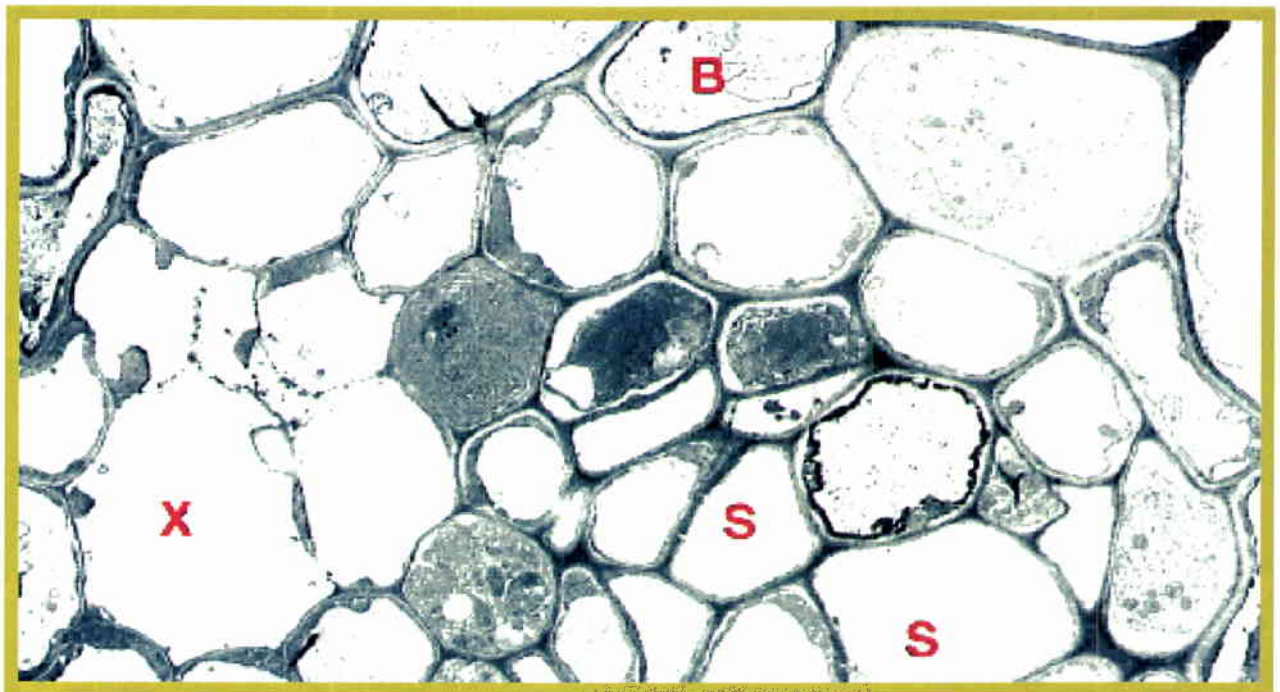


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Characteristics of the mediterranean seagrass *Posidonia oceanica* contributing to high-frequency acoustic scattering



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and Eric Pouliquen*

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acoustic scattering

Stefano Acunto, Anthony P. Lyons and
Eric Pouliquen

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Characteristics of the Mediterranean seagrass *Posidonia oceanica* contributing to high frequency acoustic scattering.

Stefano Acunto, Anthony P. Lyons and Eric Pouliquen

Executive Summary: The gas content of *Posidonia oceanica* was quantified as a function of the plant life cycle. An order of magnitude of the volume of the different elements examined and their relative importance, expressed as percentages is given. The values were considered with reference to the seasonal changes of the phenological variables on which they are dependent and to the main structural variable: leaf density.

The parameters obtained will be used as input to models which are being developed to predict the acoustic scattering properties of *Posidonia oceanica*. Mine-hunting sonar capability and performance facilitates the acquisition of accurate, large-scale data on *Posidonia* distribution, density and height. An accurate model also offers the possibility to obtain fast and large-scale information on the extent of *Posidonia* meadows and plant density and height.

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Abstract: *Posidonia oceanica* meadows are the most important ecosystem for the life cycle of coastal Mediterranean benthos with a fundamental role in the primary production of the neritic system and a decisive influence on other vegetation and animal communities. *Posidonia oceanica* meadows are undergoing a slow but progressive regression, the most common cause of which is enhanced turbidity and the consequent reduction of water transparency. The first step towards the preservation of coastal environments is to define their extent and condition. Echographic surveys allow general maps to be obtained, but accurate seafloor characterization requires knowledge of the characteristics of seabed vegetation which affect acoustics propagation.

A preliminary study quantified the gas within the leaves of *Posidonia oceanica* as a function of the plant life cycle. An order of magnitude of the volume of the different elements considered and their relative importance expressed as percentages is given. The values obtained were considered with reference to the seasonal changes of the phenological variables from which they depend and also to the main structural variable: the density of leaves.

