Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
www.hcmr.gr
DOI: http://doi.org/10.12681/mms.32324

Acoustic scattering properties of seagrass: *In*/*ex-situ* measurements of *Posidonia oceanica*

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Contributing Editor: Konstantinos TSAGARAKIS

Received: 9 December 2022; Accepted: 26 April 2023; Published online: 30 May 2023

Abstract

The marine prairies are paramount to the marine ecosystem playing a crucial role in various ways. Owing to the global atmospheric events inducing hydrospheric changes, marine seaweeds have been negatively affected and are vulnerable. Conventional methods (SCUBA), which were previously used to collect seagrasses, have become a destructive method for deriving unrecoverable damages for their stocks and have been replaced with remote sensing methods. Considering the advantages of the acoustic methods, two different *in/ex-situ* experiments were conducted to ground-truth the common seagrass, *P. oceanica*, in time and space of the Turkish Mediterranean water in 2011-2012 using a scientific echosounder with a split beam transducer operated at a frequency of 206 kHz. After the separation of the seagrass from spurious scatterers, the acoustic parameters (Sa: area backscattering strength, Sv: volume backscattering strength, and TS: Target Strength) were correlated and regressed with the biometric variables (Leaf Area Index, biomass, volume, length, width, diameter, or thickness) of different parts (leaf, rhizome, and sheath) of the seagrass. Estimation of biometrics by acoustic methods has been considered a challenge up to now. Statistical relationships between biometrics and acoustics were precisely examined for the species. The relationships were linearly established in the acoustic geometric region. There was a seasonal difference in the relationships. Their rhizomes and sheaths were considered unstable non-linear parts and remained unexplained for the acoustic response. Acoustic response to the leaf density (d, g cm^{-3}), which was a distinguished component in the reflection, was found to be dependent on the seasonal biological activities of *P. oceanica*. *Posidonia*, which has a d greater than 1 g/cm^3, produced the geometric region. The present study was the first attempt to establish the relationships of the seagrass under protection, and can decrease usage of destructive methods for future studies.

Keywords: Vegetation acoustics; meadow; acoustic-biometric relation and conversion; nondestructive method.

Introduction

An estimation of the biometric variables (LAI, BLAI, and V, see Appendix 1 for the abbreviations) of seagrass leaves, especially *Posidonia oceanica* (Linnaeus) Delile, 1813, is needed to determine the ecological status in the marine environment. This knowledge assists strategy development among ecologists, biologists, species managers, and protectors (Marba *et al*., 2002; Orth *et al*., 2006; Gobert *et al*., 2009). Seagrass meadows also function as oxygen producers, habitat and refuge, food sources, competition for invasive macrophytes, protectors of prey, and a niche for many marine organisms (Boudouresque & Meinesz, 1982; Bernier *et al*., 1997; Peirano *et al*., 2005; de Mendoza *et al*., 2018; Bonamano *et al*., 2021). The presence of seagrass has greatly enhanced invertebrate and fish production and altered food webs at low-nutrient sites in comparison to unvegetated areas (Edgar & Shaw, 1995).

Therefore, remote sensing methods are very important when studying sensitive and vulnerable seaweeds and seagrasses such as *Posidonia oceanica* and *Cymodocea nodosa* (McGonigle *et al*., 2011). Recently, some strategies were developed to avoid using the destructive methods (SCUBA to pick up the leaves that have been used in other seagrasses and macroalgae studies (Gobert *et al*., 2020; Montefalcone *et al*., 2021; Zhu *et al*., 2021). These strategies were mostly associated with the different techniques (satellite, video camera, and acoustics) (Jaubert *et al*., 2003; Robinson *et al*., 2011; Mielck *et al*., 2014; Noiraksar *et al*., 2014; Randall *et al*., 2014, 2020; Ware & Downie, 2020). Some (satellite and video-camera) techniques are limited by suitable atmospheric and sea conditions in terms of their assessment of bottom coverage (Vis *et al*., 2003; McCarthy & Sabol, 2000; Hossain & Hashim, 2019). Up to now, biometric estimation has not been achieved due to their conditional limitations and requirement of ground-truthing. Therefore, these techniques have only produced coverage and mapping of the different habitats (e.g., Fakiris *et al*., 2018; Dimas *et al*., 2022). Of the techniques, the acoustic method is faster, more precise, and easier to ground-truth the data (Brown *et al*., 2011; van Rein *et al*., 2011) and develop the algorithms to remove spurious scatterers (Urick, 2013). However, previous studies on the vegetation acoustics have remained at the assessment of percent coverage and canopy height of the seagrass (e.g. van Rein *et al*., 2011; Lee & Lin, 2018), depending on the limitation of echosounder parameters (Lurton, 2002). There were few attempts to calibrate the acoustic data to estimate the biometrics of the seagrass (Mutlu & Balaban, 2018; Olguner & Mutlu, 2020) and to identify the seagrass (Mutlu & Olguner, 2023a).

Of the seaweeds in the Mediterranean Sea, *P. oceanica* and *C. nodosa* are dominant seagrasses covering the infralittoral zone (*P. oceanica*) extending to the circalittoral zone (*C. nodosa*) (Mutlu *et al*., 2022a, b), and are ecologically important (Colantoni *et al*., 1982; Pal & Hogland, 2022). Biometric parameters play a crucial role in the determination of population dynamics, seasonality, ecology, management, sustainability, protection, and the blue carbon content of the seagrass (Colantoni *et al*., 1982; Brown *et al*., 2011; Pal & Hogland, 2022).

It is hereby worth noting that sampling more than 1000 shoots is considered a destructive sampling of this sensitive species (Mutlu *et al*., 2023); *P. oceanica* is under worldwide protection. Standardization has been developed to use non-destructive methods. For instance, sampling more than orthotropic shoots (n = 20) is considered a destructive method (Pergent *et al*., 1995). Cutting leaves above the ligule is considered a non-destructive method (Gobert *et al*., 2020). For this purpose, two different preliminary acoustic algorithms were developed (Mutlu & Balaban, 2018; Olguner & Mutlu, 2020) to eliminate the destructive sampling. Therefore, the calibration equations to convert the acoustic to the biometric measurements have been created regarding the structure of *P. oceanica*.

Because of the ecological importance and lack of historical comprehensive acoustic information on the meadows, this study aims to provide the seasonal acoustic traits and characteristics with the biometrics of *P. oceanica*. The present empirical study is the first attempt to describe the structural and acoustic energy of the seagrass and to establish regression equations between acoustic and biometric measurements.

Material and Methods

Study area

The distribution of *P. oceanica* was summarized for their population and growth dynamics in the present study area (Mutlu *et al*., 2022a) to guide the present study with the acoustic dynamics in time as follows: Shoot density was not significantly different among seasons and was above 364 ± 27 shoots m^{-2} but was different among the depths. The biometric variables decreased with the bottom depth gradient along which the number of leaves per shoot, inter-shoot distance, and morphometrical variables tended to increase. Growth of the biometrics occurred between March and August-September, followed by the mortality, regardless of its coverage area. The mortality occurred due to the highest annual salinity in late summer. A transition depth in space and month in time was assessed as 15 m and August for variation of the biometrics, respectively (Mutlu *et al*., 2022a).

Experimental data collection and analysis

The study was carried out to conduct *in*/*ex-situ* acoustic calibration of one common seaweed (*Posidonia oceanica*) on the east coast of Turkey (Antalya Gulf, East Mediterranean Sea) during six cruises (July 2011; December 2011; January 2012; March 2012; April 2012; August 2012) (Fig. 1). Two different empirical studies for

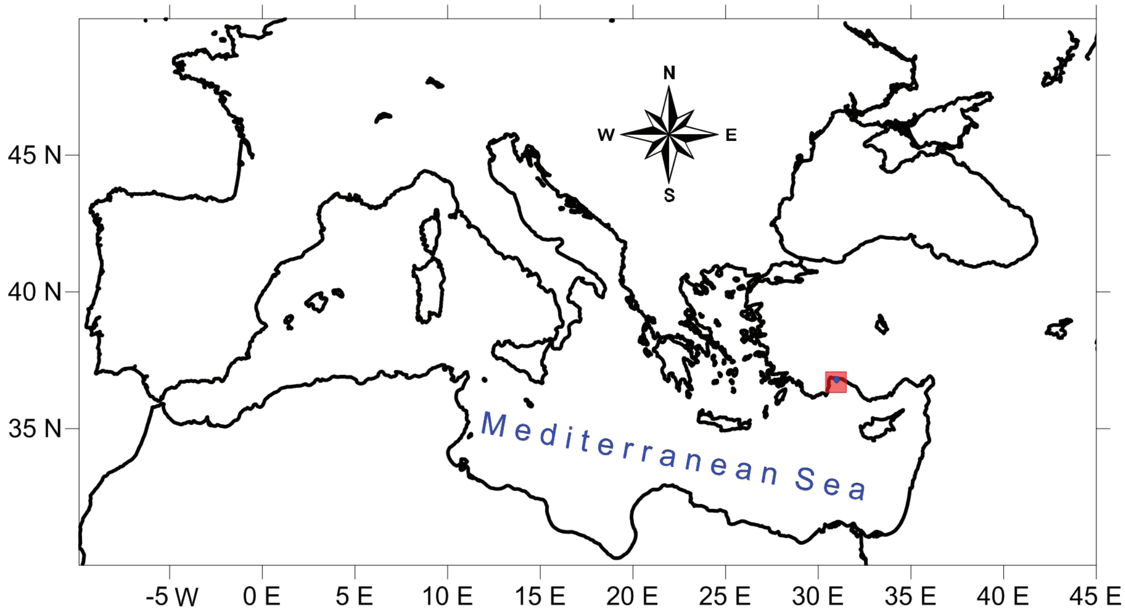

Fig. 1: Study area (red rectangle), and experimental location (blue circle) of seasonal “leaf” and “cut” experiments for *Posidonia oceanica*.

the biometrics-acoustics relation were set namely as follows: “Leaf experiment” and “Cut experiment”.

