on theoretical prolate spheroid shapes, taking into account different sizes based on the computer tomography scan measurements.

Table 6. Acoustic parameters settled for Kirchhoff ray mode model

Parameters	**Values**
Speed of sound in water (ms^{-1})	1509
Speed of sound in fish body (ms^{-1})	1570
Speed of sound in swimbladder (ms^{-1})	345
Density of the water (kgm^{-3})	1030
Density of fish body (kgm^{-3})	1070
Density of the swimbladder (kgm^{-3})	1.24

A difference between the two species is evidently looking at the preliminary target-strength pattern within a step of 1 kHz broadband spectra in Figure 1, where the wide divergence resulted between 70 and 120 kHz. Moreover, a shift in the peak of TS between the two species based on fish tilt angle and frequencies was detected. The latter corresponds to about 2° for all frequencies except for the 38 kHz, where the chub mackerel achieves the higher TS value close to 90° of fish tilt angle as shown in Figure 1. Generally, the peak TS values coincide with 80° of fish tilt angle which, based on the swimbladder angle relative to the fish axis measurements (*S. colias* meanΘ = 11.41; SD = 1.8; *T. mediterraneus* meanΘ = 10.9; SD = 2.3), reflects a swimbladder perpendicular to the acoustic pulse. At 38 kHz the small size of most *S. colias* specimens can lead to a conversion between a Geometric region to a Transition region, where the backscatter of the target is less directive.

In summary, our results give the first theoretical insight into the use of broadband backscatter in the Mediterranean Sea, demonstrating the potential of this approach in distinguishing between species as already proved by other authors in different areas [11]. Moreover, our findings on the swimbladder angle relative to the fish axis and the related backscatter are similar to the results reported in the literature for all frequencies [12], [13].

More work is needed for the application of FEM on real 3D triangular mesh shape swimbladder morphology which can be used to assess the species-specific acoustic reflectivity in a wider range of fish tilt angles.

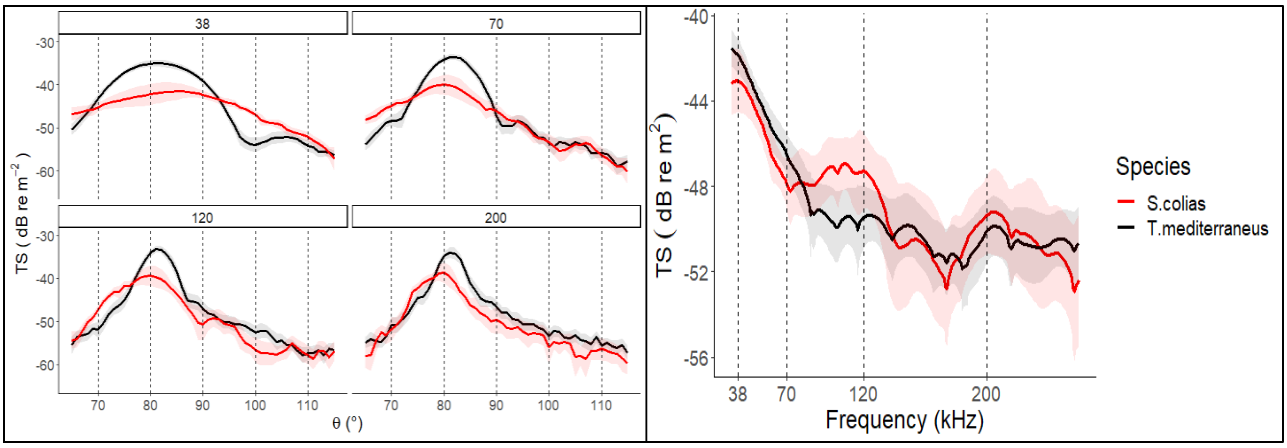

Figure 1. Right panel: species-specific broadband curve. The nominal operating frequencies used in fisheries acoustics (38, 70, 120 and 200 kHz) are shown with vertical dashed lines. Left panel: mean TS vs tilt angle at each discrete frequency (38, 70, 120 and 200 kHz). The 95% confidence intervals are shown by the shadow areas around the mean (solid lines).

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Application of analytical approaches to characterize the target strength of pelagic fish in the Mediterranean Sea

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Application of analytical approach to characterize the target strength of pelagic fish in the Mediterranean Sea

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Abstract.

The lack of data on the species-specific Target Strength (TS) on ancillary species limits the application of acoustic surveys to assessing their abundance and distribution worldwide. The TS values of *Scomber colias* and *Trachurus mediterraneus* in use in the Mediterranean Sea rely on studies conducted on other species in the Atlantic and Pacific oceans. Nevertheless, the application of backscattering models offers the possibility to overcome the absence of empirical data handling the parameters that most affect the TS. X-Ray scans were performed on 82 specimens to get digital representations of the swimbladder and the fish body which were used as input for the application of Kirchhoff Ray Mode model to measure the TS as a function of frequency and tilt angle. The morphometric differences between the two species produced divergent relative frequency response and broadband TS patterns. Moreover, comparing the results with one ex-situ experiment, we found a good agreement considering a mean tilt angle of -10°, standard deviation = 12°. Our results provide the first theoretical insights into the use of backscattering models as a tool to distinguish between

species in the Mediterranean Sea by acoustic method, increasing the knowledge of acoustic reflectivity of ancillary species.

Keywords: fisheries acoustics, target strength, backscattering models, Mediterranean horse mackerel, Atlantic chub mackerel, swimbladder

Introduction

Atlantic chub mackerel *(Scomber colias)* and *Mediterranean horse mackerel* (*Trachurus mediterraneus)* are two pelagic species found in the temperate waters of the Mediterranean and Atlantic Seas (Froese and Pauly, 2022). They play an important ecological role in the pelagic niche since they are mainly plankton and small fishes feeders that contribute to linking the lower and the higher level of the trophic chain (Sever et al., 2006; Šantić et al., 2013; Bourg et al., 2015;). However, they are generally considered ancillary species in the Adriatic Sea (Angelini et al.,2021). In the Mediterranean Sea, they have low economical value and they mostly represent bycatch and discard of the small pelagic fishery (Tsagarakis et al., 2012; Carbonell et al., 2018). Yet, in recent years *S. colias* and *T. mediterraneus* assumed certain importance as commercial food sources, as demonstrated by the expanding landings. *S. colias* showed an increasing trend from 2005 to 2019 in the Adriatic Sea and the same trend was registered from 2010 to 2019 in the entire Mediterranean Sea (FAO-GFCM, 2021). Otherwise, in some areas of the Eastern Atlantic, it became a target species, marked by an exponential increase in landings during the last 15 years (ICES, 2020). *T. mediterraneus* accounts for around 1.3% of all landings in the Mediterranean, with total catches of about 45,000 tons in 2016–2018 (FAO, 2020), and for a substantial portion of landings in the south-western Mediterranean (Carbonell et al., 2018) and the Adriatic Sea (Šantić et al., 2003).

The aforementioned data underlines the importance that the species covered by this study is progressively gaining in Mediterranean waters. Nevertheless, they have not been considered target species for fisheries in this basin, and thus for scientific assessments as well: only recently, a stock assessment was performed on Mediterranean horse mackerel (Angelini et al., 2021) thanks to the data collected during the regular acoustic survey carried out annually in the Adriatic Sea within the framework of the EU Mediterranean International Acoustic Survey (MEDIAS) project (Leonori et al., 2021), whereas, in the Atlantic Ocean the stocks of these species are assessed yearly on the basis of acoustic surveys accomplished by the International Council for the Exploration of the Sea (ICES). The authors complain that survey and commercial catch data on these species are often not reliable for different reasons, such as the lack of geographical coverage, misidentification of species, and gear selectivity (STECF, 2016; ICES, 2020). When acoustic survey time series are available, the poor and heterogeneous knowledge of the species-specific target strength (TS) of these secondary species do not allow us to make absolute estimates of species biomass (ICES, 2020). Both STECF and ICES stress the importance of monitoring the status of the *Scomber* and *Trachurus* genus in the Mediterranean Sea and Atlantic Ocean.

Acoustic survey has the potential to provide reliable fishery-independent data since it is viewed as one of the highly effective approaches for assessing the distribution and abundance of pelagic species (Simmonds and MacLennan, 2005). However, the conversion of volume backscattering strength, provided by the surveys, to an absolute biomass estimate requires knowledge of the species-specific acoustic backscattering cross-section. This is expressed in terms of target strength relation: $TS=10\log_{10}(\sigma/4\pi)$ in which sigma is the amount of incident wave reflected by the cross-section of a single target and includes the scattering properties of the species convolved with behaviour (Foote, 1987; Ona, 1990; Simmonds and MacLennan, 2005). *In-situ* and *ex-situ* methods are suitable for the measurement of the species-specific TS in the natural environment and controlled conditions (Henderson and Horne, 2007; Kubilius and Ona, 2012; O'Driscoll et al., 2018; Salvetat et al., 2020), but only the use of backscattering models allows us to properly predict theoretical backscatter from accurate measurement and setting of organism anatomy, material properties, swimbladder morphology, tilt angles, and frequencies (Jech et al., 2015). TS is affected by acoustic frequency, fish body length, orientation (tilt angle), depth and physiological factors (Nakken and Olsen, 1977; Hazen and Horne, 2003). Furthermore, the backscatter is mostly attributable to the dimensions and shape of the swimbladder, which is responsible for up to 95% of the backscatter of a fish (Ona, 1990). Therefore, in fish species identification and assessment using scientific echosounders, the knowledge of cross-sectional area, volume and tilt angle of swimbladder is of primary importance.

The first published models were based on simple geometrical shapes such as finite cylinders and ellipsoids solved through analytical solutions. The approximation of the swimbladder to a prolate spheroid was pursued by sectioning the swimbladder into a compound sets of finite cylinders solved by a Kirchhoff ray mode model (KRM) (Jech et al., 1995; Furusawa, 1988). Subsequently, complex finite surface elements were solved using numerical methods such as the Boundary Element Method which use the integral of Helmholtz equation and the Finite Element method involving the use of inhomogeneous Helmholtz equation (Francis and Foote, 2003; O'Driscoll et al., 2011). All these models are still in use, each having advantages and constraints, and the choice of model depends on the target shape, the tilt angle range investigated, data availability and power computer availability (Gauthier and Horne, 2004; Jech et al., 2015). Numerical models are computationally demanding and require detailed measurements of swimbladder and fish body characteristics. They account for the finer morphometric variation on the surface resulting in more precise and accurate measurements of acoustic reflectivity, which can help to draw broadband frequency patterns (Kubilius et al., 2020). Small alterations along the target shape cause fluctuations in echo amplitude, introducing some incoherence, leading to coherent or incoherent echo amplitude that also relies on frequency and can be detected through the use of broadband pulse (Demer et al., 2009). Conversely, analytical models approximate complex swimbladder shapes solving the backscatter from one or more simplified geometric figures but, they do not require super-computer computations and high-resolution measurements (Reeder et al., 2004). Generally, there is a good agreement between models and empirical experiments (Horne et al., 2000; Hazen and Horne, 2004; Reeder et al., 2004), but some authors found significant differences between models, *in-situ* and *ex-situ* experiments (Sawada, 1999; Hazen and Horne, 2004; Peña and Foote, 2008).

With a continuously growing interest in the use of broadband in acoustic surveys, backscattering models give insight into the feasibility of implementing broadband techniques for species identification purposes. As a matter of fact, the recent commercial availability of echosounders capable of both narrowband and broadband is leading to a shift in acoustics data collection in favour of broadband (Stanton et al., 2010; Bassett et al., 2018). One of the main advantages concerns an increase in near-frequency resolution that improves the characterization and classification of acoustic targets (Benoit-Bird and Waluk, 2020). The use of broadband pulses gives fisheries acoustics scientists the possibility to distinguish fish of different sizes belonging to the same species (Kubilius et al., 2020). However, to the best of the author's knowledge, a single work was published on broadband acoustics response of fish in the Mediterranean Sea (Palermino et al., 2022), where the multi-frequency backscatter still represents a good tool for species discrimination (Korneliussen, 2018). Moreover, backscattering models have never been used on the species dealt with in this study.

In this paper, we collected digital images of fish anatomy through X-ray scans to develop approximate analytical models in order to investigate the swimbladder morphology and compute the species-specific TS functions of *S. colias* and *T. mediterraneus* in the Adriatic Sea. Results were compared with *ex-situ* experiments conducted on the same species in the same area. We used the Kirchhoff ray mode model (KRM) to study the variation of TS as a function of tilt angle, frequency and fish length. In particular, we focused attention on the development of species-specific TS vs Total length (TL) function. Moreover, by considering different tilt angle intervals, we proposed a species-specific relative frequency response (RFI) and broadband backscatter curve. Results were compared between species providing preliminary evidence on the possible application of RFI and broadband pulses for species discrimination purposes in the Adriatic Sea.

Materials and methods

Fish sample

Fish were collected in the Adriatic Sea during the 2020 and 2021 MEDIAS survey carried out between June and July by the Acoustic Group of CNR-IRBIM of Ancona on board of R/V G. Dallaporta. The fishing operations were undertaken with a mid-water trawl characterized by an 18 mm cod-end mesh size equipped with SIMRAD's FX80 trawl sonar to monitor the behaviour of the net. The net was cast at a seabed depth ranging from 25 to 90 m at around 4 knots for ~30 min. The sites of the hauls where the fish were collected are shown in Figure 1. A data collection plan was developed as follow: once on board, active and healthy fishes were immediately transferred and held in a 200-litre tank with running seawater on board for at least 4 hours, 12 hours when possible. After this period of acclimation surface pressure, useful to avoid variability due to depth pressure on swimbladder morphology, the fish that exhibited normal swimming behaviour were anaesthetized in the tank mixing a path of 9:1 ethanol with 4 ppm of clove oil to avoid the possible release of gas during the successively freezing operation.(Javahery et al., 2012). All fishes were frozen as soon as possible at -20° in plastic bags. In order to reduce the influence of depth pressure, hauls carried out at the surface or at low depth have been primarily selected for specimen collection. The study complies with the Italian animal research legislation (D. Lgs. N. 26 of 04/03/2014).

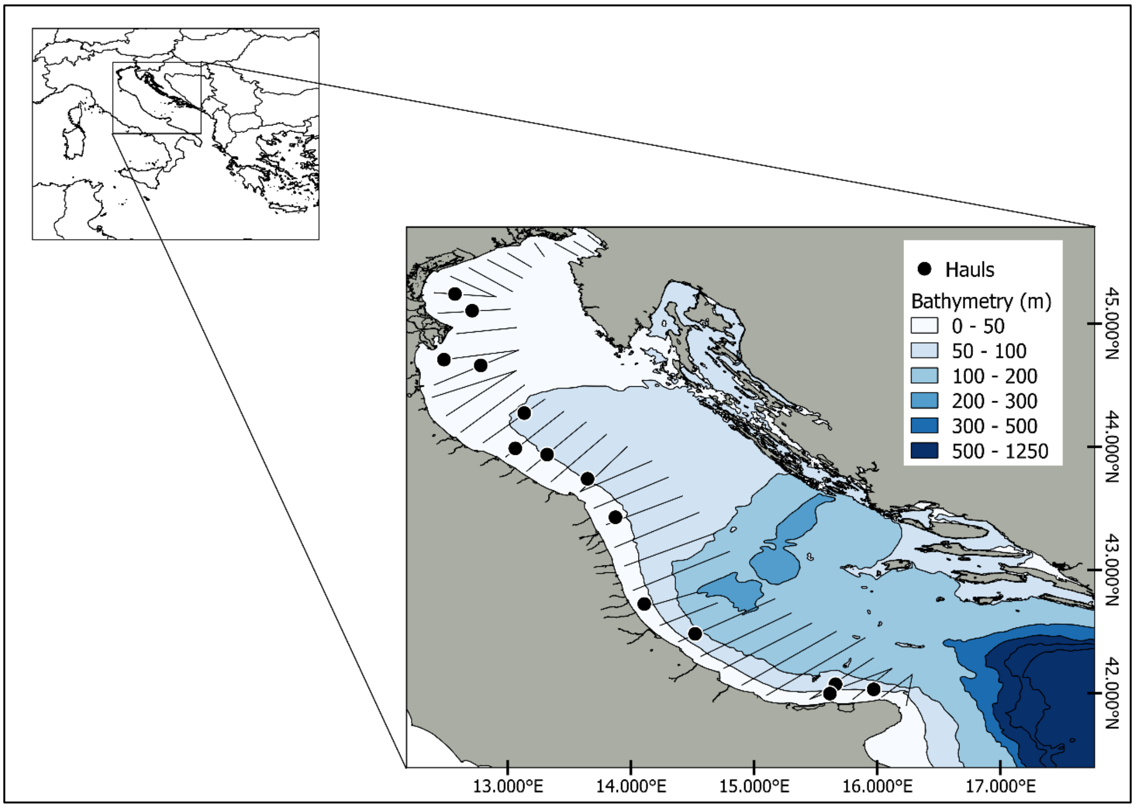

Figure 3. Net sampling positions (black dots). The figure also reports the transect plan followed during the 2020 and 2021 MEDIAS survey in the North Adriatic Sea (GSA 17).

Swimbladder measurements

A total of 82 specimens divided as follow were collected: 25 Atlantic chub mackerels (TL size range, 11-33.7 cm), and 57 Mediterranean horse mackerels (TL size range, 11.2-27.7 cm). The size range obtained was roughly representative of the one commonly found in the Adriatic Sea. Specimens were defrosted and scanned with an RT 400 HF/TS X-Ray at veterinary facilities. The fishes were placed at about 1 meter from the X-Ray source on a detector plate with known dimensions. In each session from 1 to 5 specimens were radiographed on dorsal and lateral view to collect two-dimensional projection images of the swimbladder (Figure 2). Different trials with other fishes of the same dimensions collected during the survey were carried out before image acquisition, in order to adjust instrument settings to guarantee maximum resolution. The exposure and current was 2 mA/s while the voltage was set at 52-65 (kVp) (Horne et al., 2000; Scoulding et al., 2015). A metal ichtyometer was placed in each scan for image calibration purposes.