A future step based on the present work will be the development of a model of acoustic scattering by *Posidonia oceanica* derived from a model developed for gassy sediments. The final result would enhance the capability of rapidly acquire information on the extension of meadows, plant density and height. From an operational point of view it could improve the performance of mine-hunting sonars.

Keywords: Seagrasses – *Posidonia oceanica* – Acoustic scattering – Mine-hunting

Contents

1. Impact of <i>Posidonia oceanica</i> on high-frequency acoustic scattering	1
2. Gas and cellulose in <i>Posidonia oceanica</i> leaves.....	3
2.1. <i>Material and methods</i>	3
2.2. <i>Results</i>	4
3. Conclusions	10
4. Acknowledgements	11
References	12
Annex A.....	13

Impact of *Posidonia oceanica* on high-frequency acoustic scattering

Previous laboratory and *in situ* testing have indicated that seagrasses create high levels of acoustic backscatter that interferes with object detection and identification by sonar (McCarthy, 1997). The side-scan sonar record, shown in Fig. 1, is an example of the strong acoustic scattering from *Posidonia*-covered seafloors, displaying much stronger scattering (brighter) than the sandy seafloor inside the circular regions (darker). This strong scattering, especially at the lower grazing angles, is a fact that is of extreme importance when trying to detect objects on the seafloor in shallow water and at large stand-off distances. Additionally, seagrass-covered seafloors will not be homogeneous, but will have spatial patchiness, and the current-induced motion of seagrass causes a time-varying patchiness. Clutter is the acoustic expression of the non-uniformity of this type of seafloor environment and is a major factor in reducing the effectiveness of detecting and identifying objects on the seafloor (Lyons and Abraham, 1999).

Acoustic experiments were conducted at several shallow-water sites near the islands of Elba and Sardinia, Italy, and in Saros Bay, Turkey in 1996, 1997 and 1998, to quantify scattering properties of *Posidonia oceanica* (Lyons and Pouliquen, 1998, Lyons, *et al.*, 1997a, 1997b, Lyons and Akal, 1998). The measurements were made over a wide range of frequencies (30-300 kHz) with coverage over a large range of grazing angles. The overall scattering strengths observed for dense *Posidonia* fields from the three widely separated experimental locations were quite high and consistent with average values around -23 dB seen over the range of grazing angles studied. Scattering strength values showed no frequency dependence over the frequency band studied and displayed only a slight dependence on grazing angle, indicative of volume scattering process. The amplitude distributions were often non-Rayleigh, typical of a high clutter environment, due to non-uniformity of scatterer density (patchiness) on the scale of the sonar resolution cell.

A more thorough understanding of the acoustic properties of *Posidonia oceanica* is essential for accurate seafloor characterization in many Mediterranean littoral regions and for performance optimization of mine-hunting sonars. The plant microstructure that dominates acoustic interaction has to be determined and measured in order to develop accurate scattering models that are able to predict experimental observations such as the dependence of scattering strength on grazing angle or frequency. In the following section we present some of the microscopic characteristics of *Posidonia* leaves affecting high-frequency acoustic interaction. *Posidonia* contains gas filled pockets which we assume are important to explaining the strong high-frequency scattering of *Posidonia* meadows. The presence of cellulose, which is the main constituent of a plant cell, modifies the scattering characteristics of the gas-filled channels and is therefore also of major importance.