The ship, R/V *Akdeniz Su* (26 m long), was first docked at the experimental area (Fig. 2). Overall, acoustic data was collected with a Biosonics DT-X digital scientific echosounder, which had a split beam transducer having a beam width of 6.8°, operating at 206 kHz, using a software package of “Visual Acquisition” (v. 6.3.1.10980, BioSonics Inc.). The transducer was mounted at a draft depth of 2.5 m, looking down on the starboard of the R/V *Akdeniz Su* (Fig. 2). First, water temperature, salinity, and pH were measured to enter into the “Visual Acquisition” to calculate the sound speed for each season. The echosounder was calibrated at a pulse width of 0.4 ms, referring to a tungsten spherical ball (36.4 mm in diameter) (BioSonics Inc.). The experimental acoustic data were then collected using the echosounder at a pulse width of 0.1 ms and a ping rate of 5 pings s^{-1} after the setup of each experiment. The minimum acoustic threshold was kept at –140 dB. The acoustic data was converted to Comma-Separated Value, CSV, format using the software package of “Visual Analyzer” (v. 4.1.2.42, Biosonics Inc.). The data conversion was made up of a matrix of the horizontal resolution of ping-to-ping and a vertical resolution of count-to-count (each count ~ *c.a.* one-eighth of the pulse width, 1.87 cm depending on seasonal sound speed).

A flat bottom was chosen at a depth of 15 m to perform the experiments. The tungsten ball was used to calibrate the echosounder during the field study to correct the Elementary Distance Sampling Unit, EDSU (Simmonds & MacLennan, 2005), as follows: Sv, Sa, and TS during later post processing (see Appendix 1 for the abbreviations). To sea-truth the data, biometrics of the meadow were measured as given in Figure 3.

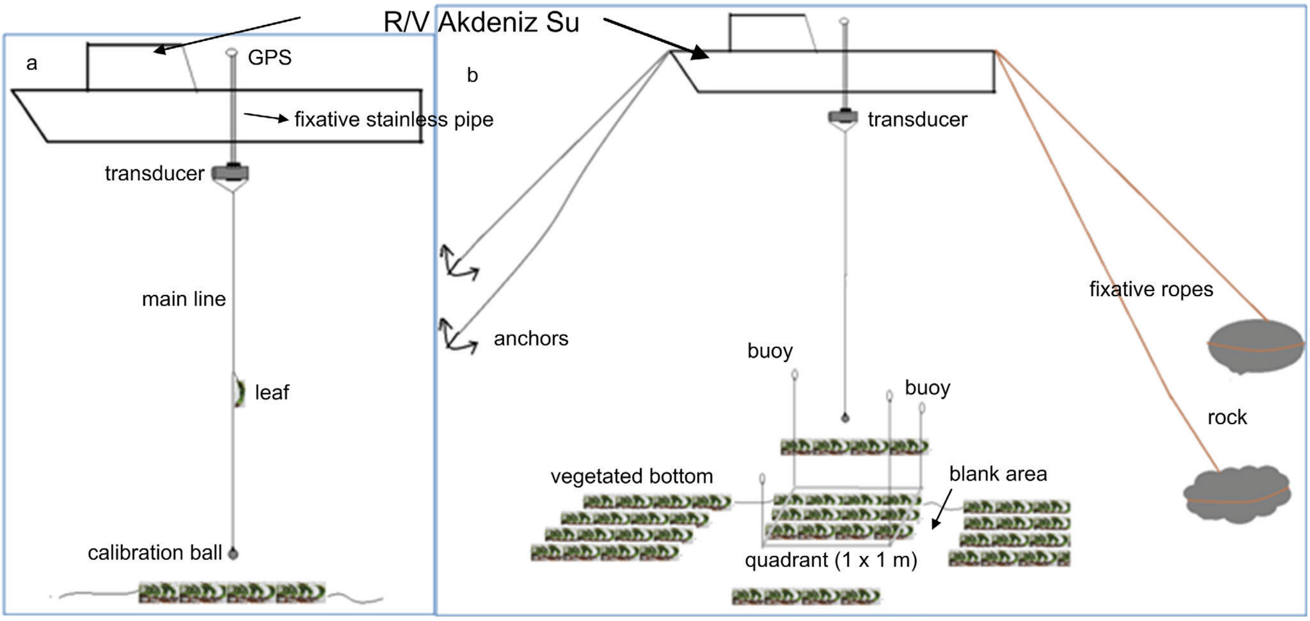

Fig. 2: *In*/*ex-situ* calibration and measurements of the seaweeds: “Leaf” experiment (a) and “Cut” experiment (b).

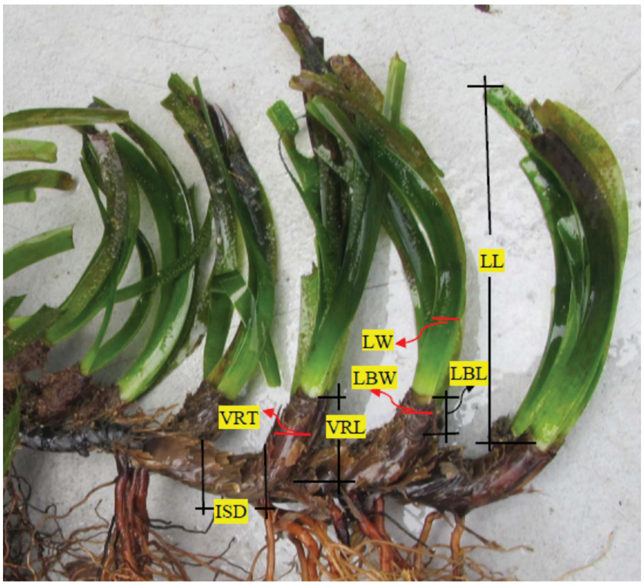

Fig. 3: Plant traits of the seagrasses; Inter-shoot distance (ISD), vertical rhizome length (VRL), vertical rhizome thickness (VRT), leaf length (LL)*, leaf width (LW), leaf base (white extension of leaf in sheath below ligule), sheath length (LBL), and sheath width (LBW) of *Posidonia oceanica*. *The leaf was measured straightened if it was curved.

"Leaf" experiment

The "leaf" experiment was designed as shown in Figure 2a. The "leaf" experiment was performed under a controlled environment and condition and was more precise than the "Cut" experiment. A length of 12 m of a nylon monofilament line (15 N, *ca.* 0.65 mm ϕ) was suspended as the main line to the bottom of the transducer at a draft depth of 2.5 m from the surface water. The calibration ball at the end of the line was used for weight and calibration purposes. Ten to twenty bundles of leaves were used for the calibration measurements during each season. Each bundle was tethered to the main line at 10 m depth (the range) from the transducer with the help of a 30 cm-nylon line (Fig. 2a). The acoustic data collection started when the bundle was positioned perpendicular to the bottom and continued for at least 2-10 mins. This provided the natural orientation of the meadows on the bottom of the sea. The bundle containing leaves and rhizomes was first acoustically measured, and the rhizomes were then cut off to only measure the leaves.

The Sv was separately measured two times using the Visual Analyzer (Sv) (Fig. 4) and an algorithm "Sheath-Finder" (Sv1) described by Mutlu & Olguner (2018). The

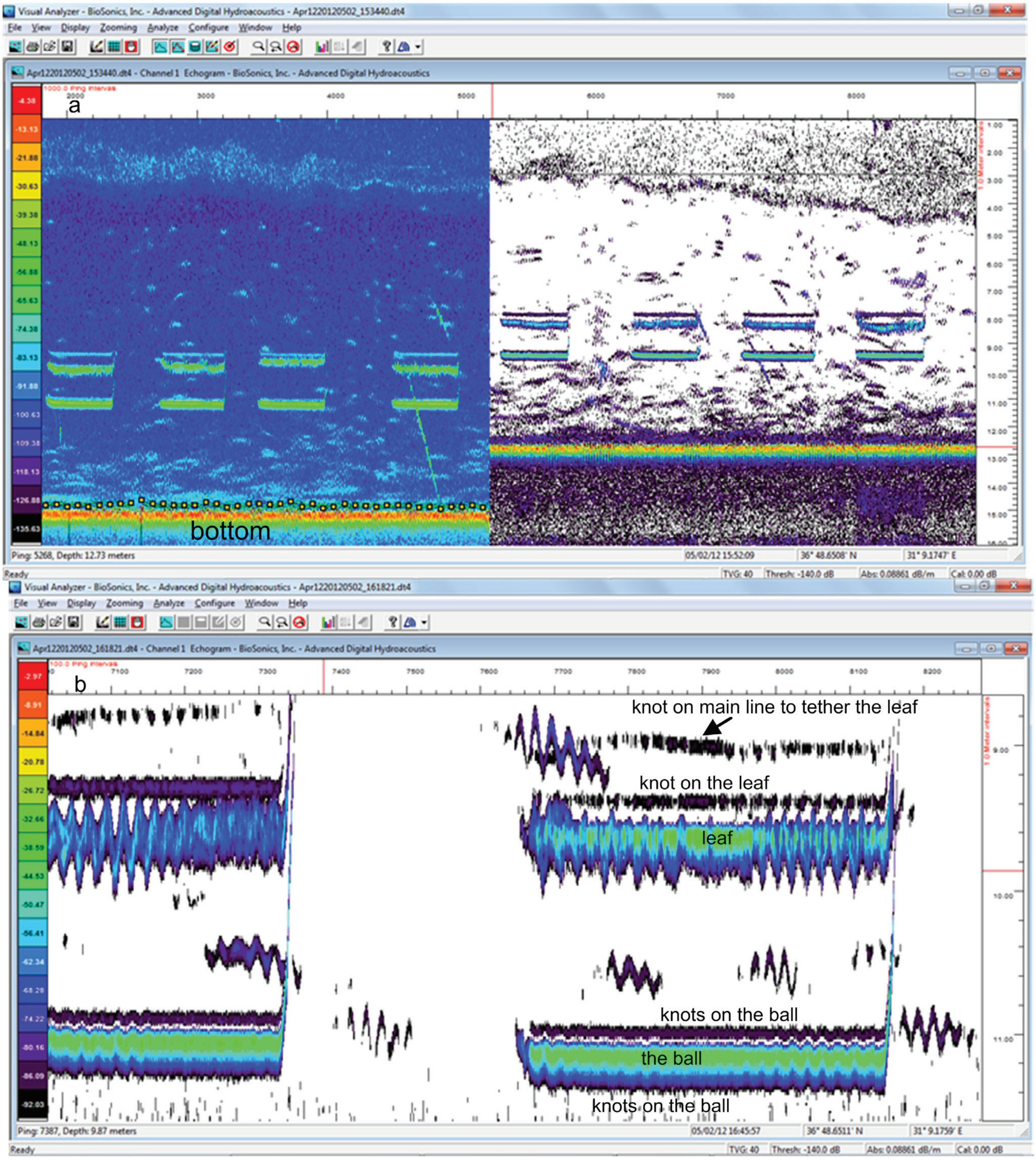

Fig. 4: Echogram of the leaf experiment during the acoustic processing by Visual Analyzer (a) and close-up view of the echogram (b).

