

Density-dependent acoustical identification of two common seaweeds (*Posidonia oceanica* and *Cymodocea nodosa*) in the Mediterranean Sea

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Abstract

The non-destructive samplings are very important in not damaging seagrasses and seaweed under protection, at the field studies. The grasses are prominent in the assessment of the ecological status of the marine environments. One of the effective non-destructive samplings was the acoustical methods which need a low level of the sea and atmospheric conditions as compared to the other remote sensing system. Like the others, acoustic data alone are inherently ambiguous concerning the identities of the scatterers and need sea-truthing at the field studies. Considering the requirements above and the advantages of the acoustical methods, an acoustical *in situ* study was conducted to discriminate two dominant seagrasses along the western Turkish Mediterranean coast in August (in the year 2012) when biometrics of both species was at maxima. Four different regions were involved in the study during data collection using a split beam echosounder operated at 206 kHz since each region had different strengths of their biometrics reflecting regional variations of the acoustical measurements. For discrimination, a statistical approach based on the acoustical parameters and their statistics was enriched and validated with multivariate analyses (Silhouette, k-means, PCA and CAP analyses). *Posidonia oceanica* was correlated with mean, median, SD and maximum value of Sa, whereas *Cymodocea nodosa* was characterized by hardness and roughness of leaf echo, followed by skewness and kurtosis of Sa. The acoustics of *C. nodosa* were related to regional differences, but *P. oceanica* was related to the depthwise difference. Both species had highly different densities (g/cm^3), followed by biomass. The present study will interactively help acoustical studies to be more common and comprehensible and the other previous studies published did as well.

Introduction

Of the seaweeds in the Mediterranean Sea, two dominant seagrasses, *Posidonia oceanica* and *Cymodocea nodosa* are ecologically very important covering the infralittoral zone (*P. oceanica* and *C. nodosa*) extending to the circalittoral zone (*C. nodosa*) (Colantoni et al., 1982; Pal and Hogland, 2022; Mutlu 2022a, b, c). Their density parameters such as leaf biomass, Leaf Area Index (LAI), and leaf length play a crucial role in the determination of their population dynamics, seasonality, ecology, management, sustainability, protection and blue carbon content (Colantoni et al., 1982; Brown et al., 2011; Pal and Hogland, 2022). These parameters were precisely estimated by a destructive method, SCUBA diving to sample seagrasses. Most seaweeds have been under protection. Therefore, the description of the destructive sampling has been specialized for the seagrass as follows; sampling more than 1000 shoots (Mutlu et al., 2022c), more than orthotropic shoots $n = 20$ (Pergent et al., 1995), and more than one-third of the sampling quadrant by cutting leaves above the ligule to recover (Gobert et al., 2020).

Consequently, remote sensing and methods are very important for studying sensitive and vulnerable seaweeds such as *Posidonia oceanica* and *Cymodocea nodosa* (McGonigle et al., 2011). The density variables of the seaweeds are important for a variety of purposes and aims of ecological studies (Colantoni et al., 1982; Brown et al., 2011; Pal and Hogland, 2022; Mutlu et al., 2022a, b). Early studies developed a variety of non-destructive methods to study seaweeds for only their distributional mapping by 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 and Downie, 2020). Recently, studies have tended to use non-destructive methods to study seaweeds (Gobert et al., 2020; Montefalcone et al., 2021; Zhu et al., 2021). Most recently, a satellite called "Sentinel 2" was launched, and the studies started to exceed the mapping by calibrating the reflectance and absorbance of the light by seaweeds with their density variables. Apart from the acoustics, methods based on images required clear air and seawater in flat conditions (Vis et al., 2003; Hossain and Hashim, 2019; McCarthy and Sabol, 2000). Another issue for all methods is the identification of species by remote-sensing data and the need for the sea-truthing of the seaweeds. Therefore, the studies were restricted to coverage and mapping of seaweeds on bottoms, and habitat typing (e.g. Fakiris et al., 2018; Dimas et al., 2022).

Of the techniques, the acoustical 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 an assessment of percent coverage and canopy height of the seagrass (e.g. van Rein et al., 2011; Lee and Lin, 2018) depending on the limitation of echosounder parameters (Lurton, 2002). There were few *in situ* attempts to calibrate the acoustical data to estimate the biometrics of the seagrass (Mutlu and Balaban, 2018; Olguner and Mutlu, 2020).

Recently, *in/ex situ* acoustical studies have increased to relate the biometrics to the Elementary Distance Sampling Unit (EDSU, Simmonds and MacLennan, 2005) of different seaweeds; Depew et al. (2009) on *Cladophora* sp., Monpert et al. (2012) on *P. oceanica*, and *Zostera marina*, Llorens-Escrib et al. (2021) on *P. oceanica*, Shao et al. (2019) on *Saccharina japonica*, Minami et al. (2021) on *Sargassum horneri*, Mutlu and Olguner (2023a) on *P. oceanica*, and Mutlu and Olguner (2023b) on *Cymodocea nodosa*. Unlike the *ex-situ* studies, the *in situ* studies needed a sea-truthing to determine the species of seaweeds. Overall, either multifrequency or statistical analyses for single frequency have been applied to classify the species (e.g. SIMFAMI 2002; Simmonds and MacLennan, 2005; Wang et al. 2018) since acoustic data alone are inherently ambiguous about the identities of the scatterers. Furthermore, the forward problem (solution) which has not been applied to seaweeds yet is very useful to discriminate the species EDSUs e.g. (Wiebe et al., 1996, 1997; Mutlu, 2005). Depending on the density and sound velocity contrast (h , g , respectively $\sim R$) of specimens the scatterers showed different acoustical properties (Bush and Hill, 1983; Randall et al., 2014; Mutlu and Olguner, 2023a, b) and reflection coefficient (R) in addition to dispersal type of the seaweeds (e.g. Mutlu et al., 2022a, b, c). Such circumstance helps the statistical treatment and interpretation of the EDSUs for the classification of seaweeds. A variety of these methods has been often used for fish species discrimination (Simmonds and MacLennan, 2005). Another acoustical method, the bottom typer i.e. Visual Bottom Typer (VBT, Biosonics inc.) was often used recently for the classification of habitat types including seaweeds (Hamilton, 2001; Penrose et al., 2005; Hamilton et al., 1999; Mielck et al., 2014; Fakiris et al. 2018; Olguner and Mutlu, 2020; Shao et al. 2021; Mutlu and Olguner, 2023a).