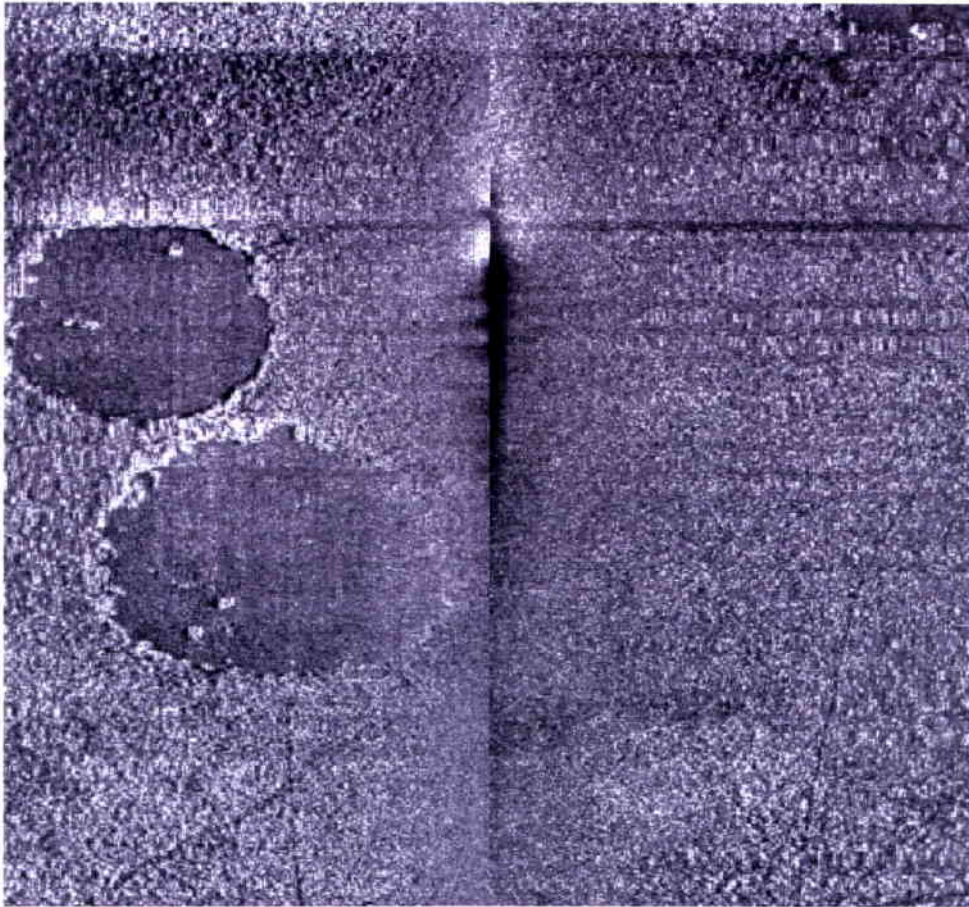


Figure 1 Side-scan sonar record of *Posidonia oceanica* field.

Gas and cellulose in *Posidonia oceanica* leaves

2.1. Material and methods

Samples of *Posidonia oceanica* were collected in October, 1998, from a natural population situated about 10 km south of Livorno (Ligurian Sea, Italy). Sampling was carried out by SCUBA diving at 10 m depth. From each of the 5 shoots sampled, an adult leaf was selected and the anatomical examination was carried out on three transverse sections of the leaf blade taken 20-50 mm above the ligula (15 observations = replicates). Sections of live tissue were mounted on slides without staining and observed through an optical microscope (40x). The thickness of sections was measured by a micrometer, and the mean number of gas lacunae (counted in the whole section) and of mesophyll and epidermal cells (counted in a portion equal to 1/40 of cross section for leaves 10 mm large) were estimated. In a section of each of the five adult leaves chosen, the dimension of five lacunae, five epidermal cells and five mesophyll cells were measured by a micrometer (5 lacunae or cells X 5 sections = 25 observations or replicates). Only the lumen of the cells has been measured in order to obtain by difference the portion of section occupied by the cell walls.

The measures obtained have been used to calculate the surfaces occupied by gas, cell walls and cytoplasm on 1/40 of leaf cross sections. Percentages of gas, cellulose (considered as the main constituent of cell walls) and water (considered as the main constituent of cellular cytoplasm) were calculated as follows:

$$\% \text{ Gas} = (\text{lacunae surface} / \text{section surface}) \times 100$$

$$\% \text{ Cellulose} = (\text{cell walls surface} / \text{section surface}) \times 100$$

$$\% \text{ Water} = ((\text{epidermal} + \text{mesophyll cell's lumen surfaces}) / \text{section surface}) \times 100$$

Mean volumes and percentages of each of the elements considered (gas, cellulose and water) were calculated, for the whole leaf and for a shoot, on the basis of surface values and of the mean leaf length and the mean number of leaves per shoot. Both annual and seasonal means were considered. Values of these phenological variables have been obtained from previous works conducted along Tuscany coasts. Different meadows of different localities have been considered: Foll1 and Foll2 in Follonica (GR) (Acunto *et al.*, 1997), Bar1 and Bar2 in Baratti (LI) (Acunto *et al.*, 1997), Liv1 and Liv2 in Livorno (Piazzini *et al.*, 1996; Castellazzi, 1991) and Elba1 and Elba2 in Elba Island (LI) (Acunto, unpubl. data) (Table 1 & 2). The mean volumes calculated for a shoot have also been used to produce a reference table that relate the seasonal changes in volumes of the different elements considered to the different class density (# of leaves/ m²) as defined by Giraud (1977) (see Table 1).

Table 1 Seasonal trend of the mean number of leaves of *Posidonia oceanica* from different meadows of different localities.

	Foll1	Foll2	Bar1	Bar2	Liv1	Liv2	Elba1	Elba2	Mean	St. Dev.
Spring	6.5	6.6	5.8	5.8	5.2	6.2	5.4	6.1	5.95	0.4957
Summer	6.4	5.6	4.9	5.4	4.6	5.9	4.8	5.8	5.425	0.62048
Autumn	7.4	7.1	5.8	7.2	6.05	6.4	6.6	7.1	6.70625	0.58459
Winter	7.7	6.5	7.3	6.4	5.85	6.9	6.7	7.2	6.81875	0.58428
Mean									6.225	
St. Dev.									0.65822	

Table 2 Seasonal trend of the mean length of leaves in centimeters of *Posidonia oceanica* from different meadows of different localities.