TS was an output of the Visual Analyzer (v. 4.1.2.42, Biosonics Inc.). The Sa was separately estimated two times using the SheathFinder (Sa) and Visual Bottom Typer (Sa1) (VBT, vers. 1.10, Biosonics Inc.) (see Appendix 1 for the abbreviations). All measurements and estimations were performed using a threshold of -95 dB.

A "SheathFinder", a compact algorithm, consists of three new algorithms to estimate the EDSU's of *P. oceanica*. The first algorithm includes the autonomous 'lost' bottom and dead zone detection to correct the real bottom. The second algorithm removes spurious targets as well as artificial and background noise, surface, volume and bottom reverberations, and interference from other acoustic scatterers. The third algorithm consists of the EDSU's estimator by fixing and detecting vertical rhizomes or sheaths in ping by ping, and then checking the vertical acoustic count samples for presence of leaf of *P. oceanica* (Mutlu & Olguner, 2018). After fixation of the entire leaf, the Sv and Sa were calculated as described by Simmonds & MacLennan (2005) to estimate the EDSUs.

During the process by the Visual Bottom Typer, VBT (Fig. 5), an algorithm, B3 (ratio of the parts of the first bottom=leaf echo), was chosen to classify the meadows (E1'/E1) (Fig. 5c-d) and to measure the echo level of the meadow by setting minimum and maximum thresholds, and minimum and maximum pulses for the leaves (Fig. 5b). The automatic target tracking was deactivated marking the leaf bundle in suspension within the acoustic beam (Fig 5b). Considering the range and beam angle, E0=E1' + E1 of the leaf bundle was converted to Sa1 (dB) (see Appendix 1 for the abbreviations). Integrated echo level (E0) was converted to area backscattering coefficient using the equation sa=E0/m^2 (area sampled by the echosounder). The sa was then converted to Sa (Sa1) in dB using an equation of 10 x $\log_{10}$ (sa).

Biometrics for each bundle were measured on board before the acoustic measurements (Fig. 3). The biometrics was organized in two categories: concentration variables (LA in cm^2 and W in g, see Appendix 1 for the abbreviations) and morphometric variables (leaf length,

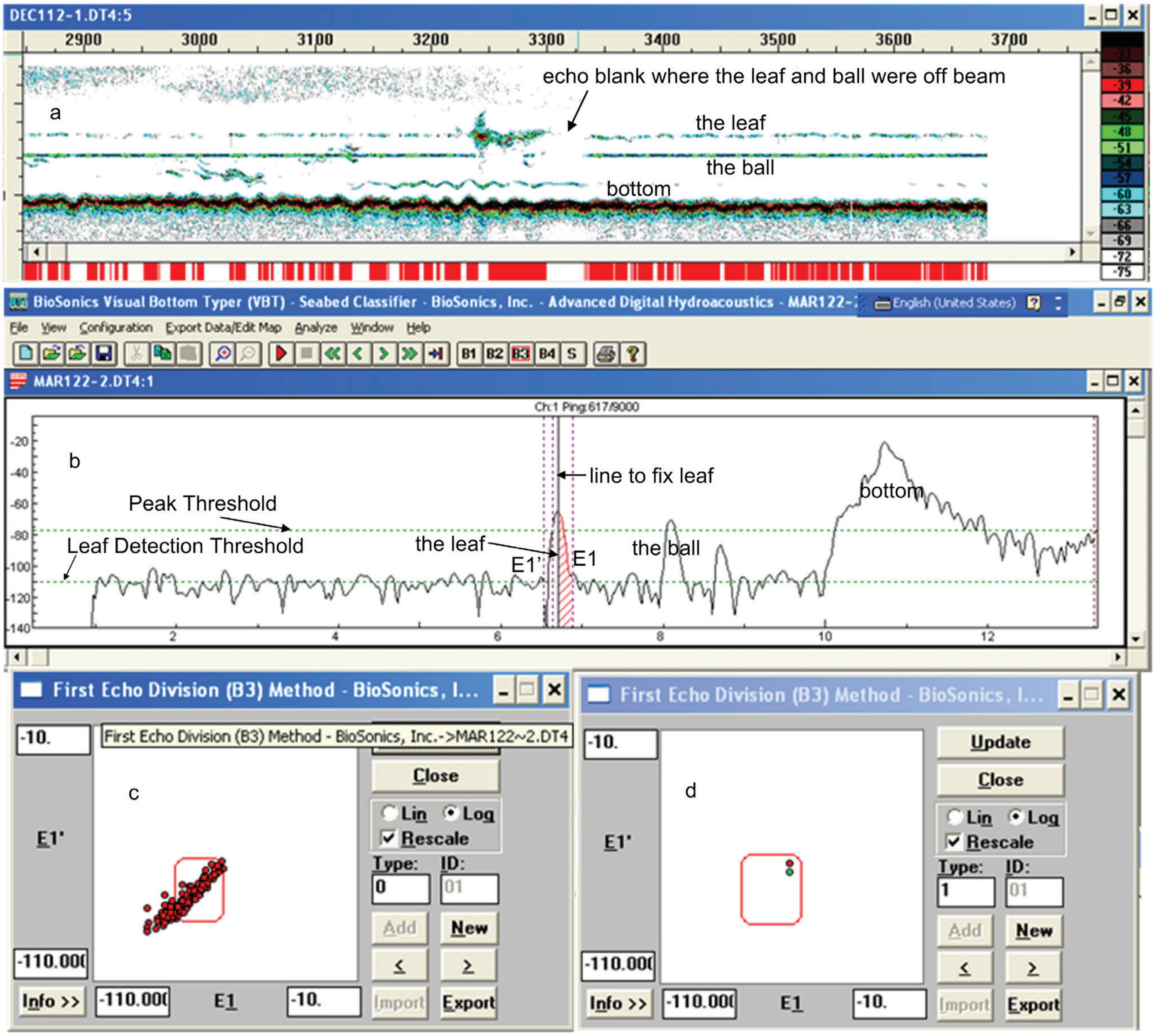

Fig. 5: Acoustic processing by the VBT; echogram (a), signal (echo) and signal parts (b), and classification of the cluster of a signal by *Posidonia oceanica* on cumulative (c) and instantaneous clusters (d) (see Appendix 1 for the abbreviations).

width, thickness, vertical rhizome length, and diameter in mm). Leaf weight was estimated from the regression equation between LA-W (Mutlu *et al.*, 2022a). Furthermore, the leaf V (cm^3) and d ($g\ cm^{-3}$) (see Appendix 1 for the abbreviations) of the leaf and rhizome were estimated. All estimations were then related to the acoustic measurements.

"Cut" experiment

Another experiment, namely the "Cut" experiment, was based on the manual dissection of a certain number of the shoots from the sampling area inside a plastic frame of 1 x 1 x 0.04 m, where each corner was designated with an egg-shaped floater (calibration=cal buoy) in suspension with nylon line having different heights. The frame material had a flesh thickness of 2 mm, and the inner part of the frame was enabled to get water. The calibration ball (ball sphere) was suspended in the center of the frame and at around 1 m above the tips of the meadows. This was performed to track the inner part of the frame by eye and acoustics (Fig. 2b, 6). The plastic frame was chosen since it is a weaker acoustic reflector, like rubber, than the meadows. The ground, up to 1 m away around the frame, was cleaned of *P. oceanica* to be a blank area (Fig. 2b).

The experiment was conducted during calm weather and sea conditions and in clear (transparent) water. The sampling area had a flat bottom of 15 m deep and 12.5 m in range (*c.a.* ~1.7 m^2 sampling area of the echosounder). Before the settlement of the experiments, the ship was fixed in place from its corners by anchoring from the "*prova*" or "*prora*" (front of the ship) and by tethering it to underwater rocks using the thick ropes from the "*puppa*" (rear of the ship). Finally, the anchorage chains were tensed (Fig. 2b).

First, the acoustic measurement was done, and then the SCUBA divers picked up a certain number of shoots inside the frame. The dissection or rarefaction of the shoots was completed by repeating process 5-6 times until nothing was left inside the frame (Fig. 2b). Each acoustic measurement was performed for 5-10 mins. Between dissections, we waited for suspended materials and air bubbles from the SCUBA divers to disappear to have clear meadows.

On board, the meadow samples were then preserved in borax-buffered 5% formaldehyde for later biometric measurements at the lab.

An algorithm, "SheathFinder" (Mutlu & Balaban, 2018), processed the acoustic data for each dissection. Unlike the leaf experiment, only Sv and Sa were estimated to relate to the biometric measurements (Fig. 6) (see Appendix 1 for the abbreviations).

In the present study, the weight and biomass of *P. oceanica* were considered by estimating them from the LA of the leaves instead of the leaf length. Therefore, the leaf weight (WL and WLA) and biomass (BL and BLAI) were comparable between estimates of biometrics of leaf length and LA as given in Figure 7, respectively. One of the individual leaf weights or biomasses of *P. oceanica* could be chosen during future studies to relate Sa or Sv to the proper weight or biomass using the equations in Figure 7 since the LA was considered in the present study.

Statistical analyses

A parametric correlation coefficient (Pearson's product moment) and linear model of regression equations were used to explain the relationships between biometric and acoustic variables at $P < 0.05$. For significance, a t-test was used for the correlation and regression coefficients (a, intercept, and b, slope of the equations).