In the present study area, biometrical distribution and ecological dynamics of *P. oceanica* (i) and *C. nodosa* (ii) in time and space were as follows: i) "About one-third of the study area was inhabited by the meadow found only calcite rocks on bottoms between 0.5 m and 29 m. Shoot density was not significantly different among seasons, and was above 364 ± 27 shoots.m⁻², but was different among the depths. The density variables decreased along the bottom depth gradient along which the number of leaves per shoot, inter-shoot distance and morphometrical variables tended to increase. Inferring the dynamics of biometrics (length and width of leaf, orthotropic rhizomes and leaf sheath) and density (LAI, leaf biomass and the number of leaves per shoot), biometrics of the meadows grew seasonally between growth by March and mortality by August-September, regardless 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 as August for variation of the biometrics, respectively" (Mutlu et al., 2022a). ii) "Of *C. nodosa*, excluding the number of leaves per shoot and internodal distance, the grass densities and plant traits showed regional (four bays) and depth (5–20 m) differences; higher in Kekova and Kaş Bays, both on sit on the rim current, compared to Finike and Antalya Bays were virtually devoid of the grass and rim current (Fig. 1). This was studied at 5 m in exposure to the waves than the greater depths. The gravel content in the sediment also dictated the occurrence of the grass. Density and plant traits peaked in late autumn and early spring depending on water temperature, salinity and nutrient limitations, and were minimal during summer" (Mutlu et al., 2022b).

Leaf density (g/cm³, and shoots/m² and g/m²) and dispersal type of two seagrasses were considered as hypothesis to test relation to their acoustical measurements only for sea-truthing their biometrics (morphometrics). For this purpose, one of the common seagrasses in the Mediterranean prairies is *P. oceanica*, followed by *C. nodosa* and they are under protection. The destructive methods are strictly recommended to avoid the application of the seagrasses. Therefore, the present study has been considered conduct to separate these two seagrasses using the acoustical data. Regarding the ecological importance of the meadow prairie and the lack of historical comprehensive information based on the acoustics in the Mediterranean, this study aims to provide statistical discrimination of *P. oceanica* and *C. nodosa* using statistics of their EDSUs measurements in the field.

Material And Methods

The present study was carried out on the western coast of the Turkish Mediterranean in August 2012 to acoustically classify two common seagrasses (*Posidonia oceanica* and *Cymodocea nodosa*) by R/V Akdeniz Su. The leaves of both species were the longest in August (Mutlu et al., 2022a, b). The study was conducted on the infralittoral zone of four different areas; Antalya, Finike, Kekova, and Kaş coasts (Fig. 1). Antalya was inhabited by *P. oceanica*, Finike and Kekova by *C. nodosa* and Kaş by both *P. oceanica* and *C. nodosa*. Some other seaweed, to a lesser extent, was available in the study area but did not perform prairie on the bottom (Mutlu et al, 2022d).

Acoustic data was collected with a DT-X digital scientific echosounder with a looking-down split-beam transducer (beam width: 6.8°) operated at 206 kHz (Biosonics inc.) by Visual Acquisition (v. 6, Biosonics inc.). Before the acoustical data collection, the echosounder was calibrated at a pulse width of 0.4 msec with a tungsten ball supplied by Biosonics inc. The seaweed data was collected at a pulse width of 0.1 msec, a pinging rate of 5 pings/sec, and a threshold of -140 dB for at least 10 mins. The R/V Akdeniz Su was anchored at 24 sampling stations of the present study (Fig. 1). Each sampling station was inhabited only by either *C. nodosa* or *P. oceanica*.

The seagrasses were picked up by SCUBA divers at each station to measure their biometrics (leaf biomass, area, length, width) at Lab. During the diving, SCUBA observers noted orientation of the seagrasses as right, semi-right and flat to relate their beam patterns of the seagrasses to results of the statistical analyses (Table 1).

Acoustical data were converted to Comma Spread Verbose (CSV) format at a horizontal resolution of ping-to-ping and a vertical resolution of count-to-count (1.87 cm, one-eighth of pulse width) using a threshold of -140 dB by Visual Analyzer (v. 4.1.2.42, Biosonics inc.). The CSV data was processed through a script, namely SheathFinder (Mutlu and Balaban, 2018) to estimate Sv (dB m⁻³), subsequently Sa (dB m⁻²) of the seagrasses averaging on a bottom area of 25 m² at each station (Fig. 2).

Furthermore, Visual Bottom Typer, VBT (vers. 1.10, Biosonics inc.), a bottom-type definition program was used to estimate hardness and roughness, echo level (EL) and its fractions (Table 1) of the seagrasses classed by the B4 Fractal Dimension Method of the VBT (Fig. 3). The VBT was configured to follow automatically two seagrasses.

A data matrix was formed from the biometrics and acoustics to be involved in the statistical analyses. Besides, basic descriptive statistics were added to the data matrix (Table 1). The leaf biomass was estimated based on two morphometrics, leaf length (BL) and single-sided Leaf Area (BLAI), and their relationships between the biomasses were established for *P. oceanica* and *C. nodosa* (Fig. 4).

Table 1
Basic acoustical and biometrical variables were used in multivariate analyses to characterize *Posidonia oceanica* and *Cymodocea nodosa* during the field survey calibration using “SheathFinder” and VBT analyses.

Variables Abbreviated	Description	Units
Acoustical		
SaM	Mean Sa	dB
SaMx	Maximum Sa	dB
SaSD	Standard deviation of Sa	dB
SaSkv	Skewness of sa	
SaCur	Kurtosis of sa	
SaMed	Median of sa	dB
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
Biometrical		
LAIMn	Minimum Leaf Area Index	m ² /m ²
LAIMx	Maximum Leaf Area Index	m ² /m ²
LAIM	Mean Leaf Area Index	m ² /m ²
BLAIMn	Minimum leaf biomass based on LAI	g/m ²
BLAIMx	Maximum leaf biomass based on LAI	g/m ²
BLAIM	Mean leaf biomass based on LAI	g/m ²
BLMn	Minimum leaf biomass based on leaf length	g/m ²
BLMx	Maximum leaf biomass based on leaf length	g/m ²
BLM	Mean leaf biomass based on leaf length	g/m ²
SNMn	Minimum shoot density	shoots/m ²
SNMx	Maximum shoot density	shoots/m ²
SNM	Mean shoot density	shoots/m ²
L	Leaf length estimated from the acoustic	m
Orn	Orientation of leaves to bottom	Right, semi-right, flat
LM	Mean leaf length from the field samples	cm

One univariate analysis was applied to the biometrical and acoustical variables (Table 1) as follows; the Spearman correlation coefficients were estimated to test the significance between correlations of the biometrics and acoustics at $p < 0.05$. Statistical treatment and interpretation were subsequently performed by a series of multivariate analyses. i) A Silhouette analysis was conducted to estimate the optimum number of clusters using a normalized matrix of the acoustical data and statistics (Table 1) measured at 25 stations in August 2012 (Fig. 1). The data were normalized with a help of PRIMER 6 (vers. 6.1.13) + PERMANOVA + (1.0.3.), ii) the k-means analysis was then performed to partition the data into the optimum clusters, and to estimate their total “city-block” distance to separate the clusters, iii) the Principal Component Analysis (PCA) was applied to the normalized acoustical data to assess which acoustical variable was linearly effective to discriminate the *C. nodosa* and *P. oceanica* in the regions, and iv) Canonical Analysis of Principal Coordinates (CAP) was used to test the significance of the correlation and discrimination based on each component determined in the PCA.