	Foll1	Foll2	Bar1	Bar2	Liv1	Liv2	Elba1	Elba2	Mean	St. Dev.
Spring	39.15	41.25	53.45	45.55	46.5	48.2	63.32	55.8	49.1525	7.98925
Summer	58.55	60.5	80.9	68.15	51	55.4	82.05	74.3	66.3563	11.8033
Autumn	24.3	24.9	44.8	33.65	21.5	36.5	41.35	35.6	32.825	8.4659
Winter	26.9	27.8	28.9	29	30.5	31.6	38.05	31.4	30.5188	3.46574
Mean									44.7131	
St. Dev.									16.6427	

2.2. Results

A transverse section of the leaf blade of *Posidonia oceanica* showed an upper and a lower monolayered epidermis including a mesophyll with at least 5-6 layers of large parenchymatous cells. Gas spaces were small and the microscopic observation of live tissue makes it difficult to detect the smallest gas channels of which only those bigger than 20 μm in diameter have been considered. Values of the thickness of sections and of the number of gas lacunae, of mesophyll and epidermal cells are reported in Table 3. Table 4 reports dimensions of lacunae, lumen of epidermal cells and lumen of mesophyll cells. Epidermal cells are smaller than mesophyll ones and radially elongated. Estimates of surface area (mm^2) have been obtained by approximating lacunae and mesophyll cell's lumen as circles and epidermal cell's lumen as rectangles. Results are given in Table 5.

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Percentage of water, gas and cellulose in a 1/40 of leaf cross section resulted in 81.5, 0.6, and 17.9 respectively (Tab. 5). The proportion of the three elements considered remained the same when volumes in a leaf (Tab. 6) and in a shoot (Tab. 7) have been calculated. Estimations of water, gas, and cellulose volume contained in the leaves of *Posidonia oceanica* corresponding to each density class proposed by Giraud (1977) have been calculated from values of Table 7 and are showed in Table 8.

Table 3 Values of section thickness in millimeters (Thick.); # of mesophyll cells (Meso) and epidermal cells (Epid) counted in 1/40 of cross section; # of lacunae counted in the whole section.

Section	Thick.	Meso.	Epid.	Lacunae
1	0.235	67	26	24
2	0.23	64	29	25.5
3	0.25	65	25	21.5
4	0.2375	57	28	29.5
5	0.2275	63	30	27.5
6	0.2175	65	28	28
7	0.2125	52	26	29.5
8	0.215	69	27	26
9	0.23	54	27	26.5
10	0.2	54	25	30
11	0.195	53	26	28
12	0.2375	66	26	24
13	0.245	60	26	27
14	0.1725	54	25	21.5
15	0.1775	56	26	33
Mean	0.218833	59.93333	26.66667	26.76667
St.dev.	0.023563	5.897538	1.496026	3.172801

Table 4 Diameter (mm) measured for lacunae and lumen of mesophyll cells (Meso); dimensions (mm) of lumen of epidermal cells: height (Epid1) and width (Epid2).

Replicate	Lacunae	Meso	Epid1	Epid2
1	0.024035	0.03	0.02	0.009615
2	0.027615	0.02	0.0125	0.009615
3	0.026865	0.0325	0.015	0.008621
4	0.024545	0.0225	0.02	0.008929
5	0.024135	0.015	0.0175	0.01
6	0.02611	0.02	0.02	0.008929
7	0.024155	0.04	0.0125	0.008333
8	0.02759	0.0375	0.015	0.009259
9	0.023995	0.035	0.01	0.01
10	0.02326	0.0425	0.0175	0.009259
11	0.03036	0.0175	0.01	0.009615
12	0.02884	0.0375	0.0125	0.009615
13	0.022945	0.025	0.0175	0.009615
14	0.02638	0.0225	0.015	0.01
15	0.02564	0.0225	0.0125	0.009615
16	0.02771	0.0175	0.01	0.008879
17	0.023195	0.025	0.0125	0.009111
18	0.020985	0.0325	0.015	0.009543
19	0.02309	0.03	0.015	0.009857
20	0.030975	0.045	0.015	0.01
21	0.02257	0.0425	0.0125	0.009645
22	0.022965	0.0275	0.0125	0.008857
23	0.0309	0.0175	0.015	0.008911
24	0.024155	0.02	0.015	0.009586
25	0.02227	0.0225	0.0175	0.009645
Mean	0.025411	0.028	0.0147	0.009402
St.dev.	0.002812	0.009043	0.003003	0.000466

SACLANTCEN SM-372

Table 5 Surface areas (mm^2) of epidermal cell's lumen = Epid.; mesophyll cell's lumen = Meso.; Lacunae; cell walls = Walls. Percentages have been calculated on 1/40 of cross section (considering leaves 10 mm large) = Section surface.