Owing to a different number of dissected shoots during the cut experiment, the acoustic data were reduced (prefix with R) to get echo energy per unit of each biometric variable using the following equations:
$RSa=10*\log_{10}(sa/LAI^2 \text{ or } BLAI^3 \text{ or } V^{2/3} \text{ or } d^1)$,
$RSv=10*\log_{10}(sv/LAI^2 \text{ or } BLAI^3 \text{ or } V^{2/3} \text{ or } d^1)$,
$RTS=10*\log_{10}(\sigma_{bs}/LAI^2 \text{ or } BLAI^3 \text{ or } V^{2/3} \text{ or } d^1)$ (see Appendix 1 for the abbreviations).

A polynomial fitting model of regression equations was used to explain the relationships between biometric and reduced acoustic variables at $P < 0.05$.

Analysis of Covariates (ANCOVA) was used to test the seasonal difference in the relationships, followed by a post hoc test, Least Significant Difference (LSD), using the statistical tools of MatLab (2021a, Mathworks Inc.).

Results

There were two different experiments for characterizing the temporal acoustic properties of the meadow structure. "Leaf" experiments measured the echo strength of leaf + rhizome + sheath, leaf + leaf base (part of the leaf below the ligule), and only leaf (green part). The "cut" experiment included measurements of the backscattering strengths of only meadow leaves. All acoustic measurements were related to the biometric variables, especially LA, LAI, BLAI, and WLA, and d of the meadow (see Appendix 1 for the abbreviations). During the experimental seasons, the meadow or leaf was free or devoid of epiphytes, especially calcareous algae which could contribute to the acoustic energy from the leaves (Mutlu *et al.*, 2022a, b).

"Leaf" experiment

To characterize the acoustic traits of the entire structure (leaf + rhizome + sheath) of the meadow, the experiment was conducted only in December (minimum length of rhizome), January (growing), and July (maximum) (Mutlu *et al.*, 2022a). The leaf + leaf base (leaf base: white extension of leaf in sheath below ligule) experimental study was repeated in January, March, April, July, and December, according to the seasonal population dynamics of the meadow (Mutlu *et al*, 2022a). The leaf + leaf base

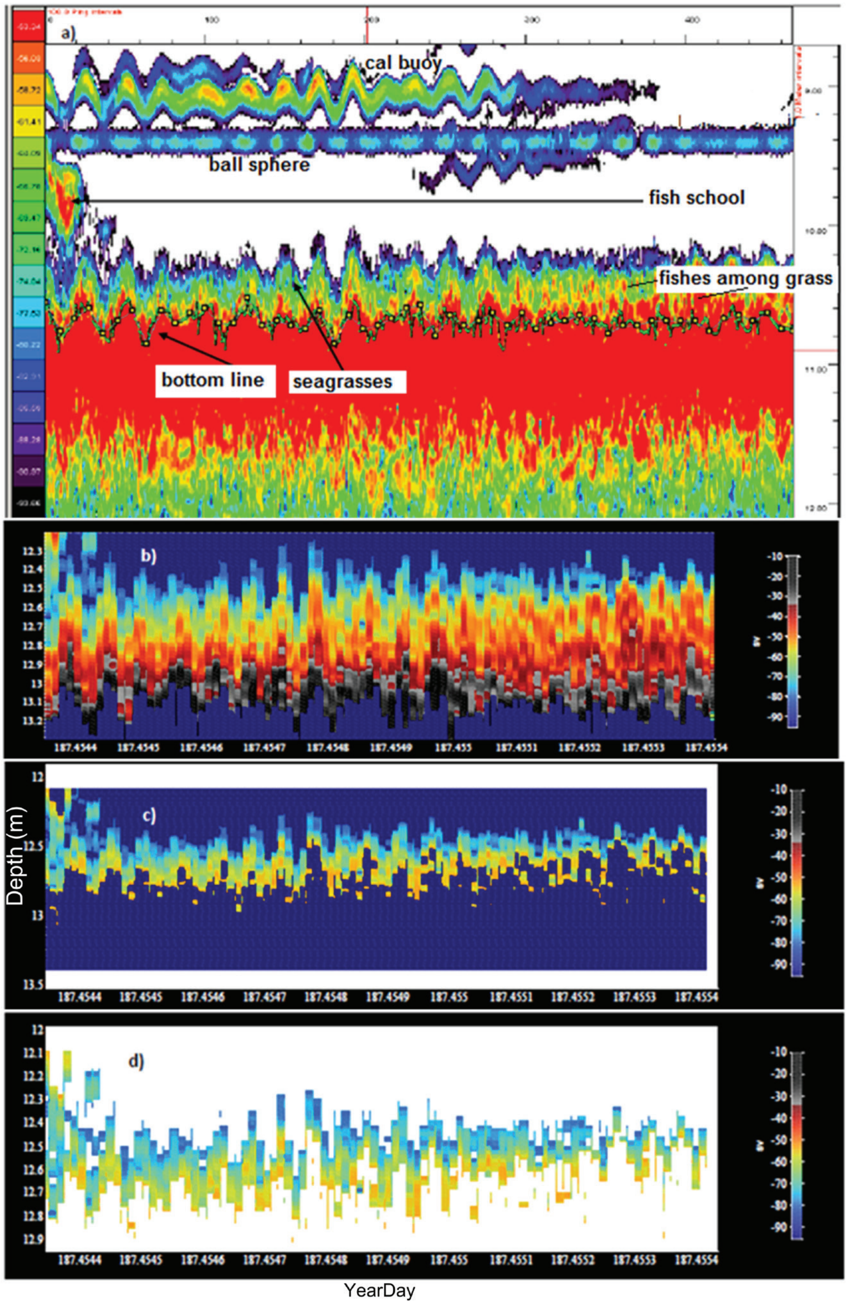

Fig. 6: Unprocessed echogram by Visual Analyzer (a), removal of weak scatterers and bottom echo (b), dead zone and orthotropic (vertical) rhizome of *Posidonia oceanica* (c), and strong scatterers among the seagrass by SheathFinder (d). Depth in range from the transducer.

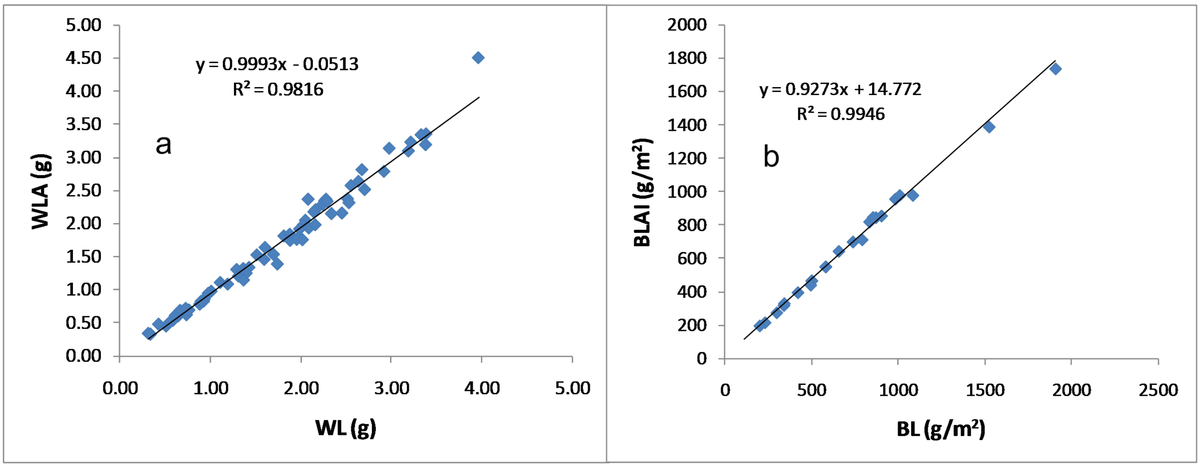

Fig. 7: The linear regression equations to estimate individual weight (a) and biomass (b) based on leaf length (L) and leaf area (LA and LAI) for *Posidonia oceanica* during "leaf" (a) and "cut" experiments (b), respectively (see Appendix 1 for the abbreviations).

was prominent for the acoustic studies to convert the relative energy to the absolute biomass of the meadow. The empirical study for the leaves excluding the base was performed in March and April before a period when the intensive calcification and photosynthetic activities had just started in the meadow (Enriquez & Schubert, 2004).

Leaf + rhizome + sheath

Maximum Sv was measured as around -50 dB, Sa as -40, and TS as -68 dB (Fig. 8). The backscattering strengths were at maxima in December and at minima in July and January. This experiment was conducted only in July 2011 and December and January 2012. However, there was no significant correlation between LA and WLA and backscattering strengths, respectively, with few exceptions at $P < 0.05$, like the regression equations (Fig. 8).

Such insignificant relationships could be explained when examining the density of the meadow structure with the acoustic strengths (Fig. 9). The temporal acoustic energy increased with the density (*d*) of up to 1.5 g cm^{-3}. This seemed to be related to the acoustic response of the frequency to the density. The density up to 1.5 g cm^{-3} fell within a Rayleigh region and then dropped within the geometric region of the acoustic frequency response (Fig. 9). This suggested that the density-related contrast plays a crucial role in the acoustic reflection coefficient.

Leaf + leaf base

The empirical result of this structure of the meadow was preferentially recommended for a conversion equation from the backscattering strengths to the absolute biomass. Overall, there were significant correlation coefficients between the acoustic parameters and LA and WLA at $P < 0.05$ (Fig. 10). Their regression coefficients were also significantly established and linearly fitted at $p < 0.05$. The backscattering strengths were found to be higher in December and April than in January, March, and July (Table 1, Fig. 10). However, there was only a significant difference in the relationships with the TS among seasons (Table 1).

To better understand seasonal differences, a post hoc test was applied to the relationships (Fig. 11). There were indeed significant seasonal differences in a coefficient and intercept (*a*) of the regression equations between Sv, Sa, Sv1, and LA and W, respectively, when there were no significant differences in a regression coefficient, slope (*b*), at $P < 0.05$ (Fig. 11). Unlike the slopes, the intercepts of the relationships with Sv, Sa, and Sv1 were significantly higher in December and April than in January, March, and July (Fig. 11). Unlike the ANCOVA, the post hoc test showed that there were significant differences regarding both coefficients among the seasons.