Spearman correlation, Silhouette, and k-means analyses were done using the statistical tools of MatLab (2021a, MathWorks). The rest of the analyses were done using the PRIMER 6.

Results

Echograms of *C. nodosa* and *P. oceanica* were visually appeared to be highly different on comparison with their acoustical reflectance (Fig. 2). This derived statistical derivation and discrimination for the following statistical analyses.

Figure 5 showed the results of the Silhouette analysis; the total city-block distance was at maxima for the analysis with 2 clusters, followed by 3 clusters. The minimum distance was estimated for 4 clusters. Therefore, the optimum number of cluster was estimated to be 3 since one of the clusters in the 2 cluster did not exceed 0.5, and the distance which has negative distance was the lowest for 4 clusters as compared to the other clusters.

The acoustical data was well partitioned into 3 clusters (Fig. 6). Regional and specific discrimination was observed according to the locations of *C. nodosa* and *P. oceanica* with few exceptions (Figs. 6, 7a). Data scattering on the k-means plot has well coincided with local and specific labels. Regional differences of both species occurred in the plot (Fig. 6), and this was better explained with the PCA (Fig. 7).

PCA showed there were specific on PCA1 and regional discrimination on PCA2. On the PCA1 axis, two species were separated and explained by a percent variance of 39.5% (Fig. 7). A critical variance of 95% for the best explanation reached at about PCA5 (Online Resource 1). The hardness and roughness were descriptive parameters to correlate with *C. nodosa* whereas the EDSUs and their statistics were prominent parameters to correlate with *P. oceanica* on PCA1, and partly on PCA2 (Figs. 7, 8, Online Resource 1). Besides, *C. nodosa* had regional discrimination between Finike-Kekova and Kaş. On the PCA2 axis, the seagrasses were differentiated into particulate regions for *C. nodosa*, and this difference was explained with a variance of 24.5% (Fig. 7). Acoustical properties of *C. nodosa* were different in Kekova-Kaş compared to Finike. This difference was derived from the high skewness and kurtosis of the EDSUs in Kekova-Kaş (Fig. 8, Online Resource 2).

Furthermore, the difference in inter-species and –regions was best explained by standard deviation, mean and maximum of Sa (Fig. 8, Online Resource 2). Mean Sa was correlated with PCA1 whereas maximum and standard deviation of Sa were commonly identical variables for both PCA1 and PCA2 implying that there were regional and species differences owing to dispersal type of the seaweed (Fig. 8, Online Resource 2).

The bottom depth was not so much effective in the regional difference of each seagrass concerning their acoustical characteristics (Fig. 9) even though the bottom depth was found to be the main factor to change the biometrics for both species (Mutlu et al., 2022a, b). An identical variable was found to be leaf density between *C. nodosa* and *P. oceanica* which had lower density (Fig. 9). This supports effective acoustical variables that were correlated with hardness and roughness between the species. Leaf length and LAI were partially correlated with the configuration of the PCA solution (Figs. 7, 9). However, the orientation of the seagrasses on the ground seemed not to be related to the PCA results (Online Resource 3).

To find out the best component explaining the biometrics, the CAP showed that there was a significant difference in variation ($tr(Q_m'HQ_m)$), and correlation (δ^2) of the acoustical variables with the axes of the PCA ($tr(Q_m'HQ_m) = 1.058$, $P = 0.027$, and $\delta^2 = 0.834$, $P = 0.001$) (Fig. 9). This significant correlation validated the specific and regional discriminations plotted on the PCA at $p < 0.05$ (Fig. 7).

In general, SaM and SaMed were significantly and positively correlated with most of the biometrics at $p < 0.05$ whereas E12 (hardness) was negatively correlated with the biometrics, followed by E0 (seaweed echo level) (Table 2). Leaf length measured from the acoustics and SCUBA was significantly correlated with most of the acoustical variables at $p < 0.05$ (Table 2).

Specific correlation analysis showed a better explanation for regional differences in contrast to the PCA solution (Fig. 7, Appendices 4, 5). The E0 and E12 were positively and negatively correlated with the biometrics of *C. nodosa*, respectively whereas the S was negatively correlated with the biometrics of *P. oceanica* contrary to the PCA correlations (Fig. 7, Appendices 4, 5).

Table 2

Spearman correlation coefficients between the biometrical and acoustical variables from 25 stations (see Table 1 for the abbreviations of the variables). Bold coefficients are significant correlations at $p < 0.05$.

	LAIMn	LAIMx	LAIM	BLAIMn	BLAIMx	BLAIM	BLMn	BLMx	BLM	SNMn	SNMx	SNM	L	LM
SaM	0.401	0.439	0.432	0.343	0.410	0.382	0.442	0.535	0.518	0.193	0.258	0.246	0.634	0.335
SaMx	0.349	0.067	0.151	0.337	0.064	0.147	0.305	0.134	0.189	0.213	-0.015	0.054	0.561	0.506
SaSD	0.217	0.216	0.217	0.190	0.194	0.183	0.233	0.293	0.277	0.294	0.187	0.234	0.461	0.438
SaSkv	-0.164	-0.331	-0.287	-0.124	-0.304	-0.260	-0.220	-0.339	-0.293	-0.022	-0.216	-0.168	-0.100	-0.020
SaCur	-0.085	-0.276	-0.233	-0.047	-0.250	-0.205	-0.152	-0.282	-0.237	-0.001	-0.195	-0.151	-0.003	0.078
SaMed	0.482	0.497	0.504	0.409	0.453	0.445	0.542	0.606	0.603	0.072	0.165	0.135	0.665	0.368
E0	0.504	0.264	0.342	0.538	0.251	0.361	0.427	0.222	0.304	0.488	0.267	0.351	0.486	0.076
E1	-0.121	-0.092	-0.089	-0.023	-0.056	-0.027	-0.198	-0.227	-0.214	0.320	0.219	0.255	-0.301	-0.594
E2	-0.093	-0.364	-0.296	-0.057	-0.337	-0.264	-0.195	-0.403	-0.349	0.147	-0.120	-0.025	-0.256	0.169
E12, E1'	-0.462	-0.594	-0.575	-0.417	-0.564	-0.553	-0.524	-0.644	-0.627	-0.207	-0.387	-0.335	-0.506	-0.049
S	0.322	0.067	0.098	0.288	0.018	0.069	0.339	0.134	0.157	-0.062	-0.232	-0.186	0.558	0.575