Section	Epid.	Meso.	Lacunae	Epid. + Meso.	Section surface	Walls	% Water	% Gas	% Cell.se
1	0.0072	0.0412	0.00030	0.0487	0.0587	0.0100	82.4521	0.5179	17.0299
2	0.0080	0.0394	0.00032	0.0477	0.0575	0.0097	82.4738	0.5623	16.9639
3	0.0069	0.0400	0.00027	0.0472	0.0625	0.0153	75.0924	0.4361	24.4714
4	0.0077	0.0351	0.00037	0.0432	0.0594	0.0162	72.1446	0.6299	27.2255
5	0.0083	0.0388	0.00035	0.0474	0.0569	0.0094	82.7834	0.6130	16.6036
6	0.0077	0.0400	0.00035	0.0481	0.0544	0.0062	87.8378	0.6529	11.5093
7	0.0072	0.0320	0.00037	0.0396	0.0531	0.0135	73.7964	0.7040	25.4995
8	0.0075	0.0425	0.00033	0.0503	0.0537	0.0035	92.9274	0.6133	6.4593
9	0.0075	0.0332	0.00034	0.0410	0.0575	0.0164	70.8039	0.5843	28.6118
10	0.0069	0.0332	0.00038	0.0405	0.0500	0.0095	80.3190	0.7607	18.9202
11	0.0072	0.0326	0.00035	0.0402	0.0487	0.0086	81.6823	0.7282	17.5895
12	0.0072	0.0406	0.00030	0.0481	0.0594	0.0112	80.5471	0.5125	18.9403
13	0.0072	0.0369	0.00034	0.0445	0.0612	0.0168	72.0496	0.5588	27.3915
14	0.0069	0.0332	0.00027	0.0404	0.0431	0.0027	93.1235	0.6321	6.2444
15	0.0072	0.0345	0.00042	0.0421	0.0444	0.0023	93.8983	0.9429	5.1589
Mean	0.0074	0.0369	0.00034	0.0446	0.0547	0.0101	81.4621	0.6299	17.9079
St. Dev.	0.00041	0.00363	4.02E-05	0.00376	0.00589	0.00493	7.824124	0.12156	7.87485

Table 6 Volumes of water, gas, and cellulose in a leaf (mm^3) of epidermal cells (Epid.), mesophyll cells (Meso.), Lacunae and cell walls (Walls). Values have been calculated on the annual mean leaf length (447.131 mm) and on the mean leaf length in each season (see Table 2).

	Epid.	Meso.	Lacunae	Epid. + Meso.	Leaf volume	Walls
Annual	131.81	660.04	6.07	797.91	978.47	180.56
St. Dev.	7.39	64.95	0.72	67.34	105.36	88.11
Spring	144.89	725.57	6.67	877.13	1075.62	198.49
St. Dev.	8.13	71.40	0.79	74.03	115.82	96.85
Summer	195.61	979.52	9.01	1184.14	1452.10	267.96
St. Dev.	10.97	96.39	1.07	99.94	156.36	130.75
Autumn	96.76	484.55	4.45	585.77	718.32	132.55
St. Dev.	5.43	47.68	0.53	49.44	77.35	64.68
Winter	89.96	450.50	4.14	544.61	667.85	123.24
St. Dev.	5.05	44.33	0.49	45.96	71.91	60.14

Table 7 Volumes of water, gas, and cellulose in a shoot (mm^3) of epidermal cells (Epid.), mesophyll cells (Meso.), Lacunae and cell walls (Walls). Values have been calculated on the annual mean number of leaves per shoot (6.225) and on the mean number of leaves per shoot in each season (see Table 1).

	Epid.	Meso.	Lacunae	Epid. + Meso.	Leaf volume	Walls
Annual	820.50	4108.72	37.78	4967.01	6090.99	1123.98
St. Dev.	46.03	404.30	4.48	419.21	655.864	548.47
Spring	862.12	4317.13	39.70	5218.95	6399.94	1180.99
St. Dev.	48.36	424.81	4.70	440.47	689.13	576.29
Summer	1061.17	5313.92	48.87	6423.95	7877.63	1453.67
St. Dev.	59.53	522.90	5.79	542.17	848.24	709.35
Autumn	649.28	3251.32	29.90	3930.50	4819.93	889.43
St. Dev.	36.42	319.93	3.54	331.73	518.99	434.01
Winter	613.56	3072.45	28.25	3714.26	4554.76	840.50
St. Dev.	34.42	302.33	3.35	313.48	490.45	410.14

SACLANTCEN SM-372

Table 8. Water, gas and cellulose volumes (m^3) contained in the leaves of *Posidonia oceanica* corresponding to each density class proposed by Giraud (1977). Annual and seasonal values are given using information of Table 7.