Like the rhizome + sheath + leaf, the density of the leaf + base created the different seasonal relationships (Fig. 12). The reduced backscattering strengths on average of the density seemed to decrease with the density in fluctuations over a year. The reduced strengths were higher in April, followed by December, than in the rest of the experimental seasons. The maximum reduced strengths were found at a density of 0.6 g cm^{-3}, followed by 1 g cm^{-3} (Fig. 12). Furthermore, the reduced strengths tended to decrease with the density in each season.

Leaf

There is a significant correlation between LA, W, and Sv, and Sa1 in both seasons at $P < 0.05$ (Table 2). Furthermore, the correlation was significantly established between TS, Sv1, and LA, and W, only in April (Table 2).

"Cut" experiment

In contrast to the leaf experiment, reduced backscattering strengths of the meadow were less in December and April than in the rest of the sampling seasons and were significantly established via a linear fitting model with biometric variables of the shoot density, LAI, and biomass (BLAI) at $P < 0.05$ (Figs. 10, 13). The Sa (Sa1) were more strongly dependent on the magnitudes of the biometric variables than Sv (Sv1) (Fig. 13), whereas the EDSUs were oscillated versus the biometric variables (Appendix 2). There were significant differences among the seasons at $P < 0.05$. All relationships in April and July were significantly correlated and established at $P < 0.05$. The reduced backscattering strengths increased with the density (*d*) by 1 g cm^{-3} (Fig. 13d). The leaf density-acoustic relation explained this oscillation better; there was a sharp limitation in the increase (December, April, and August) with the *d* at 1 g cm^{-3} for this relationship (Fig. 13d). Like the experiment, this could be related to the frequency response with the density as follows: the Rayleigh region occurred in a leaf density up to 1 g cm^{-3}, and the geometrical region at the greater density (January, March and July) (Fig. 13d).

Discussion

The present study was the first attempt to detect and sea-truth the acoustic energy of the meadow, *P. oceanica*. Macrophytes are sound scatterers at 200 kHz, having an acoustic impedance of 1.026 to seawater (Kruss *et al*., 2012). Most of the acoustic studies have concentrated only on mapping, detection and bottom coverage of seaweeds (e.g., van Rein *et al*., 2011; Lee & Lin, 2018). Besides, quantification of the seagrasses is very important to monitor the ecological status and health of the marine coastal environments (Colantoni *et al*., 1982; Pal & Hogland, 2022).

Furthermore, here, we introduced the VBT method (parts or divisions of seagrass echo; Hamilton, 2001; Penrose *et al*., 2005) for acoustic and biometric quantification of the seagrasses as Shao *et al*. (2021) used the

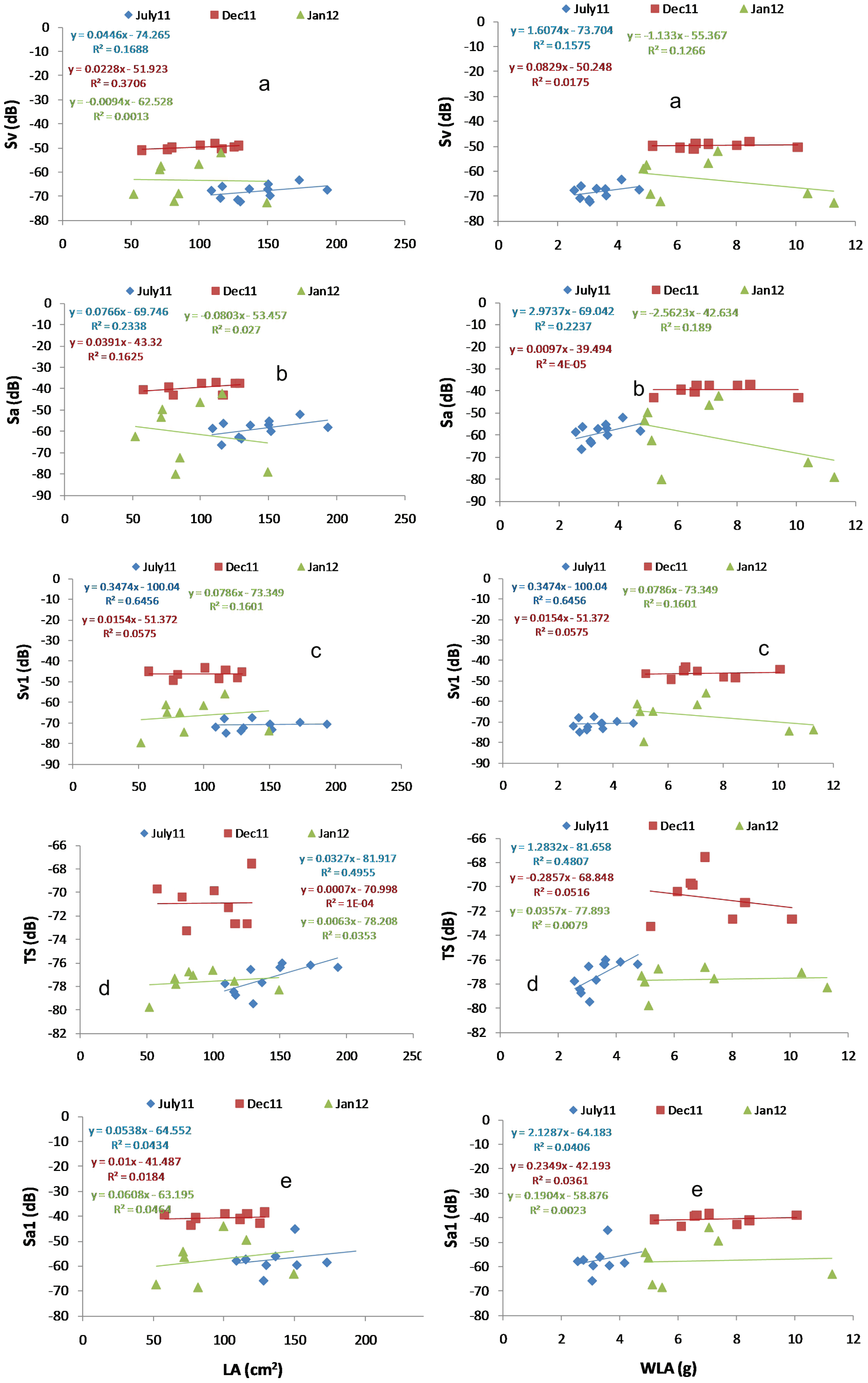

Fig. 8: Seasonal relationship between acoustic data of Sv (a), Sa (b), Sv1 (c), TS (d), and Sa1 (e) and biometric density data of LA and WLA, respectively, including the vertical rhizome and leaf of *P. oceanica* (see Appendix 1 for the abbreviations).

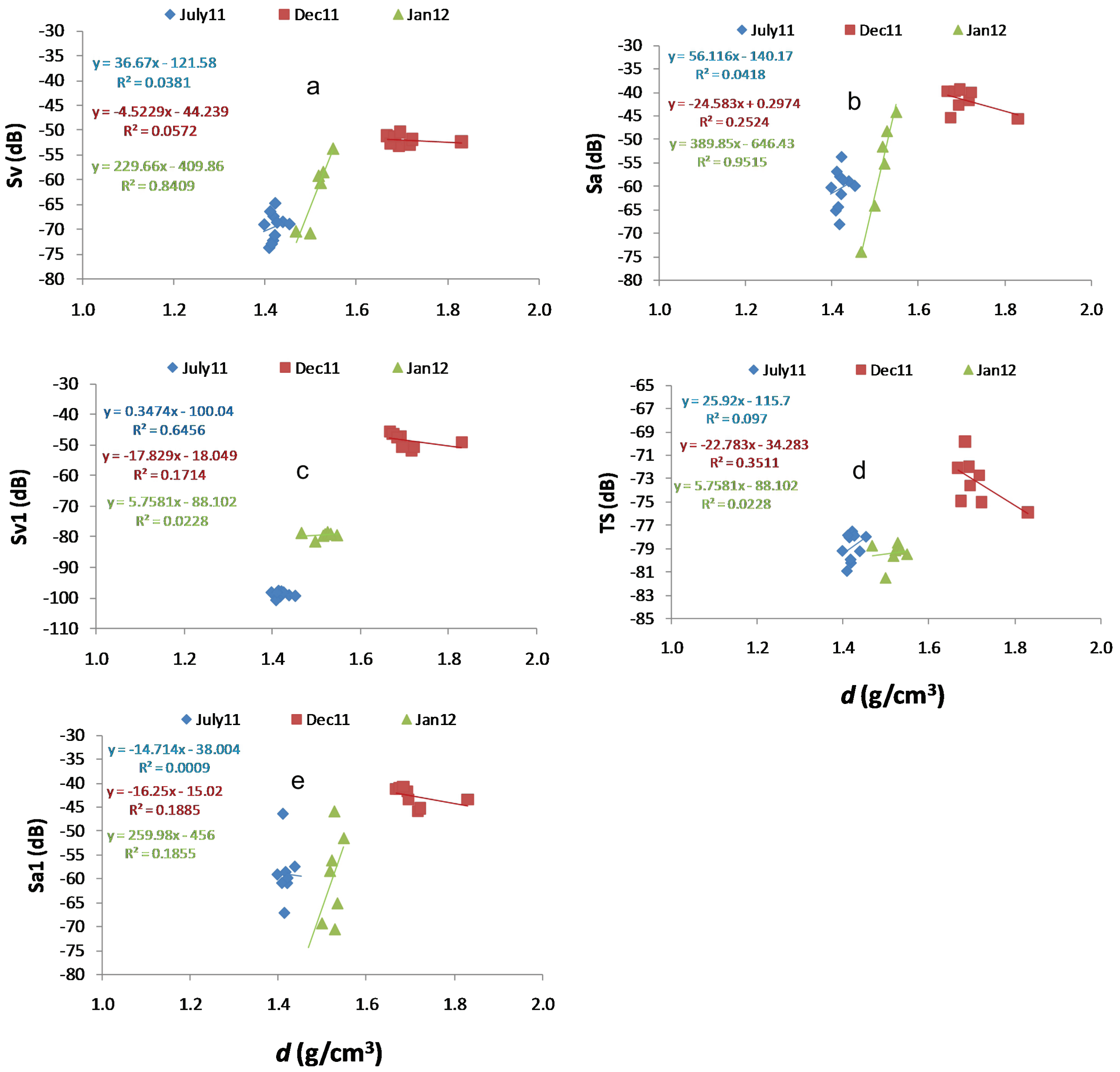

Fig. 9: Seasonal relationship between acoustic data of Sv (a), Sa (b), Sv1 (c), TS (d), and Sa1 (e), and the density, *d*, data including the vertical rhizome and leaf of *P. oceanica* (see Appendix 1 for the abbreviations).