Discussion

Such statistical techniques are often used for marine species identification based on the acoustical variables provided by echosounder, sonar, and side-scan sonar (Simmonds and MacLennan, 2005). Furthermore, some bottom typing software was used for the classification of the habitat, and species (Wang et al., 2018). During the present study, the combination of echosounder and the VBT was used to achieve the classification and discrimination of *C. nodosa* and *P. oceanica* using the statistical solution. Shao et al. (2021) used the 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 and Mutlu 2020). Fakiris et al. (2018) made acoustical segmentation and classification of marine habitats including *P. oceanica*.

The frequency response to each seaweed was found to be different (Mutlu and Olguner, 2023a, b). 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* (Mutlu and Olguner, 2023a). The dominant Sv and Sa of *C. nodosa* were estimated to vary between -45 and -50 dB, and -35 and -45 dB, respectively, regardless of the seasons (Mutlu and Olguner, 2023b). Monpert et al. (2012) showed that *P. oceanica* had 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/m² of *P. oceanica* at 200 kHz and was however reversely related to the shoot density and percent coverage (Llorens-Escrib et al., 2021) unlike a study conducted by Mutlu and Olguner (2023a).

Both species had different biometrics concerning leave length and width, the number of leaves per shoot, rhizome length and diameter, sheaths length and width, population dynamics, and dispersal type inside their patches (Mutlu et al., 2022a, b). This difference is reflected in their acoustics and statistics. For instance, the biomass of the leaves increased toward the center of patches, far away by 6 m from the patch margin of *C. nodosa* (Duarte and Sand-Jensen, 1990; Nero et al., 1989) to increase skewness and kurtosis of Sa or Sv.

Furthermore, biological activities such as photosynthesis and calcification by leaves accelerated from May to August (Enriquez and Schubert, 2004), which changed density contrast for acoustical reflection and density of leaves. For instance, in *P. oceanica*, one of the strong scatterers is the lime structure in the skeleton of the 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). Besides, some porosity occurred during the formation of the 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) to increase Sa or Sv (Mutlu and Olguner, 2023a). The calcification rate and the photosynthetic activity of the meadow were at maxima between May and August (Enriquez and Schubert, 2004). This could be variable depending on the species. 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 acoustical percent volume and biomass of the kelp species.

In conclusion, the present study was the first attempt to classify *C. nodosa* and *P. oceanica* with their biometrics and acoustical measurements using statistical analyses in the Levantine Sea. The statistical approach has been subjected to the assessment of habitat types, mapping and coverage of the seaweeds (e.g. van Rein et al., 2011; Lee and Lin, 2018; Wang et al., 2018). Sa and Sv increased for *P. oceanica* contrasted to *C. nodosa*. High skewness and kurtosis of Sa were typical to recognize *C. nodosa* in the region. This contrast was induced by the biomass of the seaweeds, followed by the leaf density. Unlike non-omnidimensional gas-inclusion organism such as fish with elongated swim bladder, orientation of the seagrasses on the ground, beam pattern was not so efficient to characterize the acoustical discrimination since such bulk of the submerged seagrasses standing on

the ground were entirely measured acoustically. The present study could be useful and considered to study bottom types, habitat types, mapping and coverage of the seagrasses. A future study would be considered and conducted to analyze their classification in different seasons and frequencies.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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Figures

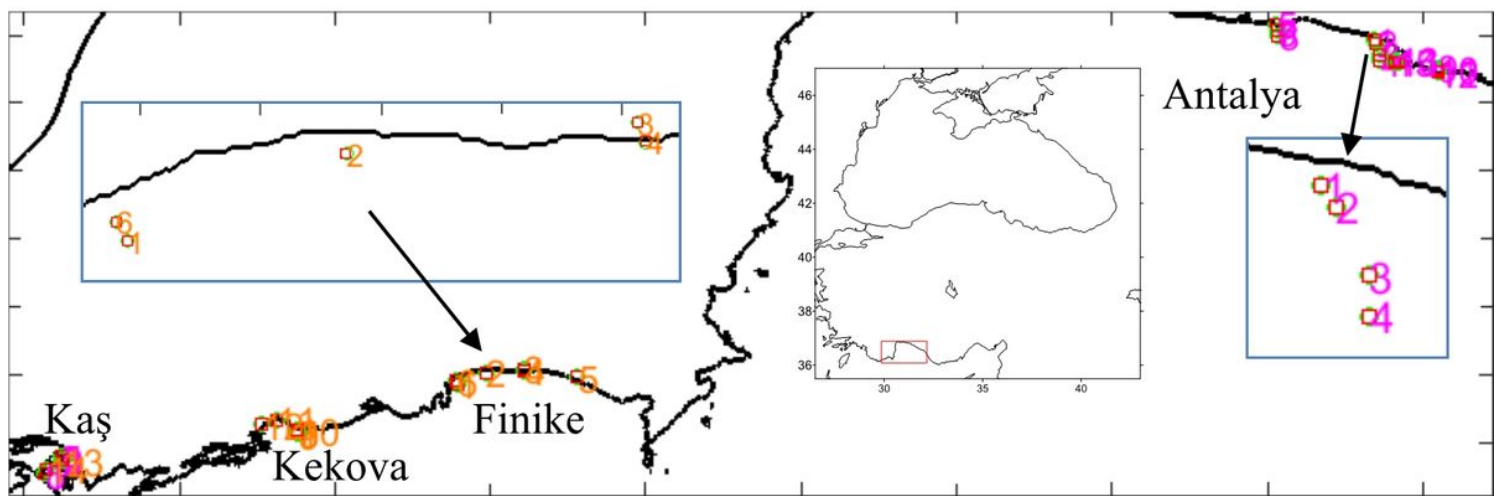


Figure 1

Study area, and field survey calibration for *Posidonia oceanica* with a number in magenta and *Cymodocea nodosa* with the number in orange in August 2012 by matching locations of acoustical measurement (green circle under magenta or orange rectangle) and SCUBA sampling (magenta or orange rectangle).