Density class	I	II	III	IV	V	VI
Shoots / m^2	> 700	400 - 700	300 - 400	150 - 300	50 - 150	< 50
Annual	m^3	m^3	m^3	m^3	m^3	m^3
Water	> 3.47E-03	1.99E-03 - 3.47E-03	1.49E-03 - 1.99E-03	7.45E-04 - 1.49E-03	2.48E-04 - 7.45E-04	< 2.48E-04
Gas	> 2.64E-05	1.51E-05 - 2.64E-05	1.13E-05 - 1.51E-05	5.67E-06 - 1.13E-05	1.89E-06 - 5.67E-06	< 1.89E-06
Cellulose	> 7.87E-04	4.5E-04 - 7.87E-04	3.37E-04 - 4.5E-04	1.69E-04 - 3.37E-04	5.62E-05 - 1.69E-04	< 5.62E-05
Spring						
Water	> 3.65E-03	2.09E-03 - 3.65E-03	1.57E-03 - 2.09E-03	7.83E-04 - 1.57E-03	2.61E-04 - 7.83E-04	< 2.61E-04
Gas	> 2.78E-05	1.59E-05 - 2.78E-05	1.19E-05 - 1.59E-05	5.96E-06 - 1.19E-05	1.99E-06 - 5.96E-06	< 1.99E-06
Cellulose	> 8.27E-04	4.72E-04 - 8.27E-04	3.54E-04 - 4.72E-04	1.77E-04 - 3.54E-04	5.9E-05 - 1.77E-04	< 5.9E-05
Summer						
Water	> 4.49E-03	2.57E-03 - 4.49E-03	1.93E-03 - 2.57E-03	9.64E-04 - 1.93E-03	3.21E-04 - 9.64E-04	< 3.21E-04
Gas	> 3.42E-05	1.95E-05 - 3.42E-05	1.47E-05 - 1.95E-05	7.33E-06 - 1.47E-05	2.44E-06 - 7.33E-06	< 2.44E-06
Cellulose	> 1.02E-03	5.81E-04 - 1.02E-03	4.36E-04 - 5.81E-04	2.18E-04 - 4.36E-04	7.27E-05 - 2.18E-04	< 7.27E-05
Autumn						
Water	> 2.75E-03	1.57E-03 - 2.75E-03	1.18E-03 - 1.57E-03	5.9E-04 - 1.18E-03	1.97E-04 - 5.9E-04	< 1.97E-04
Gas	> 2.09E-05	1.2E-05 - 2.09E-05	8.97E-06 - 1.2E-05	4.48E-06 - 8.97E-06	1.49E-06 - 4.48E-06	< 1.49E-06
Cellulose	> 6.23E-04	3.56E-04 - 6.23E-04	2.67E-04 - 3.56E-04	1.33E-04 - 6.67E-04	4.45E-05 - 1.33E-04	< 4.45E-05
Winter						
Water	> 2.6E-03	1.49E-03 - 2.6E-03	1.11E-03 - 1.49E-03	5.57E-04 - 1.11E-03	1.86E-04 - 5.57E-04	< 1.86E-04
Gas	> 1.98E-05	1.13E-05 - 1.98E-05	8.48E-06 - 1.13E-05	4.24E-06 - 8.48E-06	1.41E-06 - 4.24E-06	< 1.41E-06
Cellulose	> 5.88E-04	3.36E-04 - 5.88E-04	2.52E-04 - 3.36E-04	1.26E-04 - 2.52E-04	4.2E-05 - 1.26E-04	< 4.2E-05

3

Conclusions

This work is a first attempt to quantify those leaf components, in particular gas and cellulose, which are important in evaluating the acoustic properties of the Mediterranean seagrass, *Posidonia oceanica*. Values are obtained for these components as a function of the plant's seasonal life cycle. The estimates made, however, do not allow generalizations for the whole Mediterranean basin due to a sampling design that is not replicated enough in space, depth and season. Moreover, the microscopic observations of live tissue were not accurate enough due to difficulties in detecting the small gas channels characteristic of the species. The technique adopted produced overestimation of the size but underestimation of the number of *Posidonia oceanica* lacunae, so the consequent level of approximation obtained for percentage of gas in a section has been considered acceptable. Also, since the biggest gas channels occupy the largest volumes they will have the greatest impact on acoustic properties. Considering these limitations, an order of magnitude of the volumes of the different elements considered and their relative importance expressed as percentages has been given. The values obtained have been considered with reference to the seasonal changes of the phenological variables from which they depend and also to the main structural variable: the density of leaves. The methodology adopted for this study could be useful for evaluating the acoustic properties of other seagrasses, in particular in some species (e.g. *Zostera* spp.) in which the gas channels of the leaves are relatively large.