DICOM image files from X-Ray scans were processed using ImageJ software. The images that presented inflated and intact swimbladder were adjusted to improve the contrast between the swimbladder and fish body and when necessary the fish was rotated to get a horizontal sagittal axis. Thereafter, a grayscale threshold was set to automatically trace the boundary of the fish body and the swimbladder. Whereas the greyscale threshold was unable to accurately detect the swimbladder boundaries (especially on dorsal view), they were traced manually. We collected dorsal surface - swimbladder length (sbl), defined by the distance between anterior and posterior margins- swimbladder height (sbh), defined by the maximum thickness in the lateral aspect - swimbladder width (sbw), defined by maximum thickness in the dorsal aspect and swimbladder tilt angle (sbΘ), defined as the tilt between the centreline and the straight line crossing the latter, as shown in Figure 2. Next, from the traced dorsal surface, we computed the mean cross-sectional area, while the volume was obtained following the formula: $V = 4\pi/3\ (sbl/2)(sbh/2)(sbw/2)$. All measurements are reported in Figure 2. Next, each fish was placed in a Cartesian plane and x_j, z_j and w_j coordinates, where x_j and z_j are the upper and lower coordinates along the longitudinal axis and w_j is the width of the body on dorsal view, were collected, initially each 10 mm for the fish body and 5 mm for the swimbladder and subsequently each 1 mm for both.

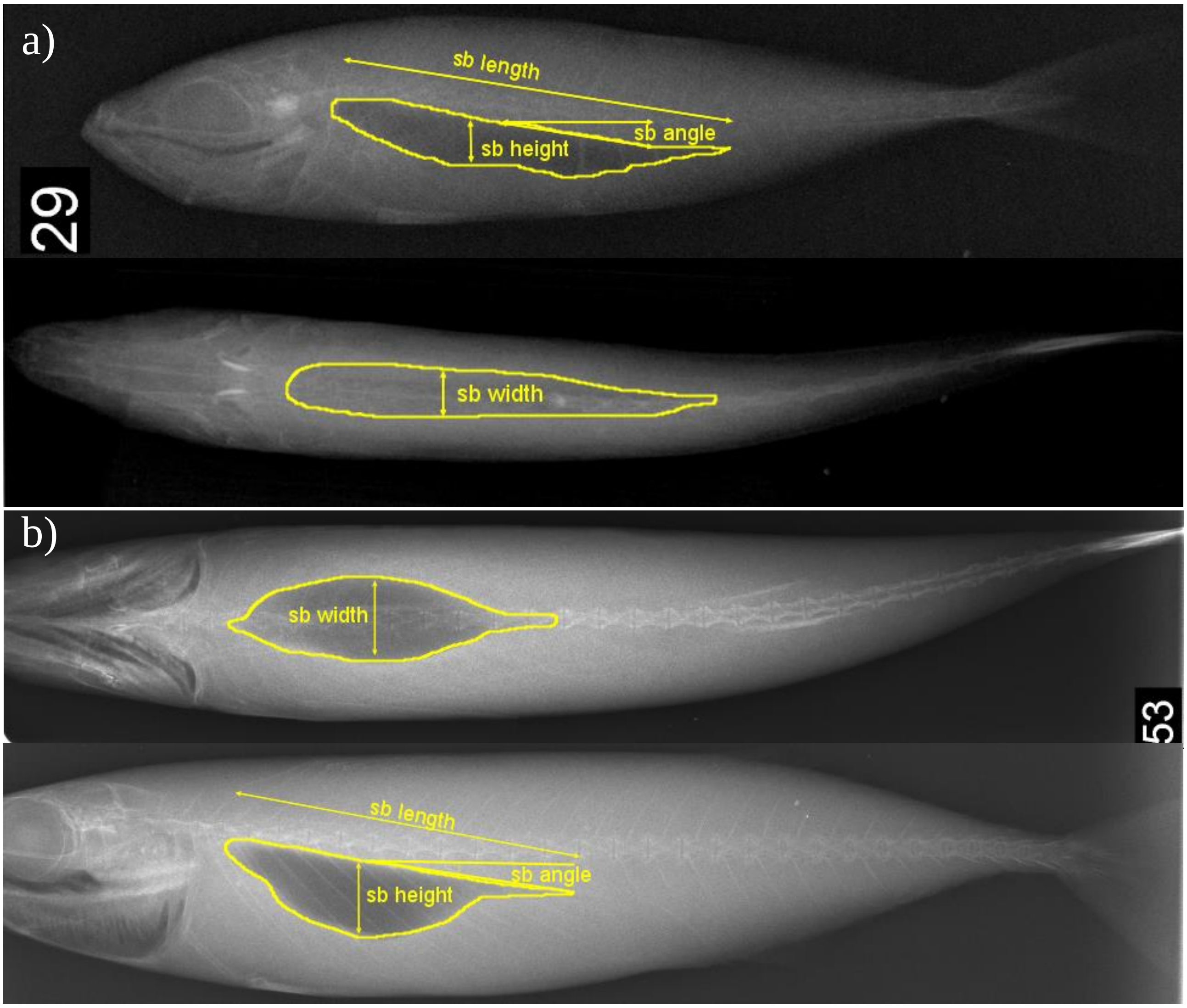

Figure 4. Soft X-Ray lateral and dorsal radiographs of *T. mediterraneus* (a) and *S. colias* (b). Swimbladder borders are shown with yellow lines, while the arrows depict the swimbladder measurements

Backscatter modelling and data analysis

Measurement results were plotted against the TL through a linear regression model. Afterwards, a t-test was performed to compare the swimbladder morphologies of the two species. Coordinates obtained from X-ray image processing of fish with inflated and intact swimbladder were used as input parameters for application of the Kirchhoff ray mode model. Kirchhoff-Ray approximation is used for computing the scattering of finite-length cylinders, summing coherently the scattering from consecutive and potentially offset gas-filled (swimbladder) and fluid-filled (fish body) stacked cylinders, which gives a 3D representation of the fish and the swimbladder. A correction for the tapers at each end of the elements is included to improve the accuracy of the model (Clay and Horne, 1994). Typical acoustic fish parameters required by the model and water parameters, computed on the basis of the environmental data collected during the survey with a CTD (SEABIRD 911 PLUS) probe, are given in supplementary materials Table S1. KRMr package was used to apply the KRM model on fish and swimbladder shapes. An analytical model on a theoretical sphere was used as benchmark for KRM backscatter results. A theoretical sphere along with the fish body and swimbladder shape collected for a *S. colias* specimen were employed to assess the KRM performance by comparing the results obtained from coarse and refined slice thickness in a range between ƛ/10 and 2 mm. Moreover, the ensemble influence of slice thickness and tilt angle was performed. Finally, the TS of a three-dimensional prolate spheroid with semi-major ($sbl/2$) and semi-minor ($\sqrt{(sbh * sbw/4)}$) axis modelled following the size of a typical swimbladder size of a small *S. colias* specimen, was computed by solving the inhomogeneous Helmholtz equation in the Acoustic modules of COMSOL Multiphysics 6.0 software through the Finite Element Method (FEM) in broadband, in order to validate the result of the KRM model.

The backscattering cross section was computed as a function of the tilt angle strictly within a range between 65° and 115° because, as demonstrated by Macaulay et al (2013), at a high off-broadside tilt angle the KRM model becomes not accurate. The corresponding backscattering cross section in linear domain (σ_{bs}) was averaged for each chosen Gaussian tilt angle interval and then logarithmically transformed to TS values. The following tilt angles distribution was chosen to represent near-normal and abnormal swimming behaviour of fish: 90°±5°; 90°±10°; 90°±20°; 101°±12°, 88°±13°; where 90° is dorsal incidence. An orientation of 88° with a standard deviation of 13° has been considered as a normal fish orientation under natural conditions in this study, mainly based on previous researches carried out on *Trachurus* species and other fish with swimbladder (Fassler et al., 2013; Kawauchi et al., 2019; Madirolas et al., 2017; Peña and Foote, 2008). The other tilt angle intervals are intended to represent the increasing swimming-orientated direction of fish. A tilt angle of 90° with a standard deviation of 10 and 20 was set as suggested by Membiela and Dell'Erba (2018) for fish spread at different depths. We then we added another tilt angle interval to compute the TS considering an abnormal fish swimming behavior. The mean tilt angle of 101° with a standard deviation of 12° was set adding the mean and standard deviation of the swimbladder related to the fish body angle computed during the swimbladder measurements. Corresponding mean TS values for each tilt angle interval were then regressed in function of fish TL using the standard model: $TS = m \, log \, L + b$ and the model proposed by Foote (1987): $TS = 20 \, log \, L + b_{20}$. Mean σ_{bs} from the normal tilt angle interval (88° s.d. 13°) and at a broadside angle at 38 kHz were related to the results of the other discrete frequencies usually used during acoustics surveys (70, 120, 200 kHz) to compute the relative frequency response following the formula: r_i(f) = σ_i(f)/ σ_i(38) (Pedersen et al., 2004).

Results

Swimbladder morphology

5 specimens for each species out of the 82 fish subjected to X-ray scan showed a deflated swimbladder. The data analysis on the remaining animals gave the values reported in Table 1. The wide fish size range, especially for *S. colias*, occasioned a high gap between minimum and maximum values of swimbladder measurements. Despite the similar mean TL, the swimbladder of *T. mediterraneus* is on average 20 mm longer, 1 mm smaller and 2.3 mm tighter compared to *S. colias*, which in turn leads to different volume and area.

Table 7. Morphological characteristics of fish body and swimbladder of *T. mediterraneus* and *S. colias*

Measure	*T. mediterraneus*			*S. colias*		
	Mean	**min**	**max**	**Mean**	**min**	**max**
Total length (cm)	15.4	11.2	23.1	14.4	11.1	33.7
Sb length (mm)	50	35.4	77.7	30	6.7	93.8
Sb width (mm)	4.8	2.7	7.4	7.1	3.6	22.4
Sb height (mm)	4	2.3	6.9	5	2	18.7
Volume (mm^3)	562	150.7	1833	2027.7	74.2	20628
Area (mm^2)	198.1	68.7	475.4	184	45.3	1132.5
Sb ϴ (°)	10.9	5.54	14.8	11.4	6.34	14.3

Swimbladder length, height, width, volume and area increase proportionally to the total length. The regressions depicted in Figure 3 clearly show a significant positive relationship characterized by a high level of significance ($p \leq 0.001$) and good fit of all models ($r^2 \geq 0.95$). Therefore, in order to remove the effect of length, the residuals of the models were kept as relative swimbladder size for comparison between the two species' morphologies. Once the length effect was removed, the t-test revealed high divergence in swimbladder length, height, width, and volume ($p \leq 0.001$ in each case) while a lower but still significant difference characterized the area ($p \leq 0.05$). Overall, the swimbladder of *T. mediterraneus* appears elongated, pear shaped and slightly compressed laterally with a thin posterior end that increases towards the head up to a wide frontal region. The haemal spines, clearly visible inside the cavity, generally make swayment on the swimbladder surface. Conversely, *S. colias* displays a spherical-like swimbladder characterized by narrow anterior and posterior regions defined by large width and height proportionally to the length as shown in Figure 4.

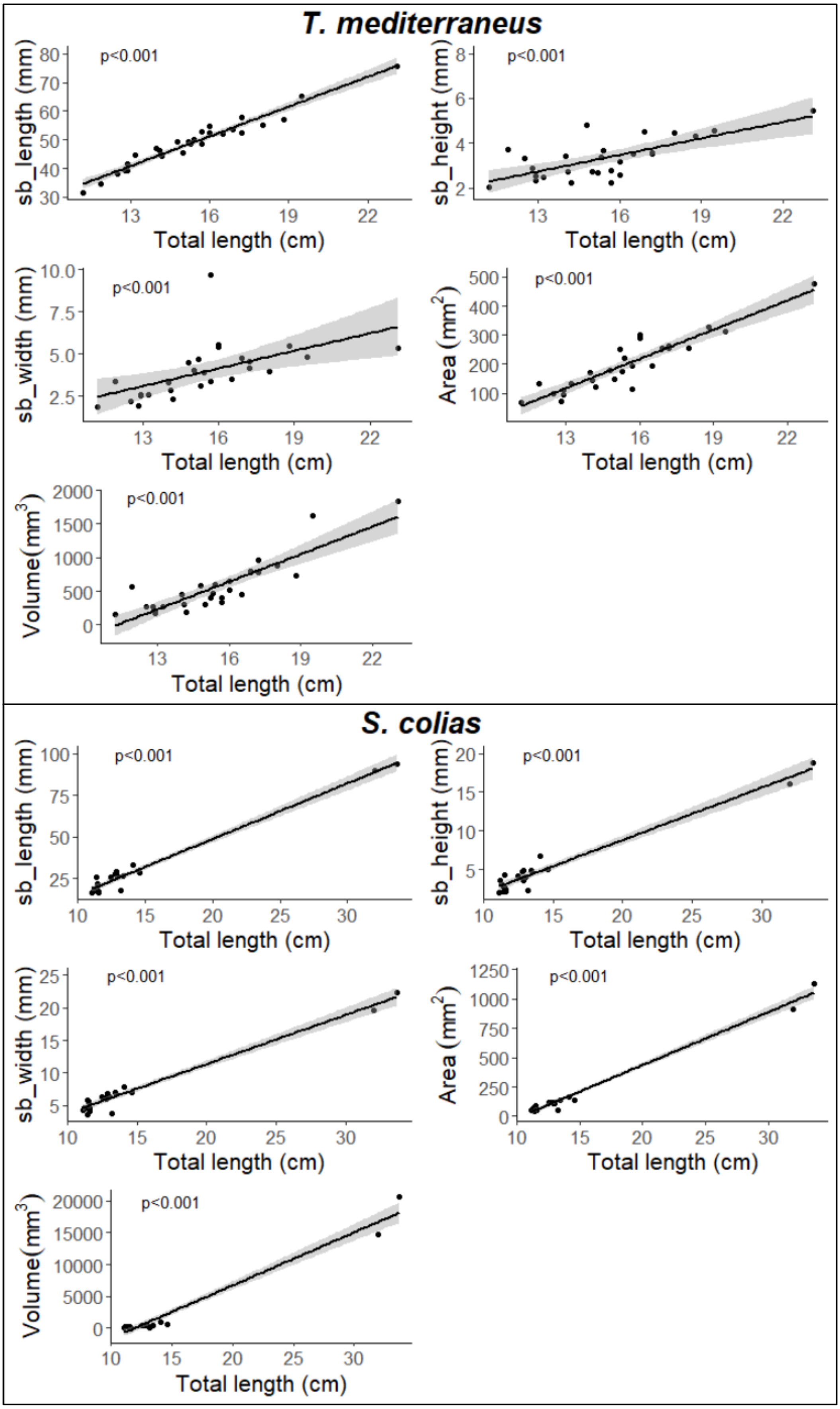

Figure 5. Linear relationships between swimbladder length, height, width, area and volume and total length of *T. mediterraneus* (top) and *S. colias* (below). The shadow areas express the 95% confidence intervals.

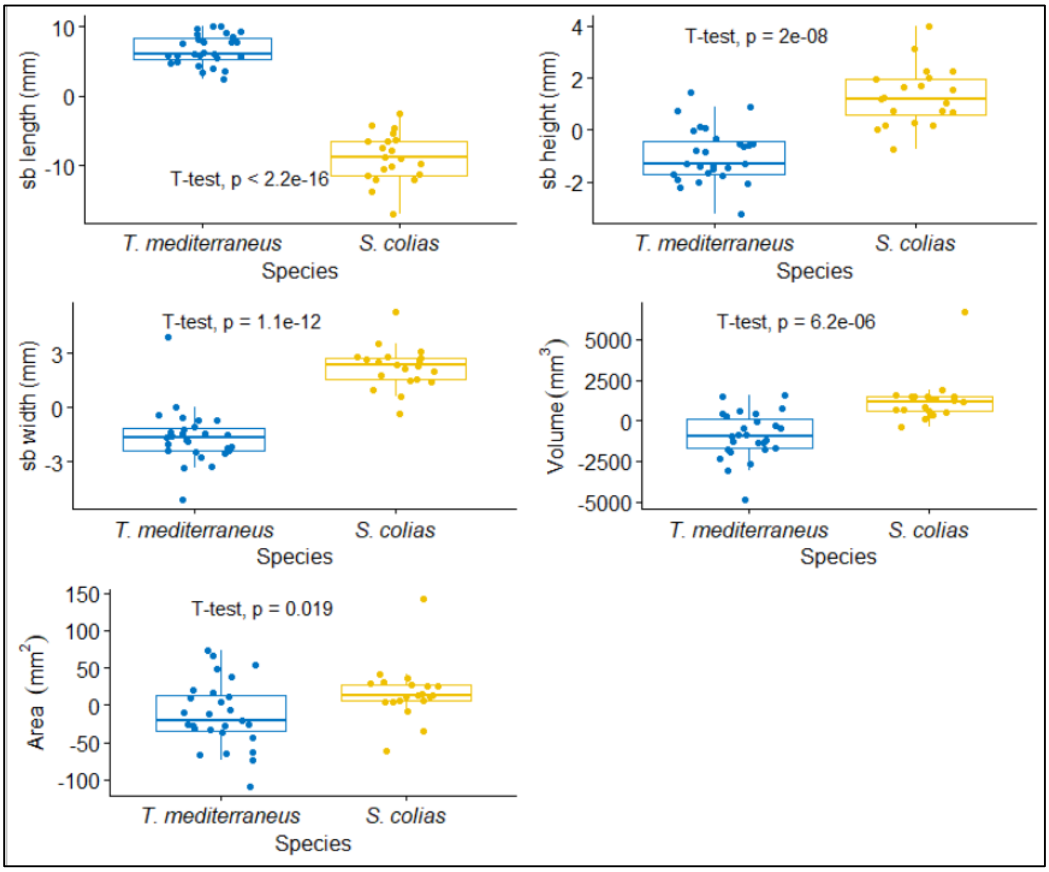

Figure 6. Comparisons between dimensionless swimbladder morphological characteristics of *T. mediterraneus* and *S. colias*. The p-value resulted from the Student t-test are depicted. The x axes report the values of the residuals resulting from the linear regression models shown in Figure 3. The distribution of data is illustrated by a boxplot showing the medians (horizontal lines), percentiles (box borders), and 5 - 95% percentiles (vertical lines)

KRM accuracy

The KRM model employed in this study shown a good agreement with analytical model (see Figure S1 in supplementary materials). Figure 5 shows the results of the KRM model in broadband considering a broadside angle of a single 11.5 cm specimen of *S. colias* of as an example. A small fish was preferred, as a trade-off between representation and computational time needed to solve the equations. The model was applied to refined and coarse measurements, firstly only on the swimbladder (length = 19 mm) and later by removing the swimbladder from the fish body and finally summing the TS backscatter from the union. The results showed differences up to 0.4 dB in the swimbladder. Notably, over 120 kHz the TS curve obtained with slice thickness of 2 mm is closer to the one obtained with λ/10. No differences were detected for the fish body, while considering the whole fish, TS resulted higher for the 2 mm slice thickness at high frequencies. TS curve undertaken detectable changes among cylinder size only at steeper negative angles (-25°) (more details in Figure S2-S3). Conversely, significant differences up to 10 dB on the backscatter were detected between 2 mm and 1 mm slice thickness computations for a sphere of 0.2 mm radius, while there were any variations between ƛ/10 and 1 mm except for the fluid sphere over 220 kHz (see Figure S4 for further details). For these reasons, in the subsequent analysis, we used a slice thickness of 1 mm as a good trade-off both for swimbladder and fish body measurements. The KRM model was also tested on a prolate-spheroid against the FEM model (Figure 6). The prolate spheroid semi-major and semi-minor axis were defined based on the swimbladder dimensions of a small specimen (semi-major axes = 0.015 m; semi-minor axes = 0.002 m) from one species covered by this study, in order to limit the CPU necessary for computations.