VBT for their classification. This method was previously achieved for the classification and partly quantification of kelp (Hamilton *et al.*, 1999), multispecies vegetation (Mielck *et al.*, 2014), and *P. oceanica* (Olguner & Mutlu 2020). Fakiris *et al.* (2018) made acoustic segmentation and classification of marine habitats including *P. oceanica*.

The dominant Sv and Sa were estimated to be -45 dB in January, March, July, and August, -57 to -58 dB in April and December, and -32 to -33 dB and -50 dB, respectively, for *P. oceanica*. Similar to our estimates, Monpert *et al.* (2012) showed that *P. oceanica* had a low Sv as compared to that of *Zostera marina* at 38 and 200 kHz. The backscattering strengths varied between -57 and -51.5 dB for a shoot density of 200-1200 shoots/m^2 of *P. oceanica* at 200 kHz and were, however, inversely related to the shoot density and percent coverage (Llorens-Escrich *et al.*, 2021). Shao *et al.* (2019) showed that the TS of giant kelp had a high variation with size class (leaf weight: 77-994 g, and length: 0.6-3.2 m). As observed in the present study, there was an oscillation in TS between -69.2 and -34.4 dB with the size class (Shao *et al.*, 2019). A similar resultant observation was determined for seaweed, *Sargassum horneri* (Minami *et al.*, 2021). When comparing the acoustic reflection of the seaweeds with the seagrasses, *P. oceanica* was a moderate scatter based on reduced TS referring cubic power of wet weight (*c.a.* based on leaf length) at 200 kHz (RTS = -89.1 to -85.1 dB in July, -83.6 to -72.6 dB in December, -89.3 to -79.4 dB in January, -97.4 to -80.1 dB in March, and -86.3 to -57.3 dB in April) as compared to *C. nodosa* (RTS = -57.9 to -41.2 dB) (Mutlu & Olguner, unpublished data), *Saccharina japonica* at 200 kHz (RTS = -125.8 to -124.3 dB) (Shao *et al.*, 2019), and *S. horneri* at 120 kHz (RTS = -84.7 to -62.6 dB) (Minami *et al.*, 2021). However, seasonal acoustic measurements have not been considered for the

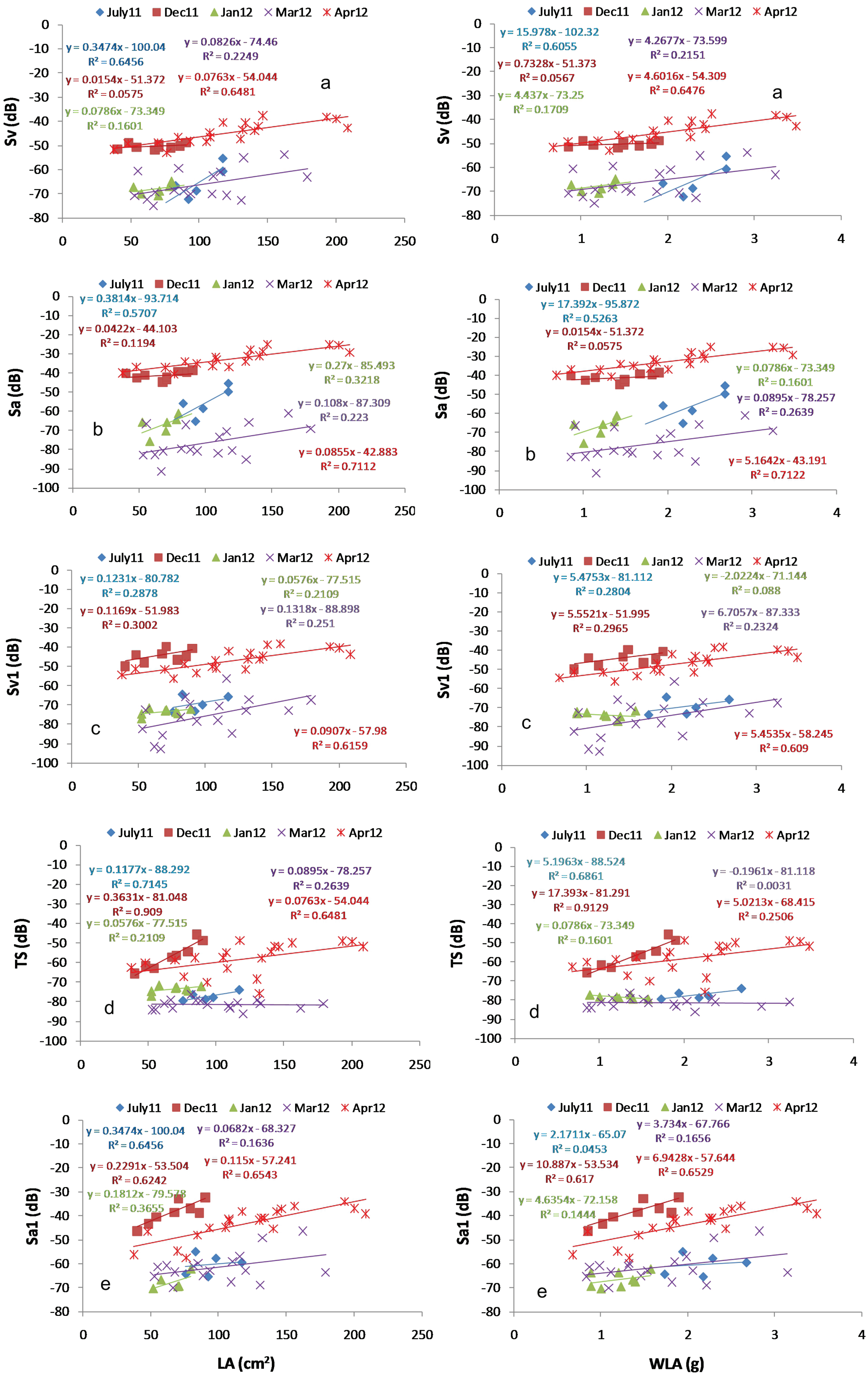

Fig. 10: Seasonal relationship between the acoustic strengths of Sv (a), Sa (b), Sv1 (c), TS (d), and Sa1 (e) and biometric variables (LA and WLA) with leaf + leaf base of *P. oceanica* (see Appendix 1 for the abbreviations).

Table 1. P values of ANCOVA for a linear relationship between the biometric and acoustic data of *Posidonia oceanica* among the seasons. The data were $\log_{10}$-un/transformed. The bold P value denotes significantly different at $P < 0.05$ (see Appendix 1 for the abbreviations).

Species, X/Y	**LA**	**WLA**	**V**	***d***
Seasonal analyses of *Posidonia oceanica*				
Sv	0.3803	0.4923	**0.0493**	0.1409
Sa	0.4766	0.6192	0.0685	0.2310
Sv1	0.9175	0.9853	0.4806	0.1636
TS	**0.0060**	**0.0059**	**0.0100**	**0.0256**
	$\log_{10}$(LA)	$\log_{10}$(WLA)	$\log_{10}$(V)	$\log_{10}$(d)
$\log_{10}$(sv)	0.3079	0.3765	**0.0197**	0.1420
$\log_{10}$(sa)	0.4742	0.5813	0.0534	0.2328
$\log_{10}$(sv1)	0.7982	0.9103	0.5372	0.2196
$\log_{10}(\sigma_{bs})$	**0.0324**	**0.0233**	**0.0215**	**0.0378**