An important future step will be the development of a model to quantify the bulk acoustic properties of *P. oceanica*, for use in scattering models of seagrass meadows. The model must include the effects of gas enclosed within cell walls on the bulk properties of seagrass, analogous to the modification of sediment bulk properties by the inclusion of methane bubbles (Anderson and Hampton, 1980a & 1980b). The final result could be a tool to improve the capabilities of acoustic instruments, including mine-hunting sonars, by enabling quantitative information to be acquired by these instruments from *Posidonia*-covered seafloors.

SACLANTCEN SM-372

4

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A.1. Introduction

Posidonia oceanica meadows are, in the Mediterranean Sea, the most widespread and widely known of the benthic vegetation communities of seagrasses (Boudouresque & Meinesz, 1982; Mazzella *et al.*, 1986; Cinelli *et al.*, 1995a). *Posidonia oceanica* is a species endemic to the Mediterranean, which grows as extended meadows of long leaves, grouped in clumps called “shoots”, in the infralittoral bottom of the basin which is, in fact, characterized by their presence (Molinier & Picard, 1952; Pérès & Picard, 1964; Den Hartog, 1970). *Posidonia oceanica* meadows are the most important ecosystem for the life cycle of coastal Mediterranean benthos, having a fundamental role in the primary production of the neritic system and a decisive influence on other vegetation and animal communities. The meadows produce organic matter at a rate of up to 16 g per m² per day, a direct and indirect source of food for many organisms and the starting point for a complex trophic web (Ott, 1980; Mazzella, *et al.*, 1995). *Posidonia oceanica* meadows function as nurseries and refuges for fish, cephalopods and crustaceans, of considerable economic importance (Kikuchi, 1974; Bell 1980; Harmelin - Vivien, 1982). The meadows are of extreme importance to littoral sedimentation, exerting a strong influence on the coastal hydrodynamic equilibrium. *Posidonia* leaves limit the energy of sea swells and currents, allow sediment accumulation, and form a natural protection belt for adjacent coasts against erosive wave action (Judy de Grissac & Boudouresque, 1985; Colantoni, 1995a). Sediment accumulation generated by the dragging action of the *Posidonia* leaves over the water movements, determines the vertical growth of the plant and allows building of the so-called “matte”. This peculiar formation, consisting of the interlacement of rhizomes, roots and sediments, causes a rising of the bottom level of the seafloor that can reach a thickness of several meters. All these evidences display the importance of *Posidonia oceanica* meadows for the maintenance of the coastal ecological equilibrium.

The *Posidonia oceanica* meadows are presently undergoing a slow but progressive regression throughout the Mediterranean (Augier & Boudouresque, 1970; Maggi, 1973; Meinesz & Laurent, 1978; Meinesz & Lefevre, 1978; Astier, 1984; Pérès 1984). There are various reasons for this phenomenon, such as mechanical damage due to trawling, repeated anchorage, dumping, landslides, explosives, hydrological changes due to modifications of the coastline caused by development activities (dams, ports, breakwaters) and discharge of sewage and industrial wastes (Maggi, 1972; Blanc & Judy de Grissac, 1978; Judy de Grissac, 1979; Ardizzone & Pelusi, 1984; Astier, 1984; Meinesz *et al.*, 1985). Natural erosion-sedimentation-erosion cycles can also play a role in the regression of *P. oceanica* meadows; these phenomena however, without notable climatic variation or human interference, would allow the meadows to subsist (Cinelli *et al.*, 1995b). The most common cause of *Posidonia oceanica* meadow regression is increased levels of turbidity and the consequent reduction of water transparency (Boudouresque & Meinesz, 1983).

Shelf seas and coastal zones are traditionally studied by *in situ* collection of plant samples and mapped by the use of direct and indirect methods (Meinesz, 1989; Acunto, 1991; Chessa, *et al.*, 1995; Colantoni, 1995b; Max & Mori, 1996). Direct methods of mapping require *in situ* observation by divers, submarines and/or optical instruments with ROV (Remote Operated Vehicles) or RCV (Remoted Controlled Vehicles) cameras (Ardizzone & Belluscio, 1988; Ardizzone, 1992). Towed devices, such as floating cameras, are also used. Indirect sampling methods employ different instruments and integrated techniques. Examples of indirect sampling mapping methodology include: drilling by dredges and buckets to map the nature of the substratum; echographic survey by high frequency echo sounders providing unidimensional bottom layouts; and echographic survey by low frequency echo sounders (sub-bottom profilers or Uniboom systems) to study deep structures (Stefanon *et al.*, 1986). Such methods are not appropriate for the mapping of benthic biocenosis as misinterpretations are possible. Acoustic systems such as side-scan sonar provide a high-resolution survey, which is the basic system for acoustic physiography allowing evaluation of surfaces and monitoring of the lower limits of *P. oceanica* beds (Newton & Stefanon, 1975; Cuvelier, 1976; Gloux, 1984; Cinelli & Piazzzi, 1991, Cinelli, *et al.*, 1992; CIBM, 1995). It is also possible to distinguish beds of *P. oceanica* from patches of other types of seagrass such as *Cymodocea nodosa* and other bottom features (Bianchi & Peirano, 1995).