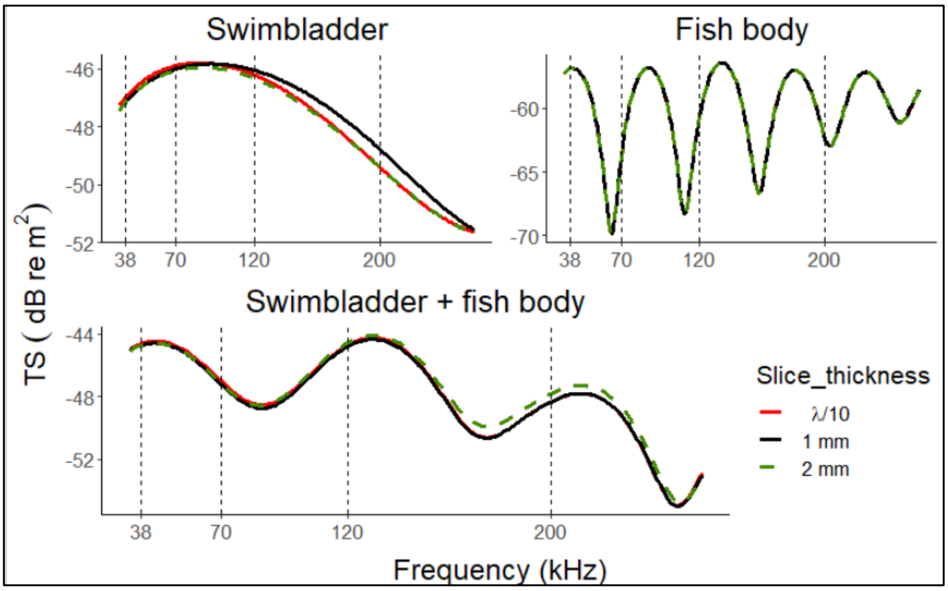

Figure 5. TS vs frequency calculated using the KRM model on swimbladder only (top left), fish body only (top, right) and the whole fish (below) of a *S. colias* specimens of 12 mm TL. The three colors point out the size of cylinder (ƛ is computed on 200 kHz). The vertical dashed lines indicate the reference frequencies more frequently used in fisheries acoustics.

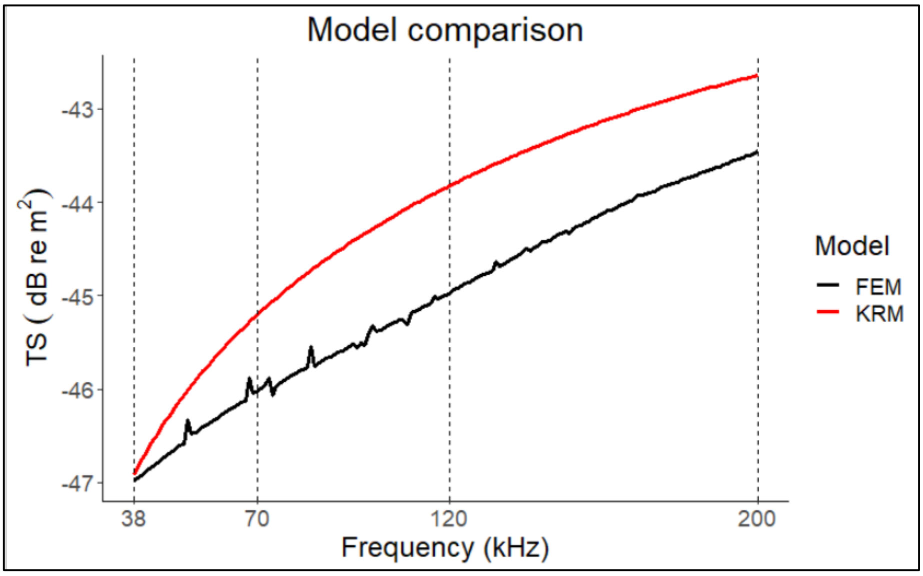

Figure 6. The TS vs frequency of a prolate-spheroid of 0.01 m semi-major axes and 0.002 m semi-minor axes calculated through the KRM model (in red) and the FEM model (in black). The vertical dashed lines indicate the reference frequencies more frequently used in fisheries acoustics.

The simulations indicated that the model agreed within 1 dB along the frequencies spectrum from 70 to 200 kHz and 0.5 dB between 38 and 70 kHz.

Target Strength analysis

Table 2 shows TS-TL function results based on five tilt angle distributions. *b* values of the standard model were lower than the b_{20} values except for a mean tilt angle of 101° with a standard deviation of 12°, which does not fit well into the linear regression for *T. mediterraneus* (r^2 ~ 0.1).

Table 8. TS-TL standard model and with slope forced to 20 by tilt angle distributions. Standard model slope (m), conversion parameters b and b_{20}, standard error (s.e.) and R^2 are shown. The values are expressed in dB re 1 m^2.

Tilt angle (°)		**TS = *m* log *l*+ *b***				**TS = 20 log *l*+ b_{20}**		
Mean	**St. dev.**	***m***	***b***	***s.e.***	r^2	b_{20}	***s.e.***	r^2
T. mediterraneus								
90	5	21.3	-65.88	1.2	0.62	-64.27	1.19	0.62
90	10	24.6	-70.12	1	0.75	-64.51	1	0.73
90	20	25	-71.58	0.78	0.84	-65.48	1.63	0.80
101	12	8.88	-56.52	1.43	0.14	-70.04	1.6	0.1
88	13	26	-71.65	0.86	0.78	-64.4	0.95	0.76
S. colias								
90	5	24.65	-71.25	1.25	0.88	-66	1.34	0.82
90	10	26	-73.2	1.26	0.88	-66.44	1.47	0.82
90	20	25.72	-74.06	1.3	0.89	-67.58	1.48	0.84
101	12	14.2	-62.54	1.16	0.74	-69.43	1.38	0.85
88	13	26.9	-74.48	1.3	0.89	-66.65	1.59	0.83

Conversely, the coefficient of determination r^2 shows a good fit in the regression models with no differences between slope fitted to the data and a fixed slope of 20. Figure 7 shows the relationship between TS and TL considering a tilt angle of 88° ± 13°. The higher standard error found for *Scomber colias* is mainly due to the lower number of samples. Thereafter, the overall mean σ_{bs} obtained at discrete frequencies 70, 120 and 200 kHz were divided by the values resulting from 38 kHz getting the patterns shown in Figure 8. At a tilt angle of 88° ± 13°, the $r_i\ (f)$ at 70 and 200 kHz settles at a ratio of ~ 0.35 and ~ 0.20 respectively for both species, whereas the main contrast concerns the results at 120 kHz, characterized by a difference of 0.30.

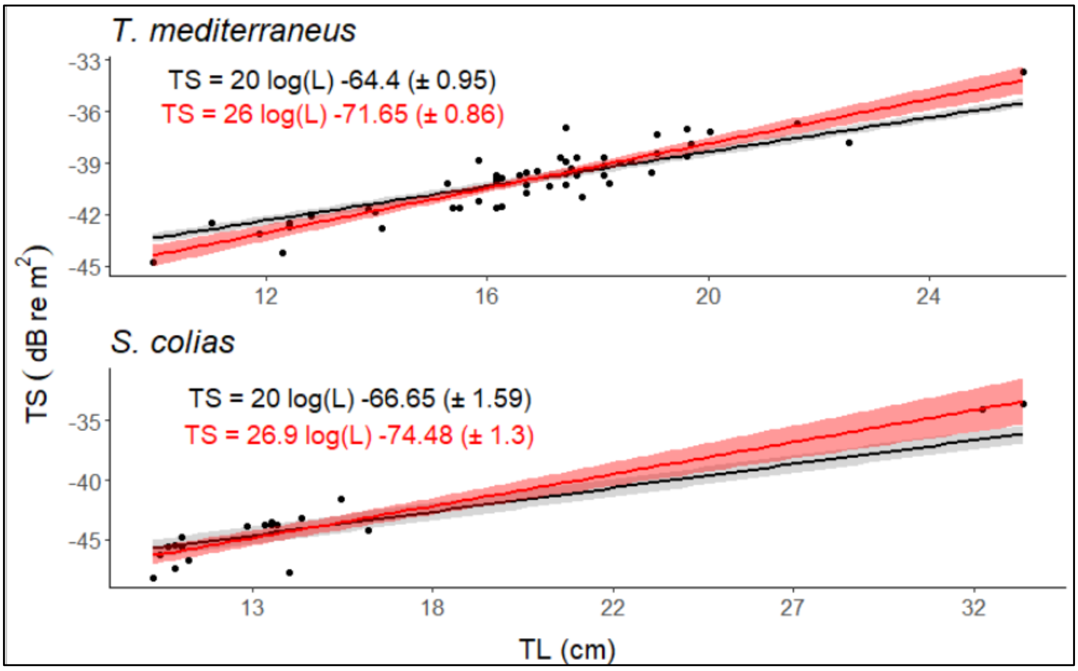

Figure 7. Target strength vs total length relationship with a mean fish tilt angle of 88° s.d. 13°. The standard model regression is shown in red, and in black the model with the slope forced to 20.

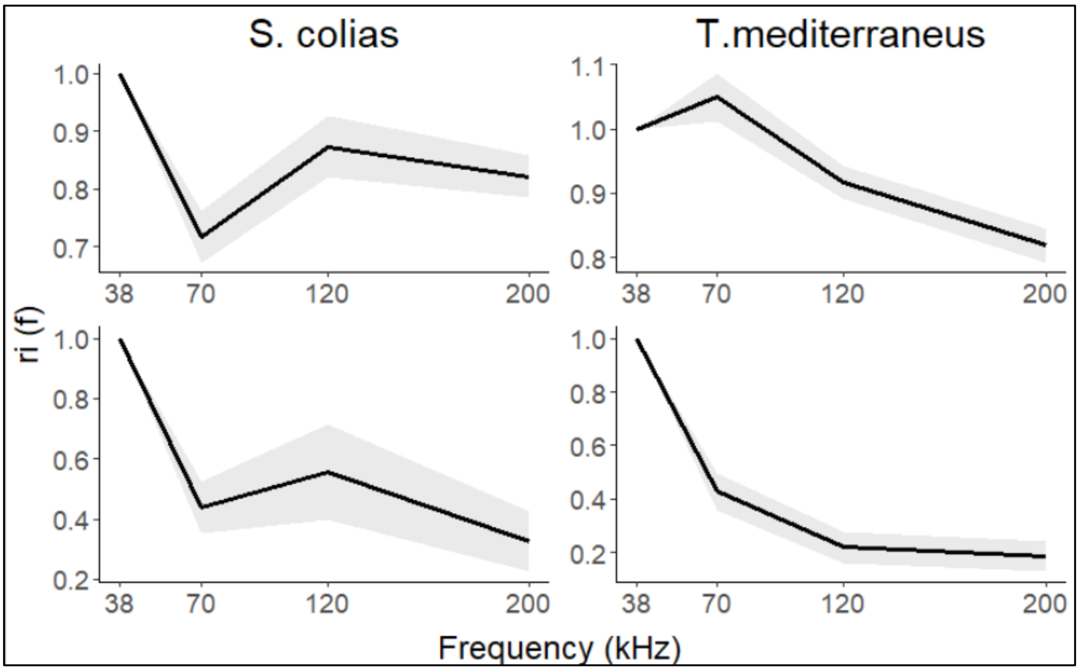

Figure 8. Mean relative frequencies responses (continuous lines) and confidence intervals (dashed areas) of *T. mediterraneus* and *S. colias* considering a mean tilt angle of 88°, standard deviation of 13° (upper panel) and broadside angle (lower panel).

At a broadside angle, as expected, the mean backscattering cross-section values at the other frequencies rose, leading to all $r_i\ (f)$ values beingover 0.7. In this case, the diagnostic frequency is the 70 kHz, which is characterized by a ratio even higher than 1, rather than the 120 kHz that yelded to similar ratios of ~ 0.90 for chub mackerel as well as for the Mediterranean horse mackerel.

However, the trend between these frequencies showed a clear downward pattern for *T. mediterraneus* and an upward pattern for *S. colias* at the tilt angles taken into account.

Discussion

The high amount of samples with intact swimbladder (77) achieved after the X-Ray scans proves the validity of the experimental plan both on physoclists (*T. mediterraneus*) and on physostomus (*S. colias*) species, which was based on the best literature on this topic (Clay and Horne, 1994; Furusawa, 1988; Yasuma et al., 2010; Membiela and Dell'Erba, 2018; Dornan et al., 2019). The X-Ray scan revealed wide differences in the swimbladder morphologies and a slight variation in the orientation within the fish body, which was partially reflected in the backscattering variation between the two species. Nevertheless, the dorsal aspect is known to be the most important diagnostic character in TS studies (Love, 1971), and in our case the dimensionless results showed similar mean-cross-sectional area values, despite the dorsal area of *T. mediterraneus* being slightly, but significant, smaller. This can explain the closeness between b_{20} values which are within 2.5 dB of variation at any tilt angles cases (see Table 2). The cross-sectional area is also a fundamental parameter for the decision of the most correct TS-TL function, especially in our case where the other dimensionless swimbladder measurements were highly divergent (see Figure 4). As demonstrated by McClatchie et al (2003), the choice of correct slope for the species-specific TS-TL function should not be done a-priori but should be based on morphological analysis, choosing the most appropriate value. For both the Mediterranean horse mackerel and the chub mackerel the mean cross-sectional area grows proportionally to the total length as shown in Figure 2. Notably, the growth is isometric, and therefore, assuming a slope value close to 20 can be considered a good approximation.

This is the second study concerning the use of backscattering models on fish species in the Mediterranean Sea; the first one described a model-based TS-TL relationship on *T. mediterraneus* and *S. colias* (Palermino et al., 2022). An advantage in modelling compared to empirical experiments is the possibility of increasing the amount of data when a limited number of samples are available, gaining species-specific TS variability and backscattering patterns (Fassler et al., 2013; Gonzalez et al., 2020). Approximate analytical models such as KRM are a good trade-off between model complexity and computational demand, which limits the application of numerical models such as the FEM model to relatively large air-filled objects (Khodabandeloo et al., 2021b), even though using an approximation model involves a decrease in accuracy especially at steeper angles and low aspect ratio (Macaulay et al., 2013). Therefore, a test on accuracy could be useful to determine the suitability of the model based on species and swimbladder morphologies. To apply the KRM model, Jech et al.

(2015) suggested dividing fish body and swimbladder into finite cylinders 1 mm thick for operational purposes, while Macaulay et al. (2013) used very thin cylinders up to 0.05 mm. However, slicing real shapes under $\lambda/10$ accuracy is in many istance time-consuming, hence we tried to test the accuracy of the model by considering coarser measurements. In the case of fish body, it did not affect the TS results likely due to the fairly small variation of the object along the x axes. Conversely, the slight differences detected for the swimbladder could affect the backscatter. Nevertheless, considering the whole fish and the results obtained on a theoretical sphere, a slice thickness of 2 mm appear not suitable, while 1 mm size can be considered highly accurate and any further effort in obtaining tiny cylinder unnecessary. We accordingly, suggest collecting a measure1 mm each both for swimbladder and for fish body for use of the KRM model. Moreover, the comparison between FEM and the approximate analytical model proved the suitability of KRM for our swimbladder shapes. Indeed, the maximum difference of 1 dB detected between them on an elongated swimbladder characterized by a semi-minor axes less than half of the length of the semi-major axes can be considered a good approximation (Macaulay et al., 2013).

New TS-TL relationships derived from models are seldom employed for acoustic abundance estimates (Fassler et al., 2013). Most frequently, models are exploited for comparison purposes with *ex-situ* or *in-situ* experiments (Hazen and Horne, 2004; Horne et al., 2000; Madirolas et al., 2017; Sobradillo et al., 2019). The relationship found in this work can be compared with the study carried out on these species by Palermino et al. (2021). During these *ex-situ* experiments, the tilt angles of the specimens were not detected, the choice of a wide range of tilt angles for model development, therefore, can help the interpretation of both model and experimental data.