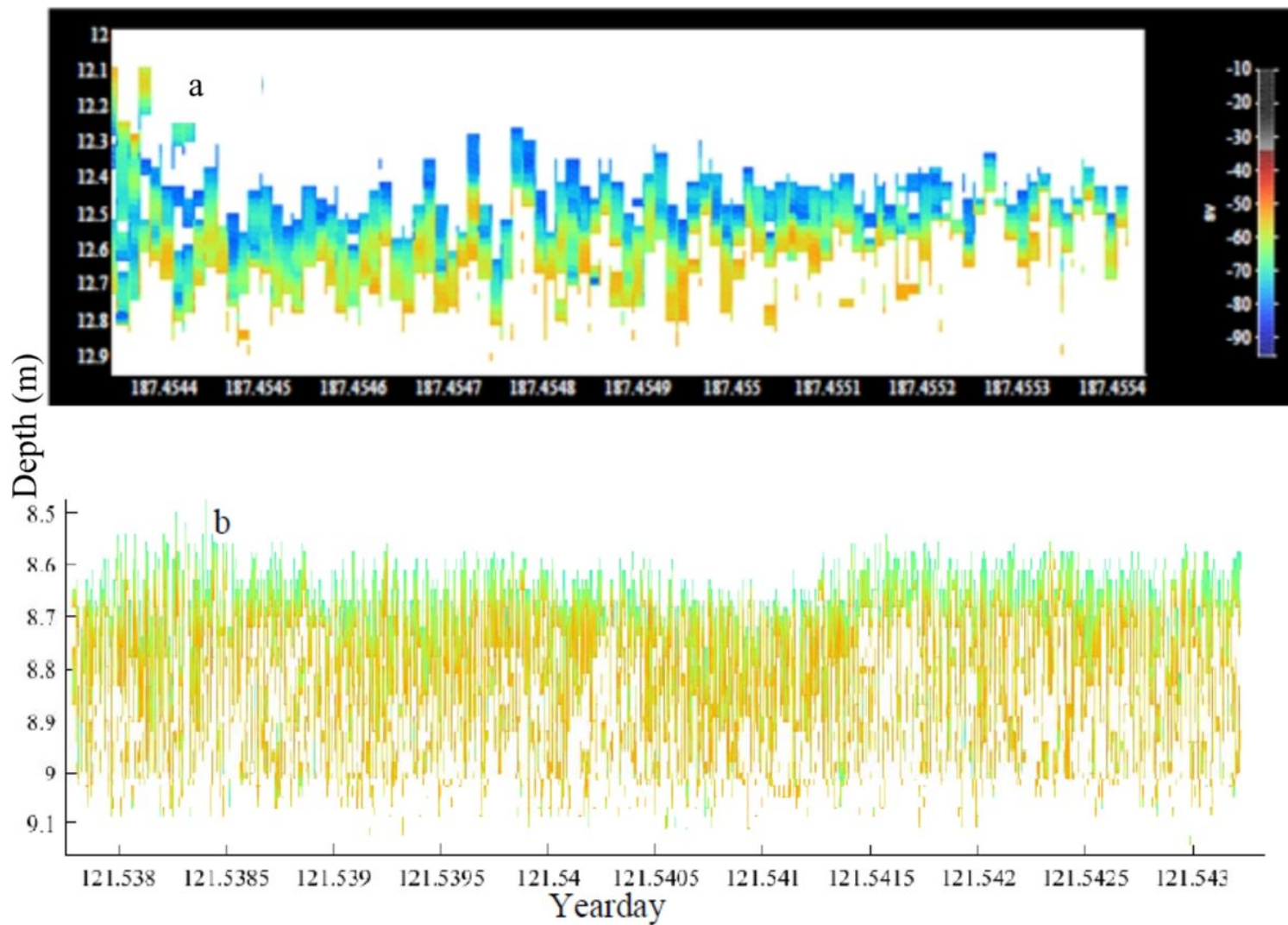


Figure 2

An example of the removal of weaker and stronger scatterers, bottom echo, dead zone, and vertical rhizome and sheath of *Posidonia oceanica* (a) and *Cymodocea nodosa* (b) by “SheathFinder” to estimate the EDSUs (Mutlu and Olguner, 2023a, b).