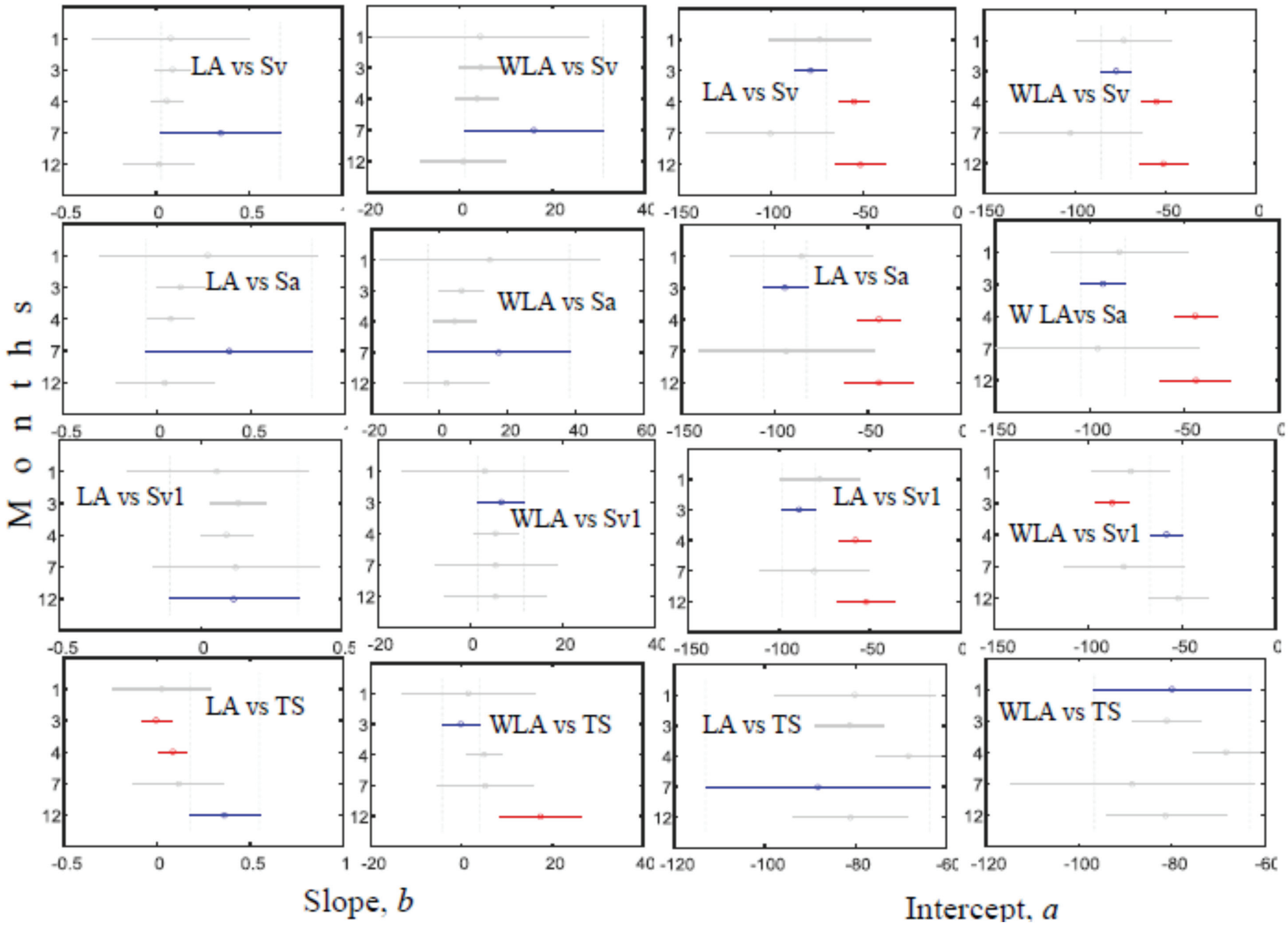

Fig. 11: The post hoc test (Least Significant Difference, LSD) of seasonal relationship based on regression coefficients between acoustic data of Sv, Sa, Sv1, and TS, biometric variables (LA and WLA) with leaf + base of *P. oceanica* (blue mark: to be tested among the months or depths, red: significantly different, grey: significantly not different between vertical discrete grey lines) (see Appendix 1 for the abbreviations).

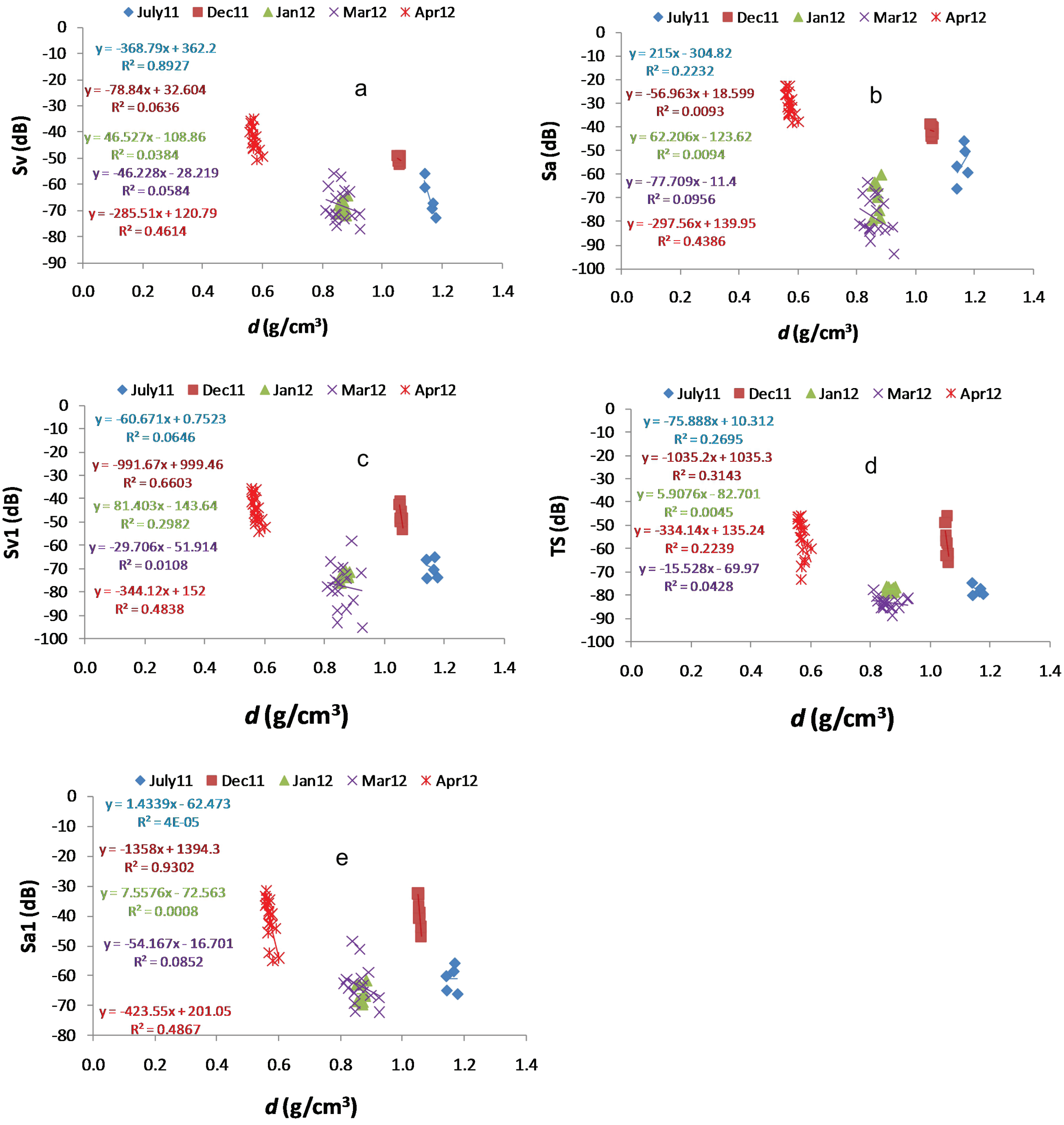

Fig. 12: Seasonal relationship between acoustic variables of reduced Sv (a), Sa (b), Sv1 (c), TS (d), and Sa1 (e) and the density, *d* of the leaf + base of *P. oceanica* (see Appendix 1 for the abbreviations).

relationships of the seaweeds by the previously published studies.

"Leaf + rhizome + sheath" measurements showed unstable and non-linear relationships between acoustic and biometric variables of *P. oceanica*. This could be related to the rhizome having vacuoles (Haznedaroğlu & Akarsu, 2009), which induced instability in the acoustic reflection. Besides, leaves of other *Posidonia* species have air-lacuna (Kuo & Cambridge, 1978; Cambridge & Kuo, 1982; Tseng, 2009). Depending on sound speed and density contrast (*h*, *g*, respectively, ~Reflection coefficient, *R*) (Lavery *et al.*, 2007), kelp, *Ecklonia radiata*, returned different echo energy from its different parts (stipe segments and blades) in the season because of the physiology of the leaves when aging (Bush & Hill, 1983; Randall *et al.*, 2014).

Regarding specimens of non-gas inclusions, one of the strong scatterers is the lime structure in the skeleton of seaweeds (Mavko *et al.*, 1998). This structure changed the reflection coefficient over density and sound speed contrast, referring to the water to carbonate (Merriam, 1999). Furthermore, some porosity occurred during the formation of calcite on the leaves (Aleman, 2004). Therefore, all changes in the structures derived seasonal differences in the backscattering strengths of leaves of *P. oceanica* (Mateo *et al.*, 1997). The calcification rate and the photosynthetic activity of the meadow were at maxima between May and August (Enriquez & Schubert, 2004). This period well coincided with a period of difference in the backscattering

Table 2. Seasonal linear relationship (Y= *a* + *b* * X) between acoustic data of Sv, Sa, Sv1, TS, and Sa1 (Y) and biometric variables of LA and WLA (X) of the leaf of *P. oceanica*. Bold R^2 denotes that there is a significant correlation (r) at $P < 0.05$ (see Appendix 1 for the abbreviations).

Season/X	LA	WLA	Season/X	LA	WLA
March			**April**		
n	16		20		
Sv			Sv		
b	0.0907	4.7999	*b*	0.0603	3.7724
a	-78.19	-77.352	*a*	-54.748	-55.058
R^2	**0.2659**	**0.2553**	R^2	**0.2931**	**0.2963**
Sa			Sa		
b	0.1268	6.6932	*b*	0.072	4.5033
a	-94.407	-93.206	*a*	-43.797	-44.16
R^2	0.222	0.212	R^2	0.385	0.3886
Sv1			Sv1		
b	0.1127	6.054	*b*	0.0065	4.0831
a	-82.433	-81.535	*a*	-56.63	-56.935
R^2	0.243	0.241	R^2	**0.3**	**0.2999**
TS			TS		
b	-0.0007	-0.0681	*b*	0.0796	4.9281
a	-80.979	-80.937	*a*	-68.404	-68.715
R^2	0.0002	0.0005	R^2	**0.4663**	**0.4607**
Sa1			Sa1		
b	0.0996	5.3018		0.0811	5.0718
a	-74.766	-73.901		-53.126	-53.535
R^2	**0.2646**	**0.2571**		**0.35**	**0.3521**

strength of *P. oceanica* from the other months of the present study. Depew *et al.* (2009) expressed similar seasonal variations in the relationships for a seaweed species, *Cladophora* sp. Blight *et al.* (2011) established non-seasonal relationships between the acoustic percent volume inhabited and biomass of the kelp species.