In the previous study, Palermino et al. (2021) found b and b_{20} values for the two species very close to each other as reported in Table 3. Our findings at an assumed normal swimming behavior of 88° ± 13° were ~5 dB higher for both species and along the tilt angle intervals, the difference is almost constant except for orientations of 90° ± 20° and 101° ± 12°, where the results are lower and closer to the b_{20} = -71.4 dB for *T. mediterraneus* and b_{20} = -71.6 dB for *S. colias* obtained in Palermino et al. (2021) (Table 3). Fish orientation is one of the parameters that influence the TS the most, especially when it is measured in unnatural and natural conditions (Ona, 1990; Horne, 2003; Simmonds and MacLennan, 2005; Membiela and Dell'Erba, 2018). It has been demonstrated that during *ex-situ* experiments fishes display a steeper angle than in their natural state which in turn can affect TS measurements (Wanzenböck et al., 2020). Despite the efforts and the novelty of the use of a piece of rope instead of a hook performed during the single *ex-situ* experiments conducted in 2013 and 2014 (Palermino et al., 2021), it is likely that the Mediterranean horse mackerel and chub mackerel specimens were constrained to a fairly abnormal swimming behavior. Consequently, the b_{20} value of

-70.4 dB for *T. mediterraneus* and -69.43 dB for *S. colias* found through the KRM model considering an abnormal tilt angle displacement in this study could be considered almost in agreement with empirical experiments within a 2 dB interval. We can assume these results as being the correct ones also for the other tilt angle intervals with an approximation of 2 dB.

Theoretical and empirical experiments proved that the peak of backscatter in fish with swimbladder is achieved at a negative tilt angle of ~ 10°, followed by a drop at steeper angles characterized by individual-fish pattern (Peña and Foote, 2008; Fassler et al., 2013; Madirolas et al., 2017). *S. colias* displays a TS pattern against tilt angle characterized by modest variation along the tilt angles reaching a peak at around -4° at 38 kHz. Conversely, the TS peak of *T. mediterraneus* was recorded close to -10° at the same frequency (Palermino et al., 2022). Some authors provided evidence of a quite steeper positive or negative tilt angle kept by fish of the *Scomber* and *Trachurus* genus in normal swimming behaviour (Fernandes et al., 2016; Mäthger, 2003). It should be highlighted that when the vessel approach fish schools or shoal during daytime acoustic surveys activity they are inclined to display an avoidance behaviour, especially in shallow waters causing an abnormal swimming behaviour leading in turn to a drop in the TS (Barange and Hampton, 1994; Hjellvik et al., 2008). However, fish orientation depends on an ensemble of natural factors such as light intensity and feeding migrations (Mäthger, 2003; Fernandes et al., 2016; Yasuda et al., 2018), and therefore the most suitable b_{20} value could vary between shifting survey conditions. The b_{20} values presented in Tables 2 and 3 at normal swimming behaviour (88° ± 13°) are closer to that of -68.7 dB now in use in the Mediterranean Sea (Lillo et al., 1996; MEDIAS, 2021). They are also close to the values detected by other studies conducted in the Atlantic and the Pacific Oceans on related species: *Trachurus capensis*, *Trachrus symmetricus mutphy* and *Scomber japonicus* (Barange and Hampton, 1994; Axelsen, 1999; Svellingen and Ona, 1999; Robles et al., 2017). Conversely, they diverge from other studies carried out on these species (Gutiérrez and Maclennan, 1998; Axelsen, 1999; Axelsen et al., 2003; Peña and Foote, 2008). Notably, the conversion parameter values obtained with a tilt angle of 101° ± 12° for *T. mediterraneus* here are closer to the results published by Peña and Foote (2008) that applied a Kirchhoff approximation on specimens of *Trachrus symmetricus murphy* subjected to MRI scanner.

The small differences could be mainly linked to the species but also to the methodology and the size of the fish. The multi-frequency approach for the identification of fish species has been applied since the early 2000s (Korneliussen and Ona, 2002; Hannachi et al., 2004; McKelvey and Wilson, 2006; De Felice et al., 2015; Leonori et al., 2021). It is now commonly used in post-processing acoustic data analysis worldwide (Korneliussen et al., 2006). However, the technique is still focused on a few target species due to the lack of data on other pelagic fish species especially in the Mediterranean Sea (Anonymous, 2012).

Table 9. Comparison between conversion parameters b and b_{20} found in this work and the results obtained in Palermino et al. (2021) at 38 kHz. The reported values are in dB re 1 m^2. Note the shortfalls that underline the b_{20} values usually employed for the conversion of backscattering volume in biomass.

Tilt angle (°)	***T. mediterraneus***				***S. colias***			
	Present work		***Ex-situ* experiment**		**Present work**		***Ex-situ* experiment**	
	b	***b*₂₀**	***b***	***b*₂₀**	***b***	***b*₂₀**	***b***	***b*₂₀**
90 (s.d=5)	-65.88	-64.27			-71.25	-66.00		
90 (s.d=10)	-70.12	-64.51			-73.2	-66.40		
90 (s.d=20)	-71.58	-65.48			-74.06	-67.58		
101 (s.d=12)	-56.52	-70.40	-64.9	-71.4	-62.54	-69.13	-63.8	-71.6
88 (s.d=13)	-71.65	-64.40			-74.48	- 66.65		

The results obtained in this study applying the relative frequency response formula pointed out a clear opposite pattern between 70 and 120 kHz, which is not affected by the tilt angle: *S. colias* showed a rising curve while *T. mediterraneus* displayed a decreasing curve. These are the first multi-frequency backscatter evidences on both species worldwide, although the relative frequency response of the congeneric species *Trachurus trachurus* has already been studied in 2005 (Fernandes et al., 2006). Our results differ from the latter, giving the possibility to distinguish between the three co-occurrence species in the Adriatic Sea via an acoustic tool. Again, the typical swimbladder morphologies could be responsible for these acoustic fingerprints. The differences in swimbladder physiology and morphological structures could justify the change in RFI between species belonging to different genuses. Conversely, the variability in condition factor and habitat preferences could justify differences in RFI between congeneric species (Santic et al., 2011). Moreover, the technological improvements undertaken by acoustic equipment during the last two decades should be underlined, as they might have determined slight differences in TS values compared to past measurements. The current use of split-beam transducer enhances the detection of the precise position of fish in the three-dimensional space being particularly suitable for this kind of studies (Korneliussen et al., 2018). In a broadband view, small variations in swimbladder shape translate into changes in the TS curve. This grant the opportunity to distinguish between species overcoming the influence of size (Kubilius et al., 2020; Khodabandeloo et al., 2021a; Khodabandeloo et al., 2021b). The frequency-dependent backscatter depicted in Figure 8 for a broadside angle reflects the broadband trend reported by Palermino et al. (2022), lending robustness to our analysis.

Conclusions

Backscatter models are not intended to replace empirical measurements, but they are useful to corroborate target strength experiments and to fill the gap in the knowledge of species that hardly fulfill the requirements of monospecific shoals of spread fish for the application of the *in-situ* method. This is the case of *T. mediterraneus* and *S. colias* in the Mediterranean Sea. The implementation of a backscattering model added essential information to the knowledge of the acoustic reflectivity of *T. mediterraneus* and *S. colias* in the Mediterranean Sea. Through swimbladder measurements, we validated the use of b_{20} instead of b during the conversion of acoustic backscatter volume in abundance for the species this study deals with. The results provided underline the importance of the use of multiple frequencies, confirming that the higher the number of frequencies in use, the higher the species discrimination power gained. The results are in agreement with empirical measurements obtained during some *ex-situ* experiments carried out in the Adriatic Sea. Nevertheless, further effort should be made to get *in-situ* measures of TS of ancillary species, since during *ex-situ* experiments the swimming behaviour may not be representative of the natural state of fish.

Supplementary material

Supplementary material is available at the ICESJMS online version of the manuscript and contains Figures S1, S2, S3 and S4 cited along the paper

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Author Contributions

Antonio Palermino: Validation, Conceptualization, Methodology, Formal analysis, Data curation, Writing-Original Draft, Visualization. **Andrea De Felice:** Validation, Conceptualization Investigation, Writing- Review & Editing, Supervision. **Giovanni Canduci:** Investigation, Writing- Review & Editing. **Ilaria Biagiotti:** Investigation, Writing- Review & Editing. **Ilaria Costantini:** Writing- Review & Editing. **Michele Centurelli:** Investigation, Writing- Review & Editing. **Iole Leonori:** Supervision, Project administration, Writing- Review & Editing, Resources.

Conflict of Interest

The authors have no conflicts of interest to declare

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Supplementary materials

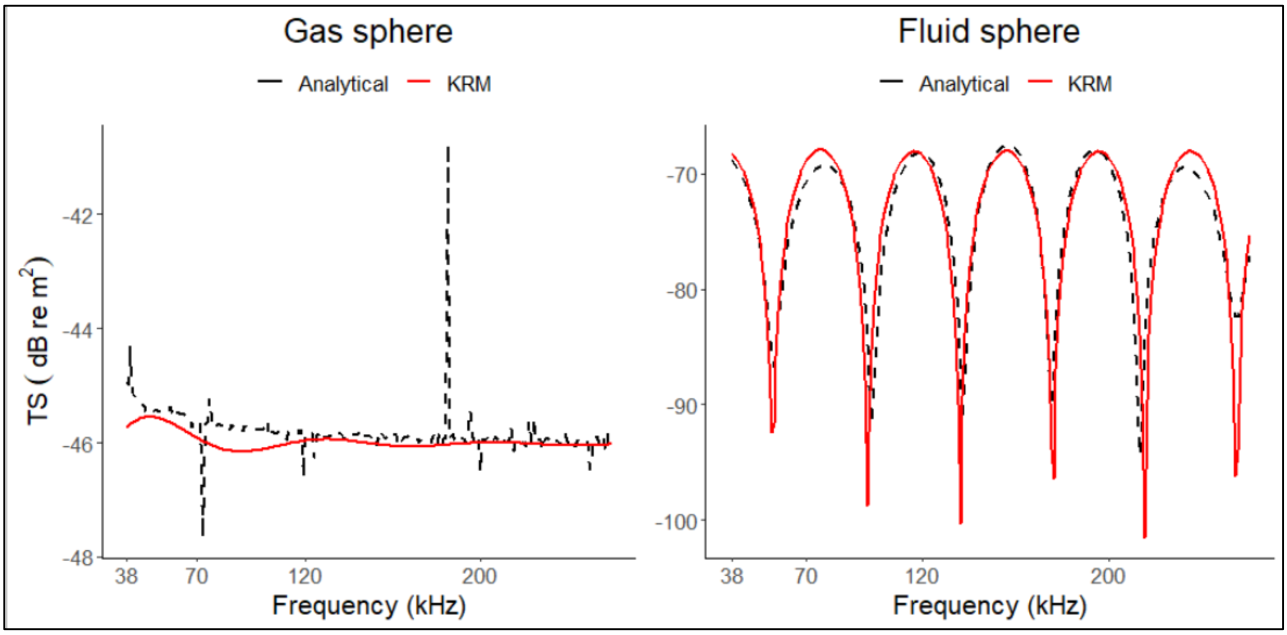

Figure S1. Comparison between analytical model (black) and KRM model(red) on a sphere of 10 mm radius in broadband spectra

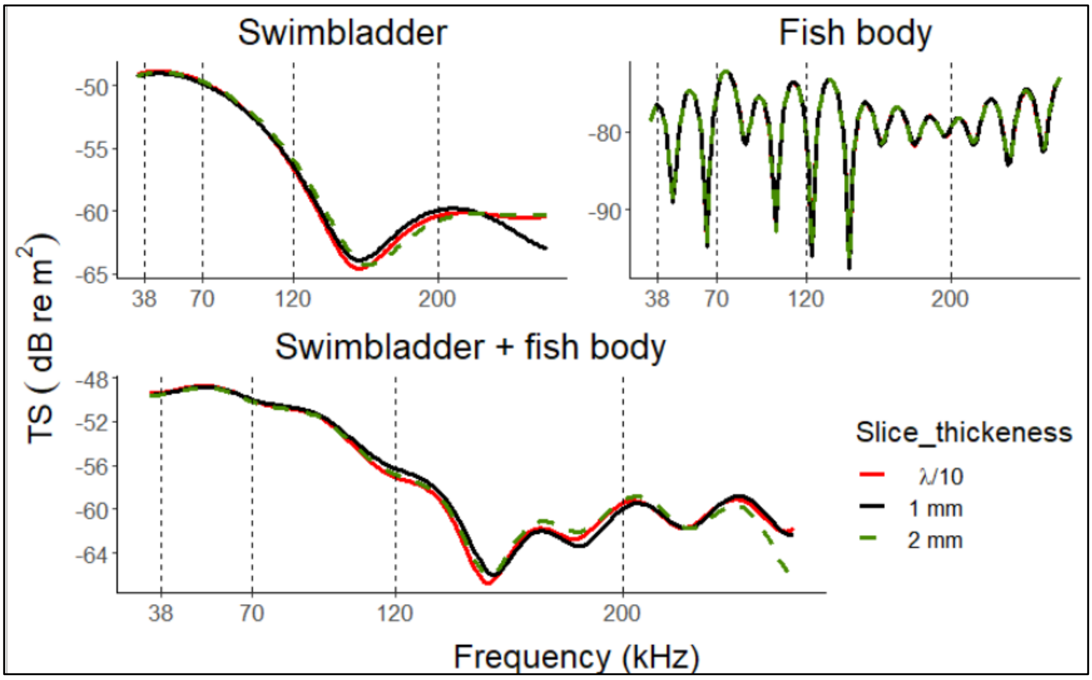

Figure S2. Broadband backscatter comparison between coarse and refine slice thickness on a *S. colias* specimen of 11.5 cm total length considering a tilt angle of + 25°

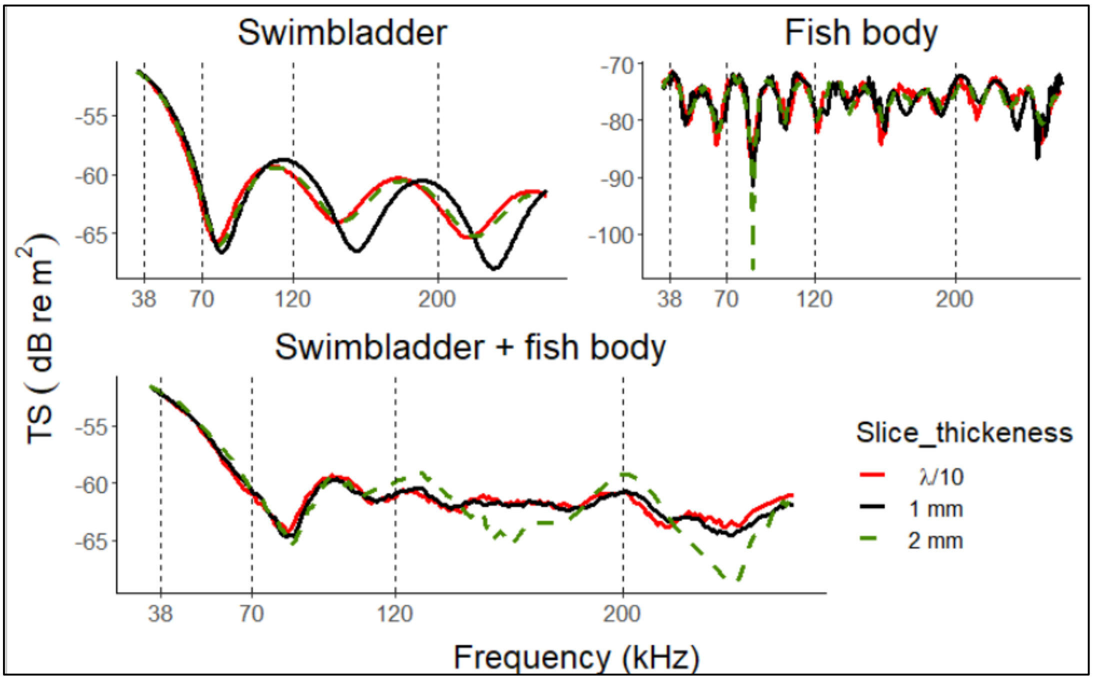

Figure S3. Broadband backscatter comparison between coarse and refine slice thickness on a *S. colias* specimen of 11.5 cm total length considering a tilt angle of + 25°

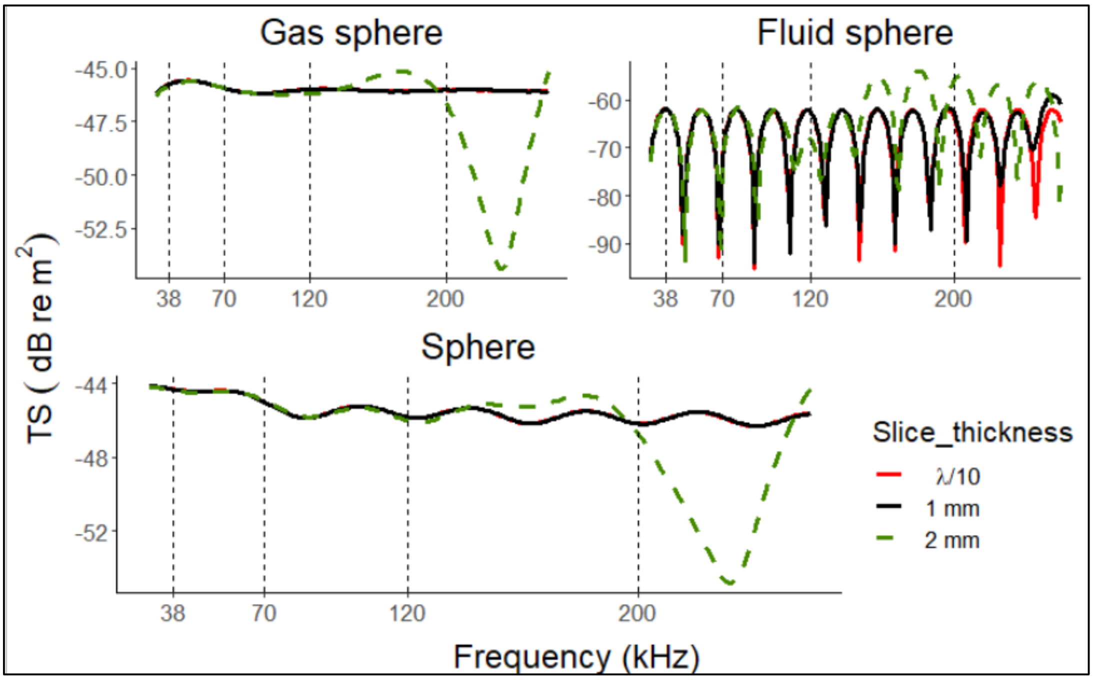

Figure S4. Broadband backscatter comparison between coarse and refine slice thickness on a theoretical gas filled sphere of 10 mm radius (top left), fluid filled sphere of 20 mm radius (top right) and the summation of both (below).