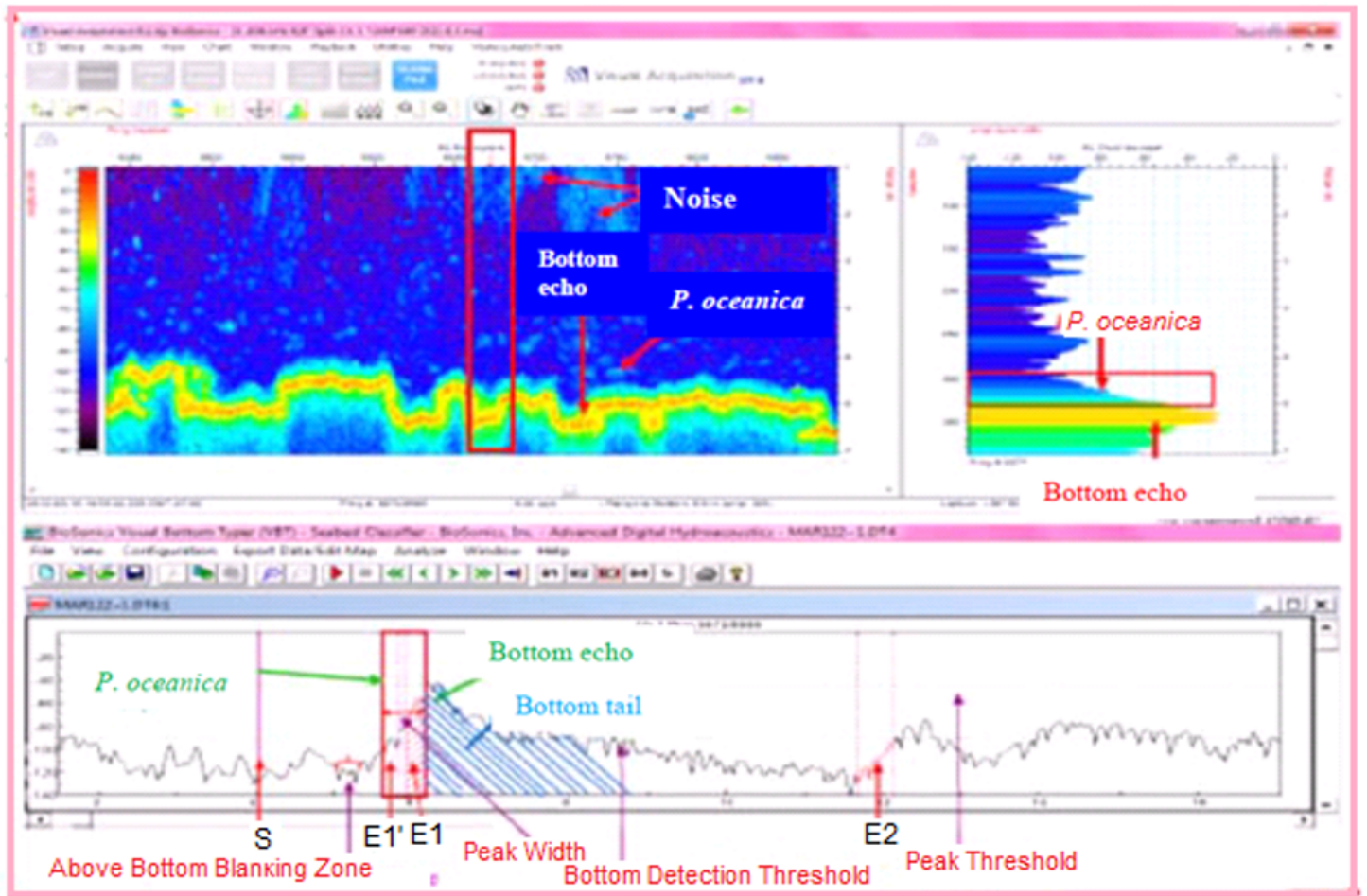


Figure 3

An echogram of the acoustical data and detection of the *P. oceanica* with the Visual Acquisition and VBT commercial software (from Olguner and Mutlu, 2020).

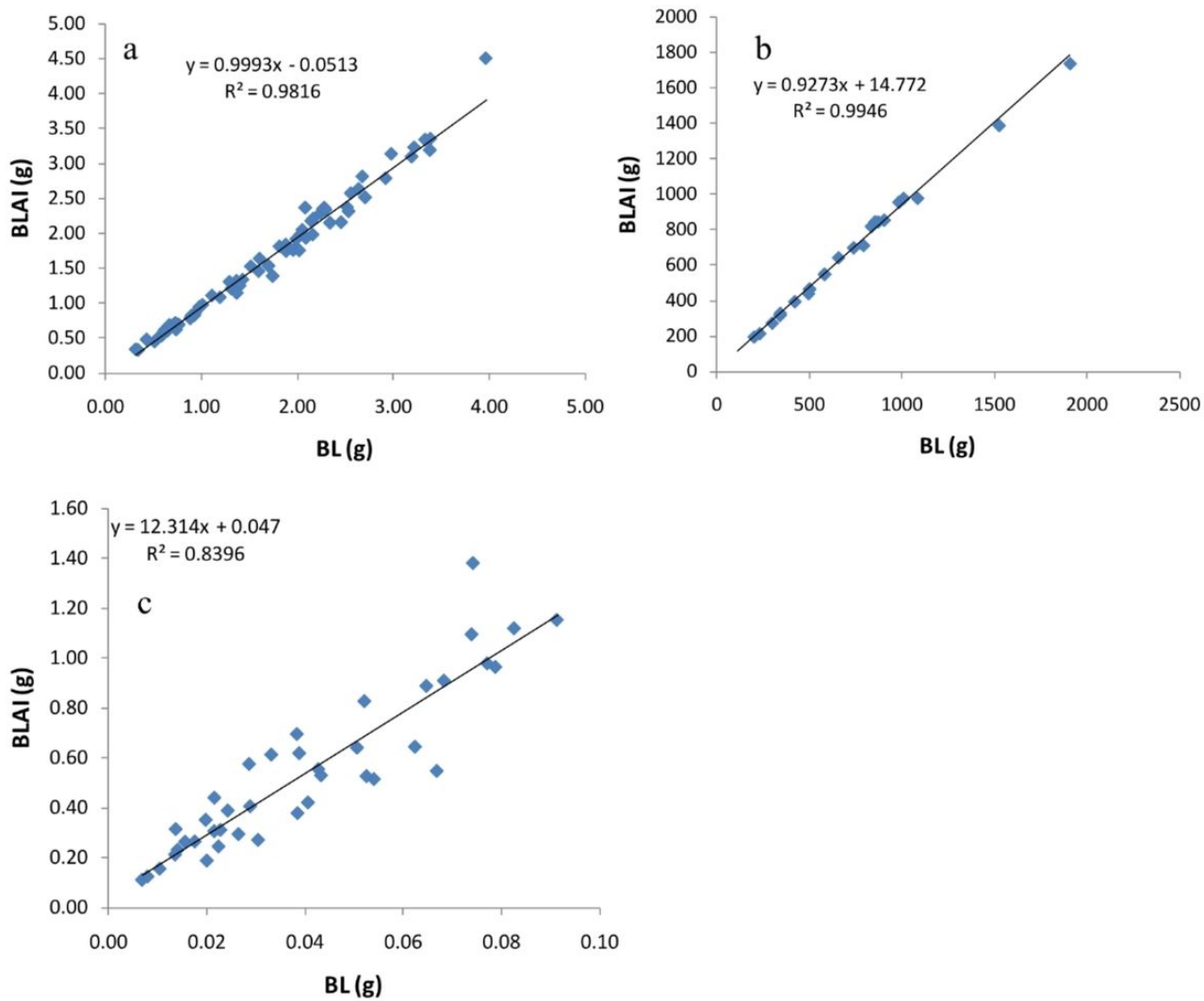


Figure 4

Regressions estimated between leaf weights based on leaf length (BL) and leaf area (BLAI) for *Posidonia oceanica* (a, b) and *Cymodocea nodosa* (c).