Furthermore, bottom types change the accumulation level of the calcites on leaves; the rock substrate stimulated more calcification on the leaves than the soft bottoms (Canals & Ballesteros, 1997). This suggests that leaves of the present study could perform calcification since *P. oceanica* was found only on rocks in the study area (Mutlu *et al.*, 2022a). Another calcite source is the calcareous red algae on the leaves. During the present study, microscopic algae were not observed on *P. oceanica* until a calcareous red alga, *Hydrolithon boreale*, was detected on the leaves in the winter and summer of 2018-2019 (Mutlu *et al.*, 2023). Seagrass, *P. oceanica*, required carbonate for the growth of its leaves (Milliman, 1993; Canals & Ballesteros, 1997). The meadow did not produce an acoustic region of resonance in the present study, but Rayleigh and Geometric regions were observed (Appendix 2). The EDSUs responded to the acoustic frequency or a function of *k* x *a* (*k*: wave number = 2Π/λ, where λ is acoustic wave length, and *a is* spherical radius of the acoustic scatterers) (Lavery *et al.*, 2007). In the present study, the frequency was fixed to be a single value, 206 kHz, hence λ depened depended on the seasonal sound speed, whereas the size of the biometric variables changed in the experiment. The *a* changed with as the leaf biometrics, hence *k* x *a* changed during the experiments by producing an oscillation called the geometric region in the frequency response. The leaf density, *d*, seemed to produce two regions: Rayleigh (*k* x *a* < 1) and Geometric (*k* x *a* > 1) regions as suggested for the possible acoustic scatterers by Lavery *et al.* (2007). Therefore, compared to the non-resonated region, Wilson & Dunton (2008) observed a resonance region in a difference of 8-10 dB for the seagrasses at lower frequencies. Furthermore, gas-inclusions in the porosity resonated with the backscattering strengths as supported by Manganini *et al.* (2019) for the giant kelp at low frequency.

In conclusion, the present study described a preliminary result of acoustic traits regarding the biometrics and their temporal relation with *P. oceanica*. The meadow is detected well by a frequency of 206 kHz to assess biomass variables, even if the higher frequency (420 kHz, Biosonics Inc.) is recommended to study the seagrass and vegetation acoustics. Quintino *et al.* (2010) recommended 50 kHz to study soft bottom classification, but 200 kHz is effective for the classification of the bottom types including seagrass. Different structural parts (rhi-

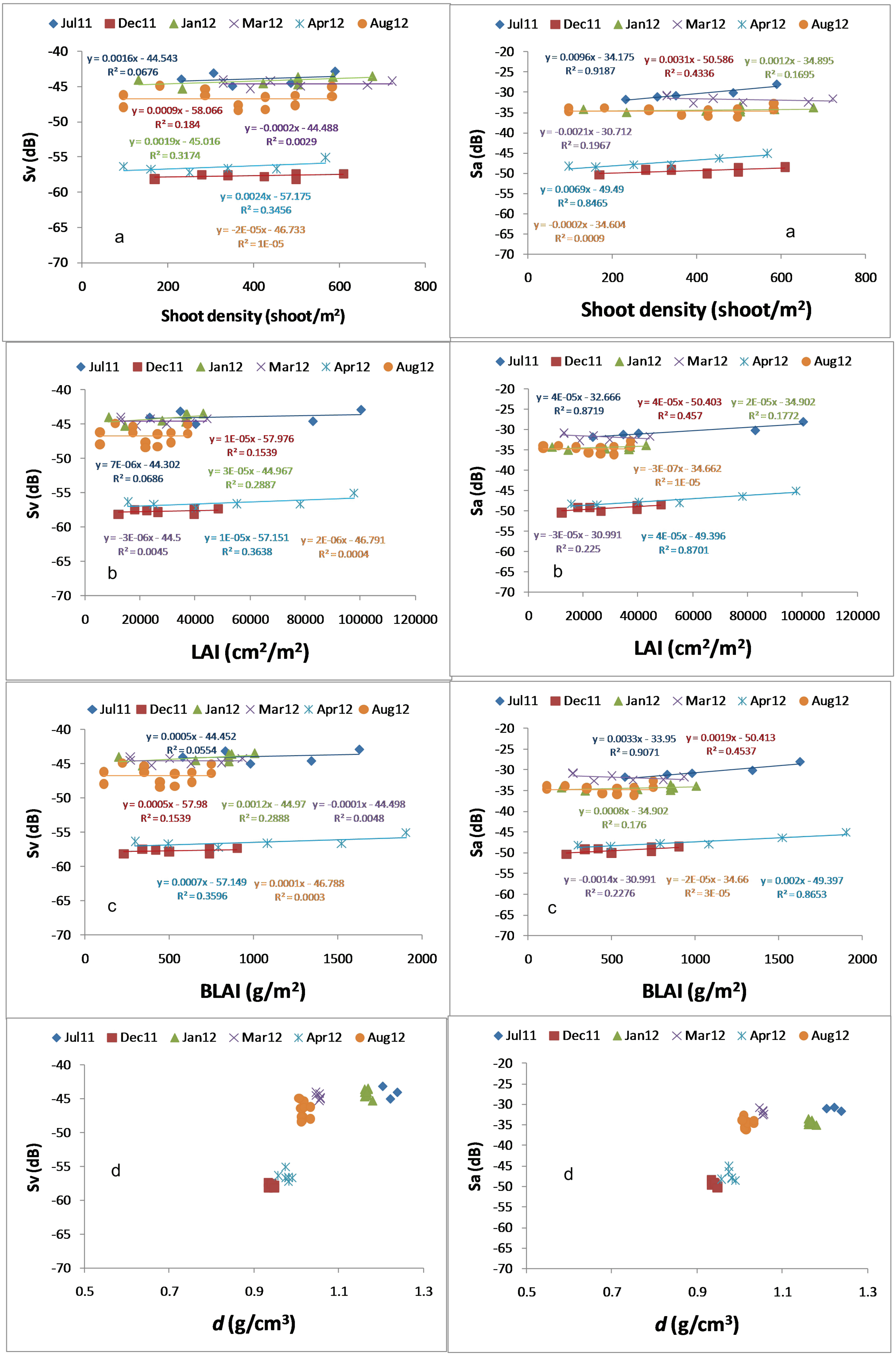

Fig. 13: Seasonal relationship between acoustic variables of Sv (Sv1) and Sa (Sa1) and the biometric variables of shoot density (a), LAI (b), BLAI (c), and reduced Sv and Sa and *d* of the leaf of *P. oceanica* (see Appendix 1 for the abbreviations).

zome, sheath, and leaf) of the seagrass backscattered different echo energies. The "Leaf" and "Cut" experiments showed different models of regressive relationships between acoustic and biometric variables. The "Leaf" experiment showed a linear relationship, and the "Cut" experiment showed a polynomial relationship due to the occurrence of geometrical oscillation depending on the biometric strength. Their estimations were also different. Two assumptions can explain the differences, i) the backscattering strengths depended on strength of biomass as a function of LAI, shoot density, and volume between the two experiments, ii) The "Leaf" experiment measured backscattering strength from individual specimens, and "Cut" experiment from large mass of the seagrass. From the "Leaf" experiment, two different equations can be used to estimate the biomass, derived from the equation Sv=TS+$\log_{10}$(N per m^3), well-known in the acoustical studies as follows:

Sv (mass)=Sv per weight+$\log_{10}$ (B per cubic meter of volume), or

Sa (mass)=Sa per weight+$\log_{10}$ (B per m^2 of area),

where Sa or Sv per weight (*c.a.* weight) corresponding to one bundle of species.

TS and Sv could be different in the experiments since the single target criteria to detect individual leaves in the bundle were effective to estimate TS. The equations of the "Cut" experiment were directly used to estimate the biometrics. Furthermore, there were seasonal differences in the relationships for both experiments. The acoustic reflection was highly dependent on the density of the leaves in time. An experimental bottom depth of 15 m was found to have a higher variation of the biometrics than the shallower and greater depths (Mutlu *et al*., 2022a). Future studies will focus on the relationships of the EDSUs and biometrics at different depths in time since there were depth-wise differences in the biometrics for each season (Mutlu *et al*., 2022a).

Acknowledgement

The present study was carried out within the framework of a project funded by TUBITAK (grant no: 110Y232). We thank Mehmet Gökoğlu and Yaşar Özvarol as SCUBA divers to supply the leaves of the seagrass on board R/V "Akdeniz Su" during the field works. We thank Tim Stanton (Woods Hole Oceanographical Institution, USA) for his valuable reviews and suggestions. We thank two anonymous reviewers for their constructive comments and suggestions.

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Appendix 1. Basic acoustical (VA and VBT) and biometric variables for "Cut" and "Leaf" experiments were used for characterizing *Posidonia oceanica* with their abbreviations in the present stud using "SheathFinder" and VBT analyses. Suffix 1 in the text of the present study for the EDSU's acoustical parameters (e.g., Sv1) estimated with VBT.

Variables Abbreviated	**Description**	**Units**
Acoustical		
VA	Visual Analyzer, Processing software	
Sa	Area backscattering strength	dB m^{-2}
Sv	Volume backscattering strength	dB m^{-3}
TS	Target strength	dB m^{-3}
sa	Area backscattering coefficient	Unitless ($m^2 m^{-2}$)
sv	Volume backscattering coefficient	Unitless, m^{-1} ($m^2 m^{-3}$)
σ_{bs}	backscattering coefficient	Unitless, m^{-1} (m^2 m^{-3})
VBT	Visual Bottom Typer, Processing software	
E0	energy of seaweed echo	Integrated Echo level
E1	energy of second part of 1st seaweed echo, roughness	Integrated Echo level
E2	energy 2nd seaweed echo	Integrated Echo level
E12, E1'	energy of first part of 1st seaweed echo, hardness	Integrated Echo level
S	thickness (length) of the seaweed layer	m
Biometric for "Cut"		
LAI	Leaf Area Index	m^2 m^{-2}, cm^2 m^{-2}
BLAI	Leaf biomass based on LAI	g m^{-2}
BL	Leaf biomass based on leaf length	g m^{-2}
L	Leaf length	cm
V	Leaf volume	cm^3 m^{-2}
d	Leaf density	g cm^{-3}
Biometric for "Leaf"		
LA	Leaf area	cm^2
WLA	Leaf weight based on LA	g
WL	Leaf weight based on L	g
L	Leaf length	cm
d	Leaf density	g cm^{-3}
V	Leaf volume	cm^3

Appendix 2. Seasonal relationship between acoustic variables of Sv (Sv1) and Sa (Sa1) and the biometric variables of shoot density (a), LAI (b), BLAI (c), and reduced Sv and Sa and density, *d* of the leaf of *P. oceanica* (see Appendix 1 for the abbreviations)