Table S 10. Acoustic parameters used for KRM computations

Model parameters	**Values**
Speed of sound in water (ms^{-1})	1509
Speed of sound in fish body (ms^{-1})	1570
Speed of sound in swimbladder (ms^{-1})	345
Density of the water (kgm^{-3})	1030
Density of fish body (kgm^{-3})	1070
Density of the swimbladder (kgm^{-3})	1.24

Characterization of European sprat acoustic backscatter

This work was presented as a poster at the ICES Fisheries and Plankton Acoustic Symposium that took place in Portland, Maine USA from 27 to 30 March 2023

Poster presented on 28th March 2023

Characterization of European sprat acoustic bakscatter

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Abstract

The identification of targets for both qualitative and quantitative observations is one of the main challenges of fisheries acoustics. The logarithmic measure of the backscattering cross-section, expressed as Target strength (TS), is a key parameter in this process. It includes the scattering proprieties of the species convolved with behaviour depending on swimbladder features, frequency and tilt angle of the fish. These determine the stochastic nature of TS that resulted as one of the main sources of uncertainty in biomass estimates. Nevertheless, only one study has been carried out on European sprat TS in the Mediterranean Sea in 1994. The application of backscattering models can achieve proper prediction of theoretical backscatter from accurate measurement and setting of the main parameters. Here we apply a frequency-domain Finite Element Method on a three-dimensional swimbladder model of sprat specimens collected during the MEDiterranean International Acoustic Survey (MEDIAS) 2021 providing for the first time the relative frequency response and broadband backscatter on the species in this basin. Moreover, an example of the application of broadband to identify swimbladder morphology characteristics is presented. Finally, a new conversion parameter (b_{20}) of -68.44 dB for sprat is proposed herein.

Keywords: Target strength, fisheries acoustics, broadband, modelling, swimbladder, *Sprattus sprattus*

Introduction

Sprat is the third species in terms of landings in GFCM area (FAO 2020). In the pelagic ecosystem, sprat plays an important tropho-dynamic role by exerting both top-down controls on zooplankton and being an abundant prey resource for piscivorous species. But, as a cold favouring species, it is threatened by the increasing sea temperature which can lead in the future to a local extinction of the species in the entire Mediterranean Sea (Schickele et al., 2021) where it inhabits Northern Spain coast, the Gulf of Lion, and the Northern Adriatic Sea (De Felice et al., 2021). In the Mediterranean Sea, it is found rarely in monospecific schools and more commonly in multi-specific schools with anchovy and sardine (Fernandes et al., 2006). The abundance of sprat is annually estimated by means of MEDiterranean International Acoustic Survey (MEDIAS) (Leonori et al., 2021), but despite its importance, only one study has been conducted on the acoustic characteristic of the species in the Mediterranean Sea in 1994 (Azzali et al., 1997), while several studies have been conducted in the Baltic Sea, North and Black Seas, (Didrikas and Hansson, 2004; Fässler and Gorska, 2009; Marinova and Panayotova, 2015; Panayotova et al., 2014). Currently, ground-truthing of acoustic targets is indispensable for the assignment of the single species amount in a certain area. Nevertheless, fisheries acoustic technologies have advanced over time opening the possibility to employ a wide range of frequencies in broadband spectra as a key tool for qualitative analysis (Korneliussen et al., 2018).

Acoustic surveys provide vast amounts of data that can potentially be used for both qualitative and quantitative observations of marine organisms over large spatial and temporal scales. Yet, one of the main challenges regarding acoustic methods is to interpret the collected acoustic data to identify the targets and provide biomass estimates (Simmonds and MacLennan 2005). The logarithmic measure of the backscattering cross-section of single targets expressed as Target strength (TS) is a key parameter and includes the scattering proprieties of the species convolved with behaviour. It depends on the swimbladder features, frequency and tilt angle of the fish (Foote, 1987; Ona, 1990; Simmonds & MacLennan, 2005). These determine the stochastic nature of target strength that resulted as one of the main sources of uncertainty in biomass estimates (O'Driscoll, 2004).

The use of backscattering models allows an accurate estimate of theoretical backscatter from accurate measurement and setting of organism anatomy, material proprieties, swimbladder morphology, tilt angles, and frequencies (Jech et al., 2015) for both qualitative and quantitative purposes. Target strength modelling of single individuals can enhance the overall abundance estimates reducing the uncertainties in acoustic data collection. Moreover, it helps the detection of species and size of the targets (Kubilius et al., 2020). A rich variety of theoretical models has been developed since 1950 (Anderson, 1950; Jech et al., 2015). They are focused on the prediction of the backscattering of bubbles and swimbladder, which alone is responsible for more than 90% of the total backscatter of a fish because of the strong impedance contrast between gas and water (Ona 1990). The swimbladder of fishes assumes complex shapes and positions depending on several factors: e.g. in physostomous fish species the volume of the swimbladder decreases with increasing water pressure when the fish descend to deeper depth (Fässler and Gorska, 2009; Gorska and Ona, 2003). It is also affected by the physiological state of the fish: several studies have demonstrated the influence of hepato-somatic index, gonad-somatic index and stomach fullness on swimbladder circularity, area and relative size (Ganias et al., 2015; Ok and Gücü, 2019). Fish orientation heavily affects the backscatter too (Membiela and dell'Erba, 2018; Reeder et al., 2004). For these reasons often, in the backscattering models, the swimbladder is conventionally modelled as a gaseous shape. The simplest way to model a swimbladder is by a regular shape, for instance, a sphere, a finite cylinder or a prolate-spheroid / ellipsoid. More complex models based on the approximation of the swimbladder to a prolate spheroid are pursued by sectioning into a compound of finite cylinders solved by Kirchhoff Approximation model (KA), deformed cylinder model (DCM) and Kirchhoff Ray approximation Mode (KRM) (Furusawa, 1988; Jech et al., 1995). However, they require an accurate representation of a real swimbladder shape which can be obtained by the dissection of the species (Ayoubi et al., 2016) or by means of X-Ray scan (Hazen and Horne, 2004; Horne et al., 2000). Even more detailed images can be obtained through Computer Tomography scans (CT) and Magnetic resonance imaging (MRI). These techniques are now in use for the implementation of complex numerical models which solve wave equation on a finite surface elements such as the Boundary Element Method (BEM), which use the integral of the Helmholtz equation, the Fourier matching model (FMM), which computes the transformed Helmholtz equation and the Finite Element Method (FEM) which involve the use of inhomogeneous Helmholtz equation (Francis and Foote, 2003; O'Driscoll et al., 2011). Numerical models are computationally demanding and require detailed measurements of swimbladder and fish body characteristics. They account for the finer morphometric variation on the surface resulting in more precise and accurate measurements of acoustic reflectivity which can help to draw broadband frequency patterns, which in turn can afford information on the target proprieties and swimbladder inequality (Khodabandeloo et al., 2021). Frequency response over broad frequency range can potentially lead to distinguishing between acoustically similar species and size groups of a single species (Benoit-Bird and Waluk, 2020; Kubilius et al., 2020). In comparison, the discrete narrowband frequency response can help species discrimination especially where split-beam transducer capable in releasing frequency modulated broadband signal are not available (Fernandes et al., 2006).

In this study, we applied a Finite Element Method (FEM) on complex sprat swimbladder morphologies obtained through CT scans. FEM model is computationally expensive but provides highly accurate estimates of acoustic backscatter as a function of frequency and tilt angle allowing the achievement of a wide knowledge of sprat acoustic backscatter and providing fundamental tools for the identification and assessment of the species in post-processing data analysis and during acoustic surveys. Moreover, we computed the conversion parameter b_{20} investigating the difference with empirical results.

Materials and Methods

Data collection and Images processing

A total of 45 specimens of sprat (total length size range 7.2-11.9 cm) were collected during MEDIAS 2021 performed in the summer month by the CNR-IRBIM of Ancona in the western side of the Adriatic Sea on board of R/V G. Dallaporta. The fishing operations were realized with a mid-water trawl characterized by an 18 mm cod-end mesh size equipped with SIMRAD's FX80 trawl sonar to monitor the behaviour of the net. The net was cast at a seabed maximum depth of 36 m at around 4 knots for ~30 min. Since it was problematic to maintain such small fish alive after capture, once on board, active and healthy fishes were immediately frozen separately in small boxes for successive analysis.

Successively all fish were defrozen and scanned with a Philip Brillance 16 P CT at veterinary facilities as shown in Figure 1. A CT scan sends radiation through the body. However, unlike a traditional bi-planar X-ray study, it offers a much higher level of detail, creating computerized, 360° views of the fish body's structures and of the swimbladder in a faster and more detailed way. Several trials with other fishes of the same dimensions collected during the survey were carried out before image acquisition, in order to adjust instrument settings listed in Table 1 to guarantee the maximum resolution.

Table 11. Philip Brillance 16 P Computer Tomography settings

CT parameters	**Value**
Slice thickness (mm)	1.25
Interslice spacing (mm)	0.65
KVp	120
mA	105
Diameter (mm)	430
Exposure time (ms)	350
Filter type	B


Figure 7. TAC Philip Brillance 16P used for sprat scan at JESIVET facilities

In each session, 15 specimens were scanned along the transversal axis obtaining a series of cross-sectional slices useful to make a 3D model of the swimbladder through image processing. After each session, the total length (TL) and the weight of the fish were collected. DICOM images were imported in 3D slicer free software. Swimbladder shapes were extracted by the auto-segmentation function in the Segment editor by adjusting the threshold to draw the boundaries between bladders and fish flesh. Then the 3D model of the swimbladder was displayed in order to check for any possible errors and eventually adjust the boundaries. Finally, a three-dimensional stereolithography (STL) file was created and imported into Gmsh software. Here the swimbladders were centred in the three-dimensional axis and any sharp edges were smoothed removing the deformities.

Backscattering modelling and analysis

The STL files from Gmsh were imported in COMSOL Multiphysics V.6 for the application of numerical Finite Element Method model. In the geometry section a water sphere was made with a radius of: $0.014 + 0.25\ [m] \times sound\ speed(cw)[\frac{m}{s}]/frequency\ [Hz]$ in order to get a water domain of at least a quarter of the wavelength at each frequency (Khodabandeloo et al., 2021) surrounded by a Perfectly Match Layer (PML) with a thickness of one eight of the wavelength. Gas domain (swimbladder geometry), water domain and PML were meshed into at least 10 small elements per minimum wavelength (200 kHz). The PML is the absorbing domain which acts as a non-reflecting boundary around the computational domain (water) to attenuate the waves hitting the boundary, where the incident pressure field and the scattered field are computed. More details can be found in "Acoustic scattering of an ellipsoid", COMSOL MULTIPHYSICS 6 Acoustic Module Library. The

Kirchhoff - Helmholtz integral equation was solved in the frequency domain for each small element computing the far field backscattered pressure:

$$P_{ext}(R) = -\frac{1}{4\pi}\int_{S(r)} e^{\frac{ik(r\,R)}{|R|}}\left(\nabla p(r) - ikp(r)\frac{R}{|R|}\right)(-n)\,dS$$

Where the incident pressure field was set at 1 Pa. All the parameters applied are listed in Table S1, while the elements of FEM model are described in Figure 2.

The backscattered TS was computed for each swimbladder 3-D model through the formula $TS = 10\,log\,10\, + (\sigma_{bs})$ [dB re 1 m^2], where (σ_{bs}) [m^2] is the backscattering cross-section of a single target, as a function of frequency (range: 38 - 200 kHz), tilt angle (range ± 30°) and total length of the fish (TL). Length normalized TS across the range of frequency was also computed following the equation: $TS = 10\,log\,10\, + (\sigma\_bs/TL^2)$. The resulting dataset was employed to assess any difference among specimens and swimbladder characteristics. Then, through the application of the following formula: r_i(f) = σ_i(f)/ σ_i(38) (Pedersen et al., 2004) we computed the relative frequency response. Successively, a standard TS-TL model: $TS = m\,log\,L + b$ and with the slope forced to 20: $TS = 20\,log\,L + b_{20}$were computed in order to obtain the conversion parameter (b_{20}) used to convert the backscattering volume obtained during the acoustic survey into abundance estimates (Foote 1987).

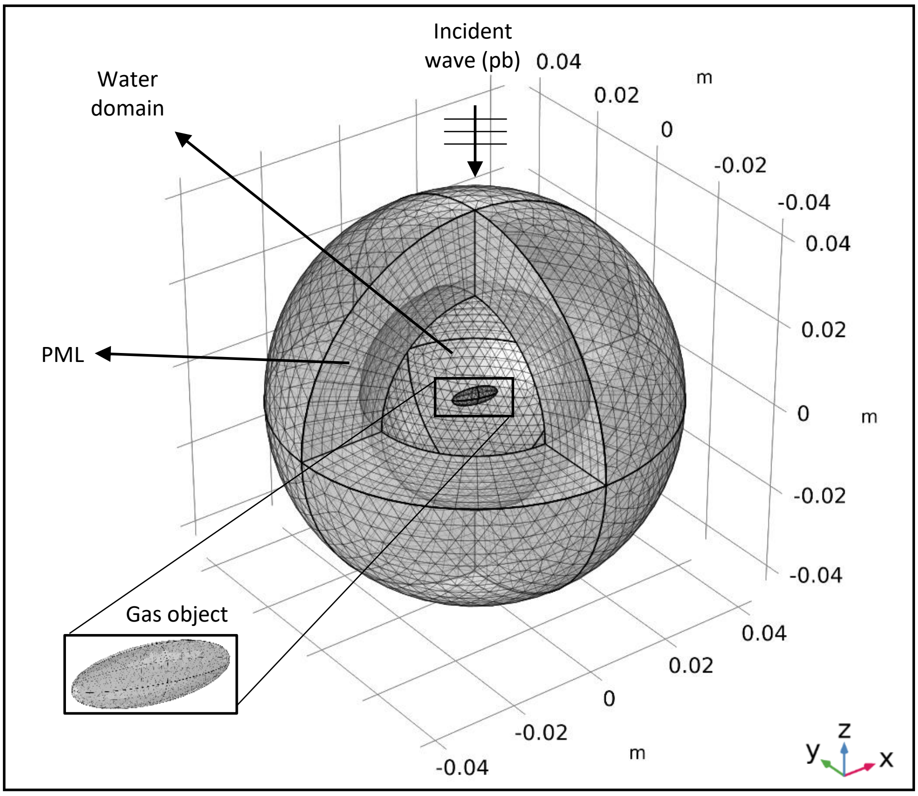

Figure 8. FEM model system showing the geometric scales (x,y,z), the incident background pressure field (pb), an ellipsoid gas object (dimension: h= 2mm, l=6mm, w=1.3 mm) simulating a swimbladder in a water domain surrounded by the Perfectly Matched Layer (PML).

Results

A total of 45 sprats were scanned, but only 13 fish presented intact swimbladders that were considered suitable for the TS analysis since the other specimens showed rupture of the outline and partially or completely deflated swimbladders possibly caused by the fishing operation, handling or preparation. Nevertheless, the remaining specimens well represent the size range of the species typically found in the Adriatic Sea in June and July. Sprat is characterized by a laterally compressed ellipsoid swimbladder which is composed of two linked chambers that the fish can inflate individually (two examples in Figure 3).

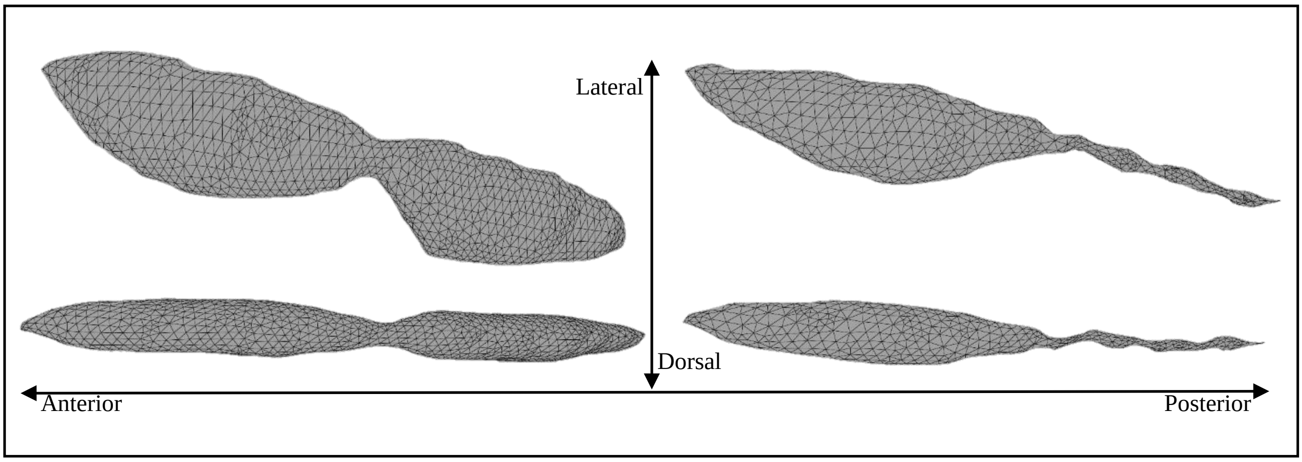

Figure 3. Examples of sprat swimbladder shape and morphology obtained through computer tomography image measurements in lateral (bottom) and dorsal aspect (top). On the left a swimbladder with two inflated chambers, on the right a swimbladder with only one inflated chamber

Four fish presented two inflated chambers while nine fish had a single inflated one. Acoustic backscatter resulted stronger at 38 kHz as expected for a fish with swimbladder, while we registered a drop of TS between 38 and 120 kHz both in the broadband spectrum and relative frequency response, followed by a stable trend up to 200 kHz (Figure 4 and 6). Successively, we investigated the possible difference in normalized TS broadband spectra between fish depicting dual inflated swimbladder chambers and fish characterized by a single inflated chamber through a simple t-student test finding a significant difference ($p \leq 0.001$; $r^2 \geq 0.95$). This distinction is underlined by the trend of normalized TS broadband spectra shown in Figure 5, where the backscatter from two inflated chambers produced higher TS between 38 and 70 kHz and characteristic null at 120 kHz. The slight variation of TS along the tilt angle interval and the shape of the curve shown in Figure 7 underline a non-geometric scattering at 38 kHz. At this frequency TS ranged from -54.18 to -47.27 dB re 1 m^2 while, TL of the analysed specimens varied from 7.5 to 11.6 cm. TS increase linearly with TL as expected and the TS vs TL regressions depicted resulted in a value of b of -65.20 dB and a b_{20} of -68. 45 dB (Figure 8).