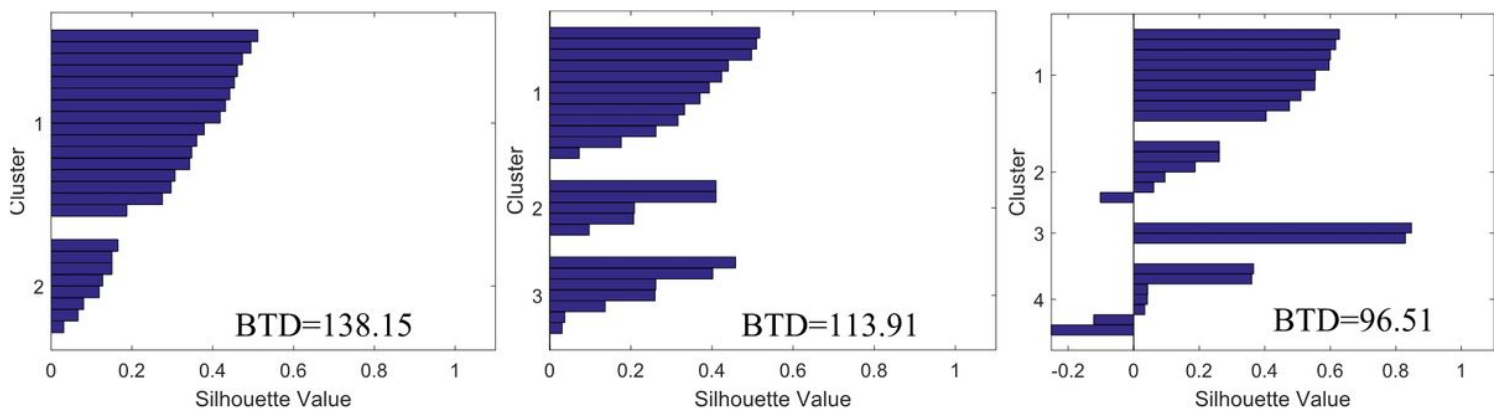


Figure 5

Silhouette analyses to estimate the number of clusters for the acoustical normalized data matrix (Table 1) (BTD: The best total distance).

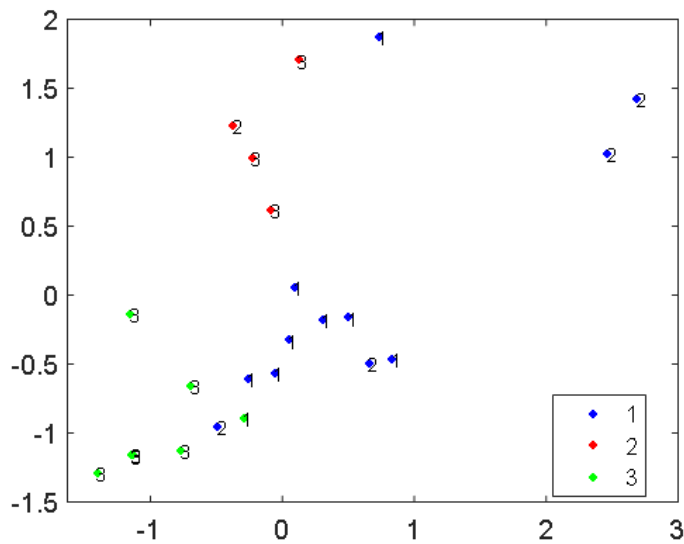


Figure 6

K-means analyses of the normalized acoustical data for 3 clusters determined by the silhouette analyses (the legend in color is a number of clusters specified by k-means, and labels (1 and 2; *P. oceanica*in Antalya and Kaş, respectively, and 2: *C. nodosa*) are species code of the seaweeds regarding the area or region (Figs. 1, 7a).

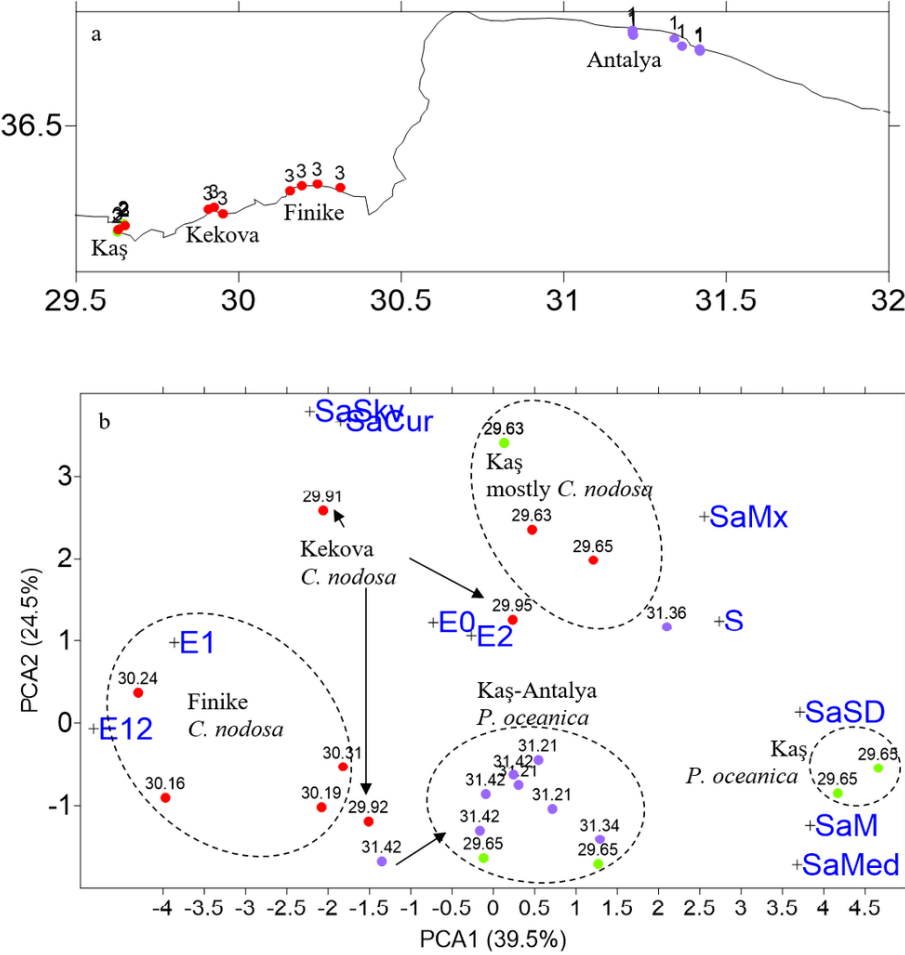


Figure 7

Classification of sampling stations with regions and seaweed species (a), and PCA solution using the normalized acoustical parameters (see Table 1 for abbreviations of the parameters) at stations labeled by the longitudes (1 and 2; *Posidonia oceanica* and 3; *Cymodocea nodosa*, and green and magenta circles denotes *P. oceanica* and red circles *C. nodosa*) (b).