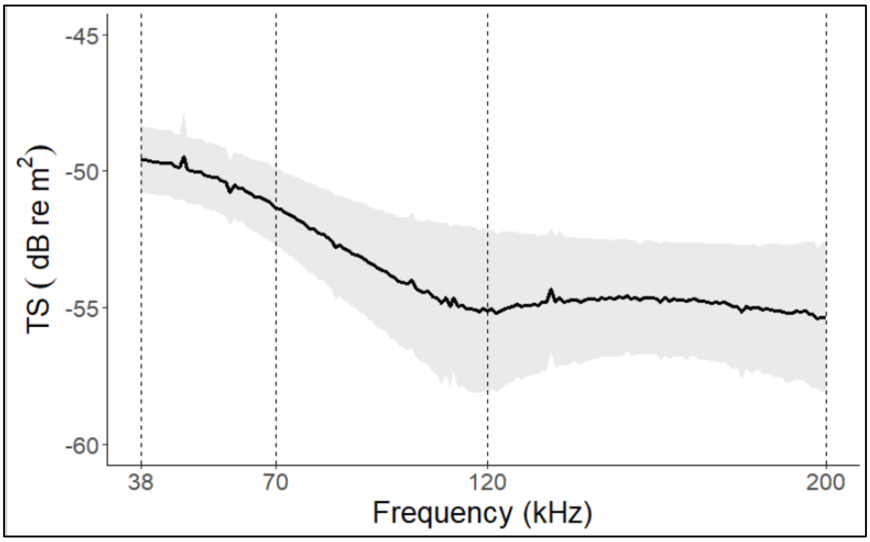

Figure 4. Mean TS broadband spectrum (continuous line) and Confidence intervals (dashed area). The most frequently nominal operating frequencies used in fisheries acoustics are shown with vertical dashed lines

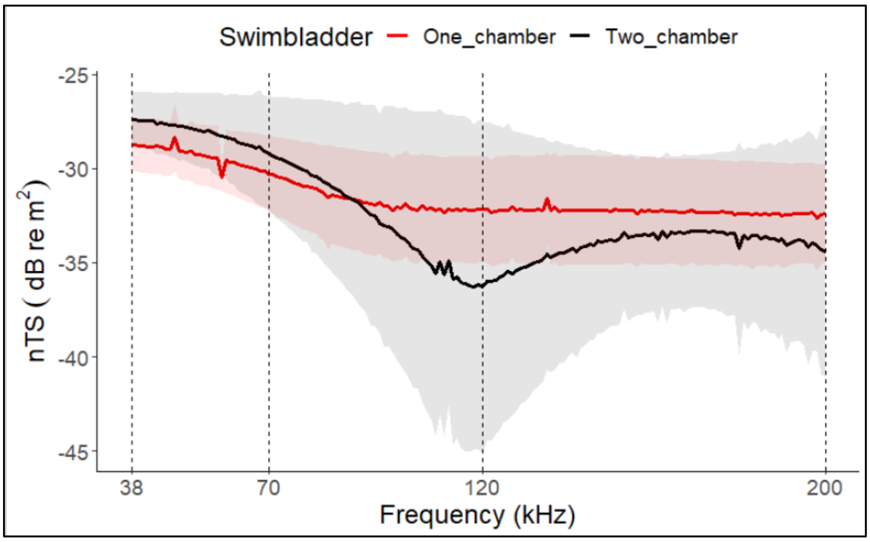

Figure 5. Mean TS broadband spectra of sprat with one inflated chamber (red line) and two inflated chambers (black line) and confidence intervals (dashed areas). The most frequently nominal operating frequencies used in fisheries acoustics are shown with vertical dashed lines

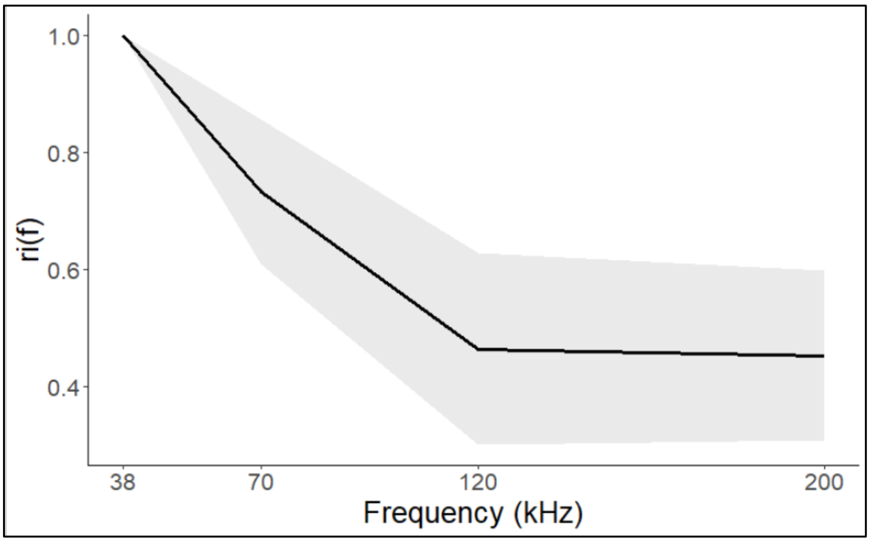

Figure 6. Mean relative frequencies responses of sprat (continuous line) and confidence intervals (dashed area).

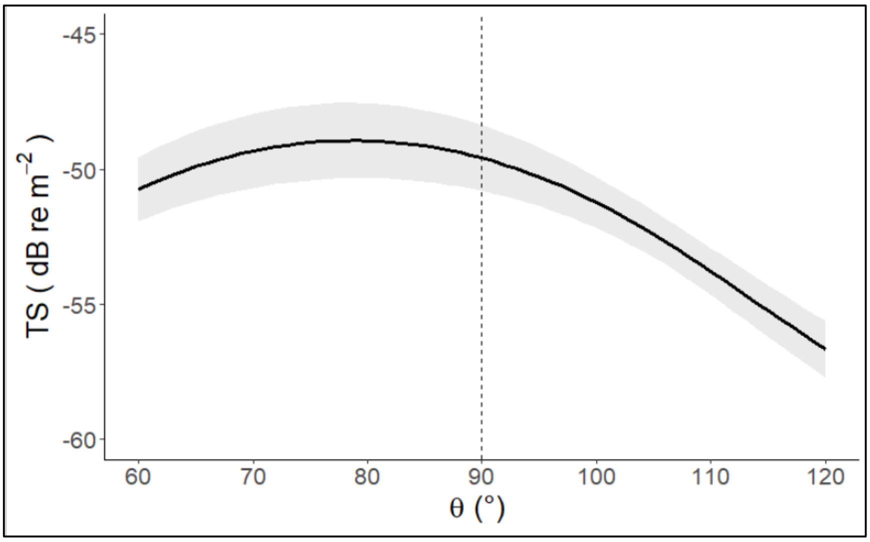

Figure 7. Mean TS vs tilt angle at 38 kHz (continuous line) and confidence interval (dashed lines). 90° mean a broadside angle perpendicular to incident wave

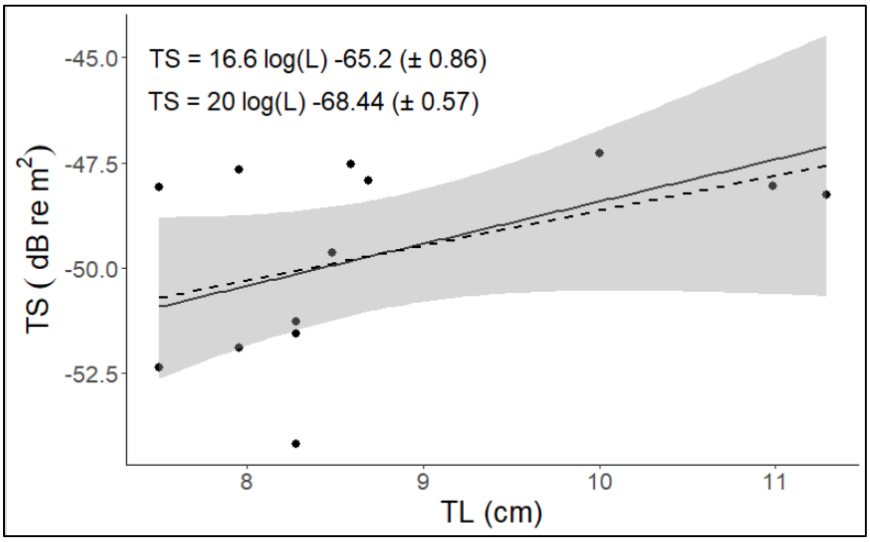

Figure 8. Target strength vs total length relationship at 38 kHz. The standard model regression is shown with a continuous line, while the dashed line points out the model with the slope forced to 20.

Discussion

Empirical techniques are considered the most reliable measurements of fish acoustic backscatter, especially, the *in situ* method because, unlike the *ex situ* approach, it collects echoes as the fish swim in the natural environment (MacLennan and Menz, 1996; O'Driscoll et al., 2017; 2018; Salvetat et al., 2020). Its main problem is the availability of monospecific hauls, which are rare in the multi-specific pelagic stock that characterizes the Mediterranean Sea (Palermino et al., 2023). Conversely, *ex situ* experiments foresee rather the use of live fish (Palermino et al., 2021) becoming complicated to apply on very small clupeids species such as sprat, which in turn are suitable for the application of numerical models due to the small dimensions. In such a situation the application of a backscatter model could be a good trade-off between the possibility to handle the main parameters which affect the backscatter and the reliability of the results. Among models, numerical ones such as BEM and FEM are advised as the best (Jech et al., 2015). They require long time computations (several hours or days for a single fish), as a consequence, approximation models are usually adopted. However, several authors demonstrated their inability in gathering finer shell variations which can affect the overall backscatter (Jech et al., 2015; Macaulay et al., 2013) unlike numerical models (Boswell et al., 2020). In this study, we demonstrated the capability of FEM model along with the exploitation of broad frequency in detecting swimbladder shape variations as shown in Figure 5. A possible larger cross-section surface and volume of dual-inflated chamber sprats could be responsible for the higher TS between 38 and 70 kHz. Similarly, the singular swimbladder morphology characterized by a shrinkage in correspondence to the connection or the absence of the latter between chambers, that we observed in several specimens, could justify the null detected at ~ 120 kHz.

Since the availability of transducers and prototypes capable to send frequency-modulated pulse, several *in situ* studies have been conducted for pelagic fish broadband backscatter measurements (Bassett et al., 2018; Benoit-Bird and Waluk, 2020; Gugele et al., 2021; Hasegawa et al., 2021; Stanton et al., 2010; Antona, 2016). Conversely, few authors employed backscattered models on real swimbladder shapes for this purpose, despite the benefits and relative easiness (Boswell et al., 2020; Palermino et al., 2022). Therefore, the broadband backscatter presented in Figure 4 is one of the first broadband studies on clupeid species. Previously, only Antona in a Master thesis investigated TS broadband of herring and Sprat in the North Sea through the *in situ* method and backscatter modeling. He found very similar TS spectra between species both characterized by a slight decrease between 38 and 70 kHz followed by a null at around 90 kHz. The overall spectrum shown in Figure 4 in this study disagrees with the results of Antona (2016). We detected a null shift by 30 kHz and in general, our TS values are lower compared to the one registered in the North Sea (Antona, 2016). The size of the fish could have determined these differences since, the aforementioned work analysed fish with an average size of 12.5 cm, well higher than the maximum TL of 11.6 cm of our specimens. Nevertheless, TS may vary among areas as reported for herring (Ona, 1990), among other species (Ganias et al., 2015; Ok and Gücü, 2019) since the physical environment could influence fish physiology and morphology (Scoles et al., 1998).

Multi-frequency characterization of scattering targets is more widespread unlike the broadband studies since they were born earlier (Korneliussen and Ona, 2002). Several studies have been conducted also on clupeids species (Fernandes et al., 2006; De Felice et al., 2015) but the relative frequency response of sprat has never been investigated. Our findings, albeit based on a model, could be useful to distinguish between the three main clupeid species which inhabit the Mediterranean Sea: *Sardina pilchardus*, *Engrauilis encrasicolus* and *Sprattus sprattus*. Sardine presents a high ratio between 38 and 120 kHz and a drop up to 0.4 ri(f) at 200 kHz, while anchovy is characterized by a sharp decrease of ratio up to 120 kHz followed by a stable trend (Fernandes et al., 2006; De Felice et al., 2015). Sprat relative frequency response trend resulted similar to anchovy but the ri(f) values at 120 and 200 kHz keep higher compared to the latter. The three clupeids show the typical trend of pelagic fish with swimbladder, described by a more or less sharp decrease of TS from lower to higher frequencies making them perfect for the assessment at 38 kHz. Generally, the shorter the ratio of the acoustic wavelength to the target length, the greater the influence of directivity (Love 1971). The computed TS for different tilt angles at 38 kHz in Figure 7 for sprat did not show any peaks, illustrating the low directivity of sprat at this frequency. It can be justified by the small dimensions of the target (swimbladder), which likely could determine more fluctuation at higher frequencies.

The lack of monospecific hauls and *ex situ* experiments carried out on European sprat in the Mediterranean Sea for biomass assessment purposes underlines the importance of using backscattering model to fill this gap (Azzali, 1997; Palermino et al., 2023). In this study, we found a new conversion factor b_{20} for sprat of -68.44 dB re 1 m^2. The regressions depicted in Figure 8 highlight a spread of TS values at the small sizes which could be attributable to specimens collected in different hauls. Notably, in the size range 7.5 - 9 cm, the five values over the lines belong to specimens collected during the same haul that was performed in shallow water with a depth of 22 m while the five values under the lines belong to another haul performed in deeper waters with a bathymetry of 36 m. Several authors have demonstrated how the increase in pressure with depth could influence the backscatter in physostomous species and in particular on clupeids (Madirolas et al., 2017; Ona, 1990). The depth dependence of TS could justify the high variability found in the present work for small-size specimens at 38 kHz. The comparison between the new b_{20} values found herein with the one now in use in the Adriatic Sea and Mediterranean Sea yields a difference of ~ 3.2 dB (De Felice et al., 2021). Nevertheless, a value of -68.44 dB re 1 m^2 fell into the range proposed by Palermino et al (2023) in a recent study conducted on two rare monospecific sprat hauls. Sometimes models application end with significant differences with *in situ* and *ex situ* measurements of the same species arising doubts about one of the two methods (Sawada, 1999; Hazen and Horne, 2004; Peña and Foote, 2008). Conversely in our case, the agreement between the model and empirical experiment gives robustness to both analyses providing a b_{20} value particularly valuable for biomass assessment purposes.

Here we provided for the first time the relative frequency response and broadband backscatter of Sprat in the Mediterranean Sea that can be implemented during post-processing acoustic analysis as a diagnostic tool for the correct identification of the species. Moreover, we obtained a new conversion parameter which is in agreement with empirical estimates and might be used as the new reference value for the Mediterranean Sea despite incorporating it into quantitative assessment effort still require a wider availability of monospecific hauls

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Supplementary materials

Table S1. FEM model settings

Model parameters	Values
Speed of sound in water (cw) (ms^{-1})	1509
Speed of sound in fish body (ms^{-1})	1570
Speed of sound in swimbladder (ms^{-1})	345
Density of the water (kgm^{-3})	1030
Density of the swimbladder (kgm^{-3})	1.24
Driving frequency (f0) (kHz)	38
PML layer thickness (m)	0.5*cw/f0
Exterior field computation (m)	1
θ(deg)	90
Φ (deg)	90
x axis directivity	Cos(θ)
y axis directivity	Sin(θ)*cos(Φ)
z axis directivity	Sin(θ)*sin(Φ)

5. Discussion

Acoustic surveys are widely considered one of the best methods for the assessment of the abundance and geographical distribution of pelagic species worldwide (Simmonds and MacLennan, 2005). However, one of the key factors for converting acoustic energy into number of fish, the target strength (TS), is seldom investigated in the Mediterranean Sea. More experiments have been carried out in the Atlantic and Pacific Oceans, although even here there is a scarcity of studies conducted on non-target species (ICES, 2022). The few studies available in the Mediterranean Sea, concern the investigation of the TS of two target species, *E. encrasicolus* and *S. pilchardus* which are overfished in this basin (Anonymous, 2012; SAC, 2022). Conversely, there are no TS studies on the bulk of non-target pelagic fish species in this basin. The lack of data would not allow a reliable assessment of these species which is fundamental for a correct management of the pelagic ecosystem in the framework of increase importance of the ecosystem-based management strategies (Effrosynidis et al. 2020, Angelini et al., 2021). Moreover, we are witnessing a shift in the commercial fish species due to the reduction of the target species' abundance (FAO 2022). Non-target species such as, *T. mediterraneus*, *S. colias* and *S. sprattus* which nowadays compose a small percentage of total fish landings in the Mediterranean Sea, may become future exploitation resources, increasing their importance, as is happening in the Atlantic Ocean (ICES 2021).

This work provides the first TS investigation of *T. mediterraneus* and *S. colias* and new measurements for *S. sprattus*. These TS values can be used in the whole Mediterranean Sea as well as in the Atlantic Ocean, due to the scarcity of studies on of TS on non-target species worldwide, to perform absolute species-specific biomass estimations (Simmonds and MacLennan, 2005). The available techniques for the TS measurements (*in situ*, *ex situ* experiments and backscattering models) have been adopted and implemented based on three sources of data: multi-frequency acoustic data of monospecific hauls, multi-frequency acoustic data of single specimens, and, morphometric and morphological characteristic of swimbladder and fish body. The experiments were designed to reduce as much as possible some of the main sources of bias in *ex situ* and backscattering models TS measurements about the: i) unnatural behaviour, ii) acoustic interference from fishing gear (e.g. the use of hook) iii) stress of animals (Henderson and Horne, 2007; O'Driscoll et al., 2018). Regarding the bias of interference of fishing gear, the attempt to use a small piece of rope instead of a hook during *ex situ* experiments brought worse results on the acoustic gain of echoes coming from the tethered fish apparatus compared to other *ex situ* experiments that involved hooks (Thomas et al., 2002; Henderson and Horne, 2007; Boswell and Wilson, 2008). On the other end, regarding the unnatural behavior and stress of animals, all fish displayed good vitality and the results clearly show the stochastic displacements of the fish demonstrated by the high range of TS values, due to its variability largely depending on fish tilt angle and roll (McClatchie et al., 1996; Horne et al., 2000). The high amount of samples with intact swimbladder, 77 on a total of 82 specimens, achieved after the X-Ray scans performed for the application of backscattering models, proves the validity of the experimental design both on physoclists (*T. mediterraneus*) and physostomus (*S. colias*) species. The protocol was developed prior to the experiments based on the best literature on this topic (Clay and Horne, 1994; Dornan et al., 2019; Furusawa, 1988; Membiela and Dell'Erba, 2018; Yasuma et al., 2010). The acclimation period to surface pressure allowed to avoid variability in the swimbladder compression due to depth pressure, while the use of anesthetic helped to avoid any contact of the fish with air avoiding any possible release of gas prior to freezing.

During the *ex situ* experiment and backscattering model, the tilt angle of fish which strongly affects TS measurements (Ona, 1990) was taken into account. Few studies have been carried out on this topic, and they are usually focused on clupeids species (Aoki and Inagaki, 1988; Huse and Ona, 1996). Nevertheless, some authors investigated the tilt angle of species belonging to the genus *Trachurus*. Peña and Foote (2008), using target tracking data during an in situ experiment, have described a mean angle between +1° and -6° with a standard deviation of 8° to 18° for *Trachurus symmetricus murphyi*, while Kamawauchi et al. (2019) during a tank experiment on *Trachurus japonicus* estimated a negative mean tilt angle of 0.53° ± 12.4. They found a TS range of ~ 30 dB for all measured fish, which is close to what I found for *T. mediterraneus* and *S. colias* during the *ex situ* experiment. Consequently, the results indicated that during the ex situ experiment performed in this work, a wide range of tilt angles and fish movements may have been acquired, which however could not be measured. Therefore, a quite normal swimming behaviour of the fish during the *ex situ* experiment was assumed, although some bias persisted related to the behavior of the fish during the TS measurements. The application of the KRM model allows the adjustment of tilt angles in a range between 65° and 115° (Macaulay et al., 2013). The following tilt angles distributions were chosen to represent near-normal (90°±5°; 90°±10°; 90°±20°; 88°±13°) and abnormal (101°±12°) swimming behavior where 90° is dorsal incidence. An orientation of 88° with a standard deviation of 13° has been considered as a normal fish orientation under natural conditions in this study, mainly based on previously studied carried out on *Trachurus* species and other fish with swimbladder (Fassler et al., 2013; Kawauchi et al., 2019; Madirolas et al., 2017; Peña and Foote, 2008). The other tilt angle intervals are intended to represent the increasing swimming orientation direction of fish. A tilt angle of 90° with a standard deviation of 10 and 20 was set as suggested by Membiela and Dell'Erba (2018) for the fish spread at different depths. Then a mean tilt angle of 101° with a standard deviation of 12°

was set adding the mean and standard deviation of the swimbladder related to the fish body angle computed during the swimbladder measurements as abnormal swimming behavior.

Due to the aforementioned issues, *ex situ* experiments and backscattering models are considered less valuable compared to the *in situ* experiments. Nevertheless, also the *in situ* measurements suffer from biases in TS data analysis concerning the acceptance of multiple targets as single target detection and the cross-comparison between TS distribution and Length Frequency Distribution (LFD) (Ona, 1990; Sawada, 1993; Demer et al., 1999; Hannachi et al., 2004; Dunford et al., 2015; Madirolas et al., 2017). To deal with the issue of single target detection, acoustic data from monospecific hauls were subjected to a filtering process employed for the selection of single targets ensuring the nearly exclusive retention of the echoes from single specimens. The application of a high-density filter algorithm over the single target detection on the two night hauls analysed yielded a reduction of ~ 11.5% of single targets. A multi-frequency method which exploits the tool of Echoview software v 10.0 and can be easily applied to every dataset was tested in addition or alternative to the high-density filter algorithm based on the principle of spatial matching criteria (Demer et al., 1999). The implementation of this filter was responsible for the rejection of ~ 69%. Nevertheless, the remaining single targets were suitable for the analysis. The multi-frequency method could suffer from the exclusion of the angular position and high distance threshold (0.7 m) between targets that may result in an overestimation or underestimation of suitable single targets retained and can justify the rejection of weaker targets. This second stage of the procedure provided the largest contribution to the rejection of the weaker targets. It affected the lower tail of the TS distribution but did not compromise the frequency around the TS modes, necessary for the theoretical computation of TS. Regarding the possible bias due to the cross-comparison between TS and LFD, a known method and a novel approach was tested: the theoretical method proposed by MacLennan and Menz (1996), based on the Rayleigh scattering theory and a new mathematical method based on the formula developed by Kasatkina (2009) according to the principle of exponential distribution of TS (Simmonds and MacLennan, 2005). In some circumstances, the echo amplitude of fish does not follow the theory (Stanton et al., 2004), and turns into a geometric scattering or a Rayleigh scattering due to frequency, incidence angle, and target size. This is attributable mainly to target orientation, which can vary significantly between species and within the same species (Korneliussen et al., 2018; Palermino et al., 2021). Conversely, in free swimming fish, the echo amplitude is mostly incoherent and the Probability Density Function (PDF) conforms to the Rayleigh PDF (Korneliussen et al., 2018). Sprat has a small and laterally compressed swimbladder, as shown in chapter 4, which generally, at 38 kHz, does not show backscatter directivity characterized by geometric spreading. Therefore, in my case, I considered the Rayleigh PDF as a good theoretical approximation to the actual trend of TS distribution, although some limitations persist (Demer et al., 1999). The matching criteria proposed by Kasatkina are useful to link a single TS to the corresponding TL from the whole dataset. Nevertheless, the results give the same importance to all TS-TL combinations. Conversely, due to the aforementioned discussion on the TS probability density function, more importance should be given to the modal numbers. Accordingly, the implementation of a weighted bootstrap on TS distribution and LFD and the corresponding TS measurements proposed herein showed that the variability of the magnitude could be taken into account, resulting in different b_{20} and TS values. Accordingly, the subsequent values are in agreement with the FEM model TS estimates of sprat backscatter giving robustness to the new method proposed herein unlike the use of the method published by MacLennan and Menz in 1996. The latter resulted less suitable in our case, thus for poor data state *in situ* approach it is preferably the application of the method proposed herein after the implementation of density filter algorithm rather than the multi-frequency threshold for single target detection.

Once the TS have been acquired taking into consideration the aforementioned key factors and its intrinsic variability, it must be scaled with the fish length through TS-TL equations 1.2.2 and 1.2.3. The b_{20} in equation 1.2.3 proposed by Foote in 1979 for clupeids and free-swimming gadoids followed the thesis proposed by Love (1971). He stated that “for an individual fish, the dorsal-aspect TS increase proportionally to the square of fish length”. Since the 90s, summarizing the relationship with a slope m = 20, become a standard when MacLennan and Simmonds (1992) suggested the use of $20\log_{10}L$ dependence of TS in the absence of data that demonstrate the contrary. Nevertheless, the choice of the correct slope for species-specific TS-TL function should not be done a-priori but should be based on morphological analysis, choosing the most appropriate value (McClatchie et al., 2003). Unfortunately, this information is rare, but through the use of X-Ray and TAC images, the problem was overcome by obtaining accurate data on swimbladder morphology of the three species covered in this study. Both the standard equation and the equation with the slope forced to 20 were computed. All the results on b obtained with the *ex situ* experiments were lower than 15.1. Moreover, in some cases, the same TS value for fish characterized by a 10 cm size difference was found. This may have been due to an incongruent growth of the swim-bladder compared to the square of fish TL, abnormal fish behaviour, high variability in tilt angle or the narrow size range considered (*T. mediterraneus*, 15.4; 31.6 - 40.6 cm, *S. colias*, 16.1 - 29.5 cm). However, as mentioned above, the cross-sectional area is also a fundamental parameter for the decision of the most correct TS-TL function. X-Ray images revealed both for Mediterranean horse mackerel and chub mackerel a proportional growing of the mean cross-sectional area with the total length. Notably, the growth was approximately isometric, therefore, assuming a slope value close to 20 could be considered a good approximation rejecting the assumption advanced as a result of the *ex situ* experiment. Also for sprat, the TS is expected to increase with increasing TL. In the *in situ* work described here, the mean TS of a sprat individual measuring 11.5 cm has overcome the one of an individual measuring 10.5 cm. Nevertheless, the few data and size range available could not allow us to prefer the use of *b* or b_{20} for this species. The b_{20} was adopted mainly for comparison purposes with values reported by other authors. Yet, the results collected from TAC images and the application of the FEM model revealed a proportional growing of the swimbladder of sprat with the increase of TL justifying the use of b_{20} instead of *b*.

X-Ray and TAC images are generally employed for the development and use of backscattering models (Boswell et al., 2020; Gonzalez et al., 2020). Nevertheless, new TS-TL relationships derived from models are seldom employed for acoustic abundance estimates (Fassler et al., 2013). Most frequently, models are exploited for comparison purposes with *ex situ* or *in situ* experiments (Hazen and Horne, 2004; Horne et al., 2000; Madirolas et al., 2017; Sobradillo et al., 2019). Comparing empirical observations and theoretical results helps to increase the amount of data when a limited number of samples are available, gaining species-specific TS variability and backscattering patterns (Fassler et al., 2013; Gonzalez et al., 2020) through the handling of important parameters such as the tilt angle and frequency. During the *ex situ* experiments on Mediterranean horse mackerel and chub mackerel analyzed in this PhD project, the tilt angles of the specimens were not detected, therefore the choice of a wide range of tilt angles during the modelling improved the interpretation of both model and experimental data.

The findings on the b_{20} values from KRM model at an assumed normal swimming behaviour of 88° ± 13° were ~5 dB higher compared to the results obtained on tethered fish for both species. The difference is almost constant along the tilt angles computations except for orientations of 90° ± 20° and the 101° ± 12°, where the results are lower and closer to the b_{20} = -71.4 dB re 1 m^2 for *T. mediterraneus* and b_{20} = -71.6 dB re 1 m^2 for *S. colias* found during *ex situ* experiment. Fish orientation is one of the parameters that most influence the TS (Horne, 2003; Membiela and Dell'Erba, 2018; Ona, 1990; Simmonds and MacLennan, 2005). It has been demonstrated that during *ex situ* experiments fishes display a steeper angle than in their natural state which in turn can affect TS measurements (Brooking and Rudstam, 2009; Wanzenböck et al., 2020). Despite the efforts and the novelty of the use of a piece of rope instead of a hook performed during *ex situ* measurements, likely Mediterranean horse mackerel and chub mackerel specimens were constrained to fairly abnormal swimming behaviour. Consequently, the b_{20} value of -70.4 dB re 1 m^2 for *T. mediterraneus* and -69.73 dB re 1 m^2 for *S. colias* found through the KRM model considering an abnormal tilt angle displacement in this study, could be considered almost in agreement with empirical experiments within a 2 dB interval. Accordingly, these results were assumed to be correct also for the other tilt angle intervals with an approximation of 2 dB. It should be highlighted that when the vessel approach fish schools during daytime acoustic surveys activity several pelagic fish species are inclined to display an avoidance behaviour, especially in shallow waters causing an abnormal swimming behaviour which in turn leads to a drop in the TS (Barange and Hampton, 1994; Hjellvik et al., 2008). However, fish orientation depends on an ensemble of natural factors such as light intensity and feeding migrations (Fernandes et al., 2016; Mäthger, 2003; Yasuda et al., 2018), therefore the most suitable b_{20} value could vary among shifting survey conditions.

In this work was carried out the first experiment in the use of a backscattering model in the Mediterranean Sea giving the first multi-frequency and broadband acoustic frequency response of *S. colias* and *T. mediterraneus* never performed. The X-Ray scan revealed wide differences in the swimbladder morphologies and a slight variation in the orientation within the fish body, which was partially reflected in the backscattering variation between chub mackerel and Mediterranean horse mackerel. Nevertheless, the dorsal aspect is known to be the most important diagnostic character in TS studies (Love, 1971). From X-Ray analysis, the results on swimbladder measurements normalized for the TL show similar mean-cross-sectional area values, despite the dorsal area of *T. mediterraneus* being slightly significantly smaller. This can explain the closeness between b_{20} values of a physostomous and physoclists species which are within 2.5 dB of variation at any tilt angles cases. The results obtained in this study applying the relative frequency response formula and analyzing patterns within a step of 1 kHz broadband spectra pointed out a clear opposite pattern, which is not affected by the tilt angle, especially between 70 and 120 kHz. Although the relative frequency response of the congeneric species *Trachurus trachurus* have already been studied in 2005 (Fernandes et al., 2006), the results shown here differ, giving the possibility to distinguish among the three co-occurrent species in the Adriatic Sea via acoustics tools (multi-frequency data). Furthermore, herein was provided one of the firsts multi-frequency and broadband characterization of sprat arising the chance to acoustically identify the species compared to the other ones studied during this work, but mostly with the other clupeids species. Indeed, in the Mediterranean Sea sprat is commonly found in multi-specific schools with sardine and anchovy (Fernandes et al., 2006)

Novel and known methods have been applied for data analysis and scrutinization. All resulted in b_{20} values different from that one currently in use in the Mediterranean Sea (Lillo et al., 1996; Doray et al., 2010; De Felice et al., 2021), see Table 5.1. The b_{20} values presented here for sprat *in situ* measurement and backscattering model are significantly higher than the -71.05 dB re 1 m^2 obtained in the neighbour Black Sea (Marinova and Panayotova, 2015; Panayotova et al., 2014) and higher than those currently in use in the Mediterranean Sea (De Felice et al., 2021), whereas they are similar to those found in the Baltic Sea (Didrikas and Hansson, 2004; Fässler and Gorska, 2009). The difference from the values of -71.7 dB re 1 m^2 currently in use in the Adriatic Sea (Azzali et al., 1997; De Felice at al., 2021) may be due to the acoustic equipment employed. This value was obtained using a single-beam echosounder on spread fish with a mean TL of 13.2 cm.

Table 5.1. b_{20} values deriving from this three years PhD project in comparison with the values currently in use in the Adriatic Sea (Lillo et al., 1996; Azzali et al., 1997) and the published values on sprat and related species to genus *Trachurus* and *Scomber: T. capensis, T. symmetricus mutphy, T. japonicas and S. japonicus* (Barange and Hampton, 1994; ICES, 1997; Gutiérrez and MacLennan, 1998; Svellingen and Ona, 1999; Axelsen, 1999; Axelsen et al., 2003; Didrikas and Hansson, 2004; Peña and Foote, 2008; Svellingen and Charouki, 2008; Fässler and Gorska, 2009; Marinova and Panayotova, 2015; Robles et al., 2018).

Species	**b_{20} currently in use (dB re 1 m^2)**	**New b_{20} (dB re 1 m^2)**			**b_{20} related species literature (dB re 1 m^2)**
		Ex situ	***In situ***	**Model**	
S. sprattus	-71.7		-67.5/ -68.8	-68.44	-65.08 / -71.2
S. colias	-68.7	-71.6		-69.73	-68.7 / -79.8
T. mediterraneus	-68.7	-71.4		-70.04	-65.2 / -76.6

A single-beam transducer provides less accurate detection of single targets, due to the difficulty of resolving the target position inside the beam (Simmonds and MacLennan, 2005). The values presented here are also higher than the historical values for physostomous clupeids given by Foote (1987) and by ICES (1983). Those experiments could now be re-evaluated in light of the recent findings, which benefit from technological advances and algorithms development (Adrian Madirolas et al., 2017; Ok and Gücü, 2019; Sobradillo et al., 2021). The current use of split-beam transducers enhances the detection of the precise position of fish in the 3D space, being particularly suitable for TS measurements (Simmonds and MacLennan, 2005). Therefore, a difference between 2.4 and 5.6 dB, found in this work, might be correct. Here it was demonstrated that different post-processing steps and models can yield a difference exceeding 3 dB. Considering the novel approach proposed herein after the application of a high-density filter algorithm as the most conservative one, a b_{20} value between -67.5 and -68.8 dB re 1 m^2 was suggested for sprat. Notably, these results are supported by the b_{20} of -68.44 dB re 1 m^2 obtained through the application of FEM model. Therefore, we recommend as new reference values for sprat TS in the Mediterranean Sea the range of values got from *in situ* experiments, although more *in situ* and *ex situ* studies could be required to fill the gap in the size range and pursue the TS of the species before its adoption.