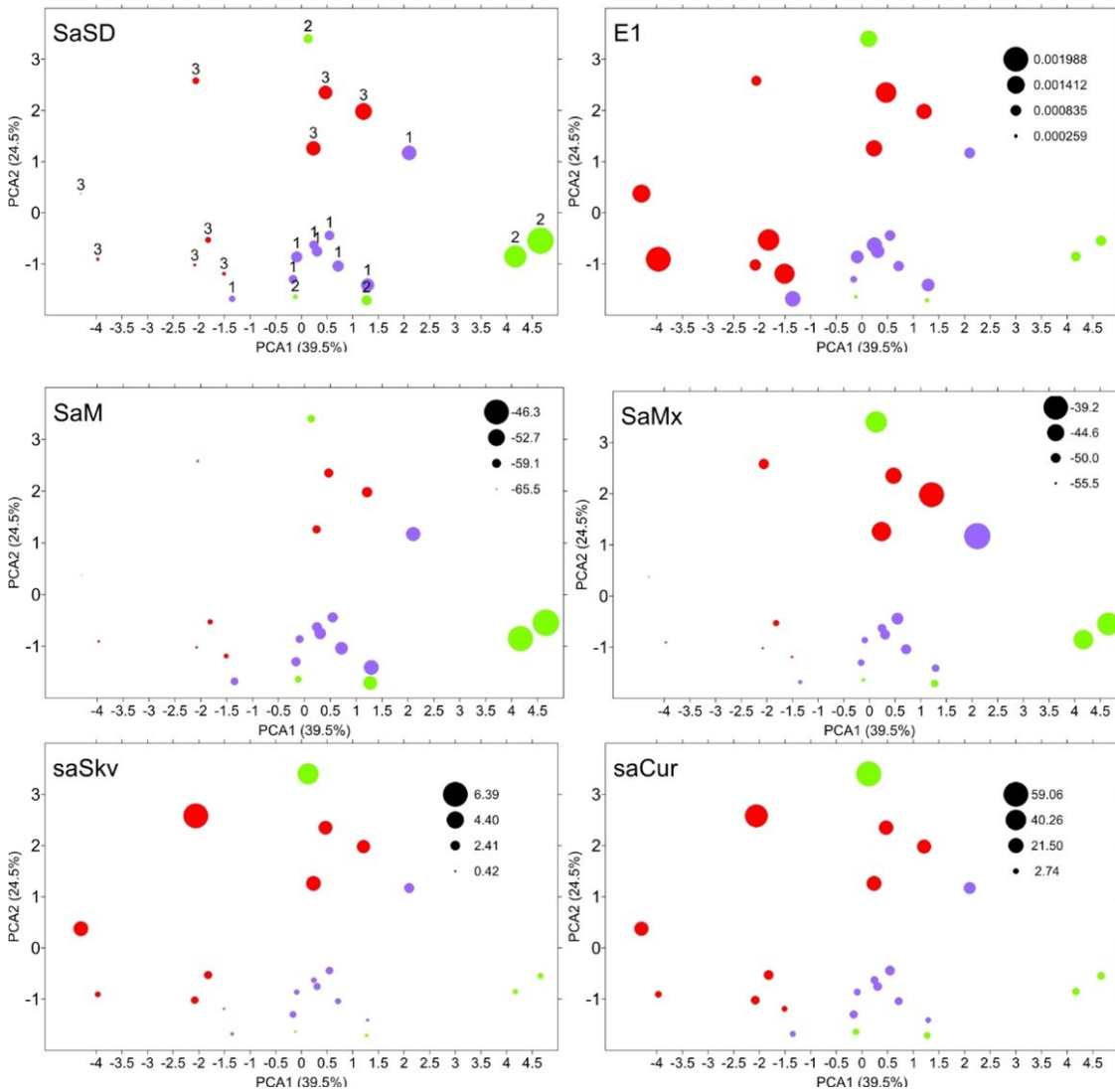


Figure 8

The determinant acoustical variables and statistics for the specific and regional discrimination on PCA solution in Fig. 7b (see Table 1 for the abbreviations of variables, and Fig. 7a for the species and locations).

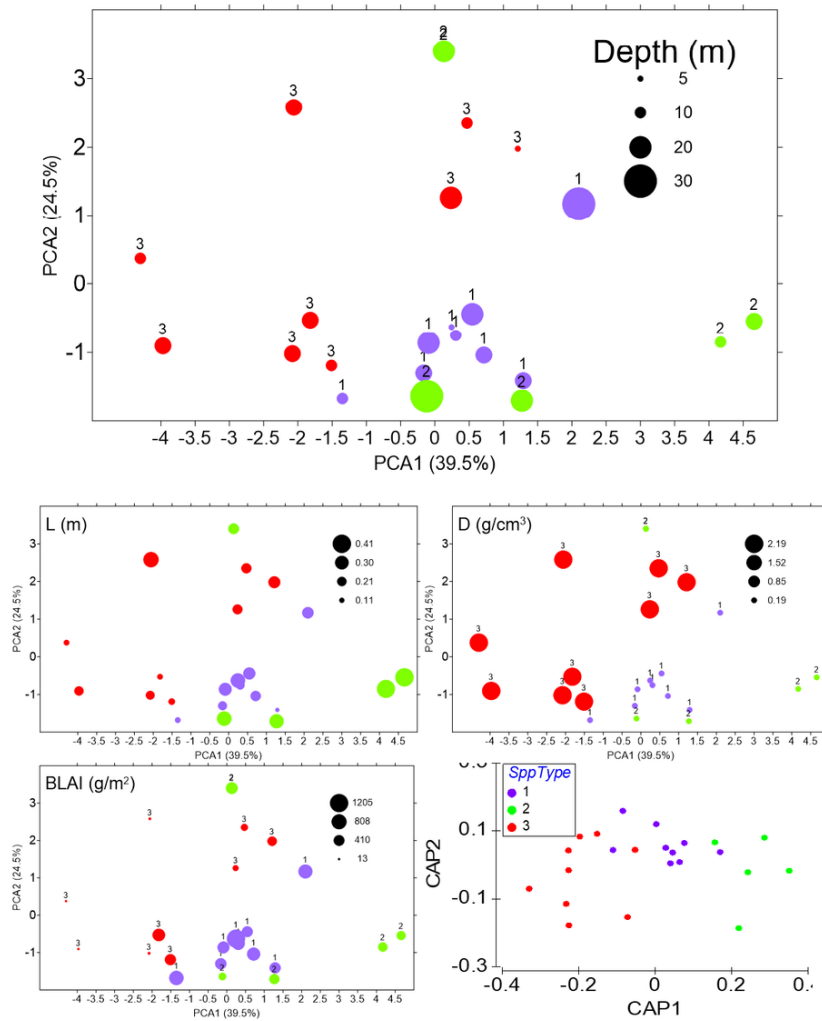


Figure 9

Distribution of bottom depth and the corresponding biometrical variables of *C. nodosa* and *P. oceanica* overlapped on the PCA (Fig. 7) (see Table 1 for the abbreviations of variables, and Fig. 7a for the species and locations) and results of the CAP to test the differences of acoustical variables characterizing two seagrass species in the different regions (see Fig. 7a for the SppType no).

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