Since there are no published data on acoustic measurement of *T. mediterraneus,* and *S. colias*, the results of this work were compared to those of the three most widely studied species, *Trachurus capensis* (Cape horse mackerel), *Trachurus symmetricus murphy* and *Scomber japonicas*. They are characterized by similar physiological and morphological features to the species covered by this study since they present the same swim-bladder anatomy (Fisher et al., 1981). Axelsen and co-workers (Axelsen, 1999; Axelsen et al., 2003) obtained very low b_{20} values for *T. capensis* in an experiment involving a submersible platform equipped with transducer that was suspended just above the fish school. Barange and Hampton (1994) documented that the escape behavior of Cape horse mackerel can influence the backscattering cross-section results. As a matter of fact, the TS distribution shifted downward during trawling operations, due to an increased tilt angle. This suggests that the b_{20} results ranging from -74.9 and -77.5 dB re 1 m^2 reported by Axelsen and co-workers may have been influenced by fish escape behavior from the submergible platform. This bias was removed in the present work by performing all TS measurements with the vessel stationary during *ex situ* experiments carried out on board of R/V G. Dallaporta. If the results of Axelsen and co-workers are excluded, the published b_{20} values of *Trachurus* range from -65.2 dB re 1 m^2 to -72.1 dB re 1 m^2 being in agreement with the resulted presented here. Conversely, only two values are available for *Scomber japonicus* at 38 kHz: -70.95 (Gutiérrez and Maclennan, 1998) and -77.6 dB re 1 m^2 (Svellingen and Charouki, 2008). The b_{20} values obtained here with KRM model considering a normal swimming behaviour (88° ± 13°) on *S. colias* and *T. mediterraneus* are closer to the that one now in use in the Mediterranean Sea of -68.7 dB (Lillo et al., 1996; MEDIAS, 2021). They are also closer to the values detected by several authors in the Atlantic and the Pacific Ocean on related species *Trachurus capensis*, *Trachrus symmetricus mutphy* and *Scomber japonicus* (Barange and Hampton, 1994; Robles et al., 2018; Svellingen and Ona, 1999). Conversely, they diverge from other studies carried out on these species (Axelsen, 1999; Axelsen et al., 2003; Gutiérrez and Maclennan, 1998; Peña and Foote, 2008), which reports values similar to the values obtained with the backscattering model considering a non-normal tilt angle of 101° ± 13° (-70.4 dB re 1 m^2) and with *ex situ* experiments (-71.4 dB re 1 m^2). Notably, the b_{20} of *T. mediterraneus* found here is similar to the one obtained by Peña and Foote (2008) by applying the Kirchoff Ray Mode model to a 3D swim-bladder shape. Similarly, the b_{20} values of -71.6 and -69.73 dB re 1 m^2 obtained in the present study for *S. colias* are comparable to that one now in use by the French Research Institute for Exploitation of the Sea (IFREMER) (Doray et al., 2010) but 2.5 and 1 dB lower, respectively, than the reference values used by the MEDIAS research groups for biomass assessment in the Mediterranean Sea.

Overall, the b_{20} results of -71.4 dB re 1m^2 for *T. mediterraneus* and -71.6 dB re 1m^2 for *S. colias* obtained after *ex situ* experiments should be preferred to the one obtained from the backscattering model at -10° tilt angle (-70.04 dB re 1m^2 for *T. mediterraneus* and -69.73 dB re 1m^2 for *S. colias*) as they are measured at sea (MacLennan and Menz, 1996; O'Driscoll et al., 2017; 2018; Salvetat et al., 2020).

Although the discrepancy and similarities with values reported in the literature could mainly be due to methodological issues, size range and limited dataset, TS may vary also among areas, as reported for instance for herring (Ona, 1990). Such variability can be associated with several environmental factors, resulting in different morphological adaptation and reflection properties (Horne, 2003; Hannachi et al., 2004; Fässler and Gorska, 2009), since the physical environment influences fish physiology and morphology (Scoles et al., 1998). Table 5.1 underlines the stochastic nature of TS and in turn the variability of b_{20}. The latter is one of the most important sources of bias in acoustic surveys (Rose et al., 2000; Scoulding et al., 2016) as demonstrated with the *in situ* experiment, since the inadequate knowledge of sprat acoustic reflectivity alone could result in a coefficient of variation (CV) of sprat biomass estimates between 16% and 18%. This uncertainty resulted in an overestimation of sprat biomass during the past years using the current b_{20} (-71.7 dB re 1 m^2) instead of the values obtained here. Then, the sprat biomass in the Adriatic Sea would be about one and a half times lower than previously estimated. It is therefore important to gain deeper insight into TS and b_{20} variability, which should be taken into account as a source of uncertainty during biomass estimates arising from acoustic surveys.

The TS variability of ancillary species could also affect the biomass estimates obtained for the other two clupeid target species in the Adriatic Sea, anchovy and sardine, because of the application of the mixed-species formula (Nakken and Dommansens, 1975). It does not necessarily affect the stock assessment of these species since the acoustic surveys are generally used as a relative time series index in the stock assessment process (Carpi et al., 2015). However, the recent introduction of Total Available Catch (TAC) in the management plan of these stocks in the Adriatic Sea (Regulation (EC) 2022/110) increased the importance of deriving precise absolute biomass estimates, an issue linked with the correct measurements of TS of all small pelagic fish species.

In order to test the influence of b_{20} on biomass estimates for the main categories of fish pelagic species which inhabit the northern Adriatic Sea area, including Slovenian waters, where sprat is more abundant (Leonori et al., 2012, 2017, 2021), the following computations were applied. The NASC allocated by haul composition through the mixed-species echo integrator conversion factor was converted to abundance in number according to the mixed-species formula (Nakken and Dommasnes, 1975), which in turn was converted to biomass in weight (kg) using the length-weight relationship parameters of each year and species. The species-specific b_{20} value of the species covered by this study in the formula was repeatedly changed, starting with the current applied values (see Table 1.6.1) and then the new values obtained herein presented in Table 5.1, while the conversion parameters of the other categories were kept fixed. The mean biomass and the overall uncertainty were estimated using the upper and lower limit of species-specific b_{20} values as input variables for each year. In

Figure 5.1, the process modifying at the same time the conversion parameter of sprat, chub mackerel and Mediterranean horse mackerel was applied considering the last period from 2013-2021. In Figure 5.1, OPS group comprise mainly *Boops boops, Sardinella aurita, T. mediterraneus, T. trachurus, S. colias, Spicara sp., Aphia minuta*. Figure 5.1 underlines the influence of TS on biomass estimates of the species deal with this study, but it also highlights the variation of biomass estimates on the other species and categories as a consequence of TS' uncertainty of chub mackerel, Mediterranean horse mackerel and sprat. Setting the b_{20} of the two mackerel species in the mixed-species formula with the new values reported herein instead of the current values slightly increases biomass along the time series due to the lower b_{20} values (-71.4 dB re 1 m^2 for *T. mediterraneus* and -71.6 for *S. colias*) compared to the values currently in use (-68.7 dB re 1 m^2 for both species). However, the subsequent biomass changes of the other categories due to the variation in the b_{20} is very low due to the small echo-integral contribution of *T. mediterraneus* and *S. colias* in the North-Adriatic Sea. In fact, they are generally scarce in this area. Moreover, the pelagic sampling is not properly suitable to capture fast swimmers influencing the catchability mainly on *S. colias* and in turn the terms w_i in equation 1.4.2, since it is well known that the catchability of *Scomber* species is low with the trawls generally employed during acoustic surveys (ICES 2021). Conversely, the adjustment of the sprat conversion parameter with the new b_{20} values resulted in a relevant reduction of the biomass of all the species, especially when the presence of sprat is high in the area of study. Therefore, the differences between tons along the categories shown in Figure 5.1 is mainly attributable to the sprat conversion parameter. Using a higher b_{20} of -68.44 dB, deriving from the model, a biomass drop of about 50000 tons for sprat, up to 50000 tons for anchovy and up to 30000 tons for sardine was obtained, while the decrease is more restrained for the other categories, in particular for chub mackerel. Summarizing, the variation and uncertainty of sprat b_{20} in the process coming from the current study, translates into a shift of biomass for all the pelagic species in the North Adriatic Sea, while the influence of chub mackerel and Mediterranean horse mackerel's new TS is less important.

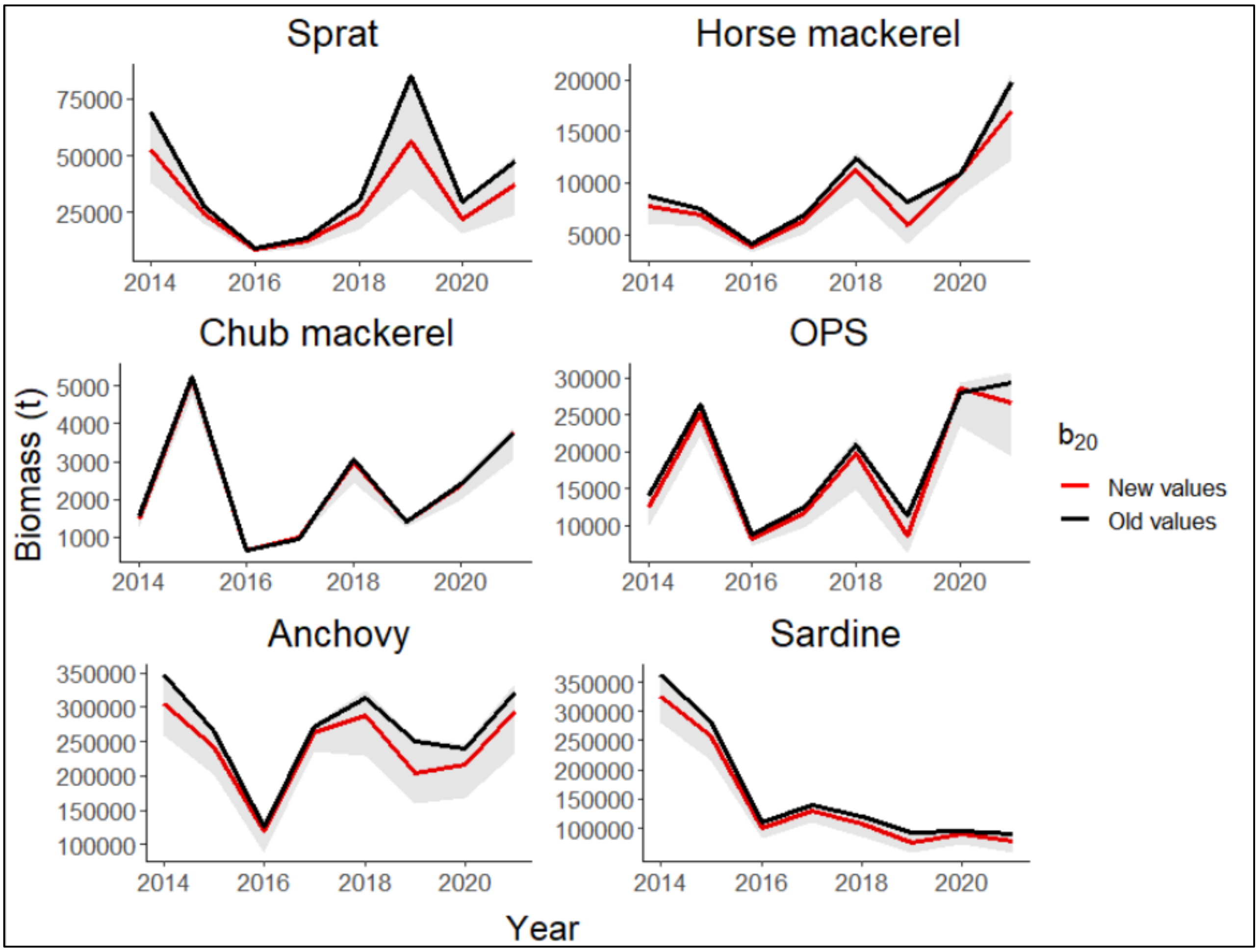

Figure 5.1 Mean biomass trend of the main species and categories assessed by acoustic surveys in the Adriatic Sea estimated using current b_{20} of sprat (-71.7 dB re 1m^2), chub mackerel (-68.7 dB re 1m^2) and Mediterranean horse mackerel (-68.7 dB re 1m^2) in Black and with the new values obtaining from empirical experiments and mathematical models (in red). The dashed areas show the biomass range resulted from the use of all the b_{20} values got in this PhD work, where the upper limit and lower limit point out the minimum and the maximum values of the three species together respectively.

6. Conclusions

The research carried out during this PhD helped to increase the knowledge of the acoustic reflectivity of non-target species in the Mediterranean Sea. New species-specific backscattering results were found. They can be considered as a starting point to overcoming the regional knowledge gap in the acoustic backscattering coefficients of non-target species. Moreover, it further confirmed the influence of TS uncertainty on biomass estimates of small pelagic fish species.

In the framework of the MEDIAS, currently, the NASC of *S. colias* and *T. mediterraneus,* collected for biomass assessment purposes, are pooled under the category "other species" along with other pelagic fish species. The b_{20} values obtained in the present study could be used for single-species assessment in the near future since any possible constraints due to the choice of the method have been taken into account. The main limitation of the *ex situ* approach used for the study of these species is related to the possible influence of the tethered system on fish swimming natural behaviour, although it can be less invasive than other methods used. The specimen size range found here may also be too narrow. Nevertheless, the size range was increased by the computation carried out with the KRM model which supported our conclusions. Computing the TS as a function of tilt angle considering also a negative inclination of the fish during the swimming movements, we observed close values with the ones obtained from *ex situ* experiment demonstrating that the *ex situ* study could have been biased by a higher displacement of a negative tilt angle of the fish during the experiment. However, the negative tilt taken into account can be associated with the behaviour of fish when the vessel approaches fish schools besides the diurnal migration from the surface to the seabed (Hjellvik et al., 2008). Our findings on the swimbladder angle relative to the fish axis and the related backscatter revealed that the acoustic echo of *T. mediterraneus* at 38 kHz is still strongly related to the fish tilt angle, conversely, the TS of *S. colias* is less affected by the fish tilt angle at the same frequency. Generally, the shorter the ratio of the acoustic wavelength to the target length, the greater the influence of directivity (Love 1971). This proves the efficiency of the use of 38 kHz in assessing the species which conversely results less suitable for *T. mediterraneus,* which might benefit from the use of 18 kHz. The presented results give the first theoretical insight into the use of broadband backscatter in the Mediterranean Sea, pointing out the potential of this approach in distinguishing between species as already proved in other areas (Ayoubi et al., 2016; Boswell et al., 2020). We presented the first TS experiments performed on the two species providing potential multi-frequency diagnostic tools and b_{20} conversion parameters to be used worldwide in the absence of other data. Altogether, our findings have the potential to be used for future biomass estimations of Atlantic chub mackerel and Mediterranean horse mackerel in the Mediterranean Sea. More work is needed for the application of numerical models e.g. FEM on real 3D triangular mesh shape swimbladder morphology which can be used to assess the species-specific acoustic reflectivity in a wider range of fish tilt angles for *S. colias* and *T. mediterraneus.* However, this requires computational power enough for solving the equations in such large swimbladders. New b_{20} values from the backscattering model and *ex situ* experiment for *T. mediterraneus* and *S. colia* are provided here but more *in situ* investigations might be useful to get further insight into the backscattering cross-section of the species retrieving the natural behavior of the fish during the survey. *In situ* experiments are difficult to perform on pelagic species since they require monospecific hauls conducted at night when fish are dispersed. The two aforementioned species often form small multi-specific schools and during the night, when several other fish species come to the surface to feed, they are mixed.

Conversely, we were able to obtain two rare monospecific sprat catches suitable for the application of *in situ* method. The comparison among methods for single target detection and cross correlation between TS and TL data carried out on sprat monospecific hauls stresses the importance of adopting a standardized post-processing protocol for the computation of equation 1.2.3: $\boldsymbol{TS = 20\,log\,l + b_2}$ **(dB re 1 m^2)**, at least at the regional level. We demonstrated that different post-processing steps and models can yield a difference exceeding 2 dB, thus compounding the intrinsic TS variability due to physiological state, wave incidence angle, depth, and frequency (Hazen and Horne, 2003; Horne, 2003). FEM model results give robustness to the new method advanced during the *in situ* post-processing data analysis, based on the mathematical computations proposed by Kasatkina (2009). Namely, the new value of – 68.44 dB derived from the analysis of CT images is in the range when using the *in situ* method. Despite the poor trawl and acoustic dataset along with the tight LFD available do not allow the use of these values for the species in the Mediterranean Sea at present, the range of b_{20} value comprising between -67.5 and -68.8 dB re 1 m^2 proposed herein for sprat may be considered as a starting point to reevaluate the sprat-specific TSs now in use in the Mediterranean Sea. By using the TS results from present work the sprat biomass in the Adriatic Sea would be about one and a half times lower than previously estimated. More monospecific hauls are needed to fulfil the size range and increase the amount of catches and acoustic data, although we tried here to fill these gaps through the application of FEM model.

The results of this PhD underline the need to include the TS variability in the estimates of uncertainty in the acoustic survey which is usually based only on geostatistical analysis which do not take into account the coefficient of variation linked to the TS (MEDIAS 2022). Nowadays, mid-water pelagic trawl catches of acoustic targets are indispensable for the assignment of the single species amount in a certain area. Nevertheless, the results on the acoustic characterization of the studied species presented here could represent a reliable tool for the improvement of the species assignment during post-processing data analysis decreasing the importance of catches for this purpose. The relative frequency responses and the broadband backscatter are objective and free of interpretation being suitable for the future application of machine learning algorithms for an automatic process for species discrimination. Nevertheless, the expertise of acoustics researchers in any specific area remains a milestone in data analysis.

Finally, the increase of knowledge on the TS of the three aforementioned species aim of this PhD thesis could favour a more reliable assessment of their populations, which is crucial for the future management of the stocks and to monitor the status of the pelagic ecosystem in the Adriatic Sea. To date, the lack of data on the TS has been one of the factors that have limited the potential of the annual MEDIAS in assessing the abundance of non-target species. Additionally, the use of new b_{20}s on sprat, chub mackerel and Mediterranean horse mackerel together determined a shift in the biomass of anchovy and sardine which might have been overestimated in the past. The results have been discussed during the last two years in the framework of MEDIAS Steering Committee. As a result, the discussion is currently open about the possibility to use the b_{20} proposed here for *T. mediterraneus*, *S. colias* and *S. sprattu* as new reference values for the Mediterranean Sea, once they would be confirmed by more *in situ* and *ex situ* data, especially for sprat, which resulted the more important species in terms of biomass uncertainty outputs. The advice provided through the stock assessment process on the target species anchovy and sardine in the Adriatic Sea could be reevaluated in light of the evidence presented herein. Finally, the three non-target species subject of this study could be added in the stock assessment process by having reliable biomass estimates from acoustic surveys useful for stock assessment model's tuning.

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