Indirect methods allow a general survey to be obtained, but accurate seafloor characterization requires knowledge of the acoustic properties of seabed vegetation. Laboratory and *in situ* testing have indicated that seagrasses adversely affect the Navy's ability to detect mines by creating high levels of acoustic backscatter and clutter that interfere with the operation of mine-hunting sonars (McCarthy, 1997). A thorough understanding of the acoustic properties of *Posidonia oceanica* is essential for optimal performance of mine-hunting sonar in Mediterranean littoral regions.

In this report we present some of the microscopic characteristics of *Posidonia oceanica* leaves. We assume that the presence of gas cavities in leaf blades and cellulose, which is the main constituent of cell walls, are important factors in explaining the strong high-frequency scattering of *Posidonia* meadows. A preliminary study has quantified air and cellulose (as percentages of the total volume of the leaf). The values obtained have been used with other biological (number and length of leaves) and structural (density of leaves) variables to produce tables of reference related to seasonal changes in leaf length and to different class density. Results from the present work will be used to develop a model of acoustic scattering from seabed vegetation.

A.2 Properties of seagrasses (with special reference to Posidonia oceanica)

A.2.1 Macroscopic features of the meadows

Posidonia oceanica colonizes mobile substrata, such as coarse sand sometimes mixed with mud, but also debris and rock. Rhizomes grow horizontally (plagiotropic rhizomes) and vertically (orthotropic rhizomes). When the plant encounters favorable

SACLANTCEN SM-372

environmental conditions it grows vegetatively, forming extensive meadows due to the plagiotropic rhizomes. The vertical growth allows the building of the so-called “matte”, consisting of interlacing rhizomes, roots and sediments, which can reach a thickness of several metres. Meadows may exist from the surface to 30 to 40 m depth, but generally along continental coastlines, where turbidity usually increases they extend from 5 to 20 m depth. A shallow and a deep boundary characterize the meadow habitat. The shallow limit, towards shore, is usually well defined, while the limit at depth can have different morphologies. According to Meinesz & Laurent (1978) the lower limit is classified in three broad categories:

Type 1: *Progressive limit*, identifiable with the presence of tracing plagiotropic rhizomes extending beyond the meadow front and oriented in the direction of the bottom slope. Matte is not present and plant covering decreases progressively with depth. This limit is governed by light availability.

Type 2: *Sharp limit*, in absence of matte the meadow ends abruptly; cover by plants goes from > 75% in the adjacent interior zone to values ranging from 75% to 50% at the limit. This limit appears to be conditioned by edaphic factors (e.g. type of sediment, features of the seafloor, etc.).

Type 3: *Erosive limit*: characterized by an erosion step in the matte that may be quite conspicuous, cover by plants can reach 100%. Considered relatively infrequent, this limit appears to be conditioned by seafloor currents eroding the meadow and preventing its progression to greater depth.

Channels and glades (round to irregularly oblong area with no *Posidonia* cover) are common in all meadows due to the action of bottom currents. In addition, small variations in seafloor chemistry or physics can affect the spread and condition of the seagrass, influencing small-scale spatial distribution.

One of the main variables measured to survey *Posidonia* meadows is the density calculated as the number of shoots per m². On the basis of density values, the meadows are ascribed to one of six classes now generally accepted in the international literature (Table 9).

Table 9. Classification of *Posidonia oceanica* meadows according to density, proposed by Giraud (1977).

Class	Description	Shoots / m ²
I	Very dense meadow	> 700
II	Dense meadow	400 – 700
III	Sparse meadow	300 – 400
IV	Very sparse meadow	150 – 300
V	Semi-meadow	50 – 150

VI	Isolated shoots	< 50
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A.2.2. Vegetative morphology and anatomy

Seagrasses generally have a similar external morphology with well-developed creeping rhizome, bearing at each node one or more branched or unbranched roots and a short, erect shoot bearing several foliage leaves, each with a sheath at the base.

A.2.2.1. Roots

The roots of seagrasses are adventitious as in all monocotyledons and arise from the lower surface of the rhizomes, generally from the nodes. The external morphology of roots often has characteristic features in different taxa, though internal anatomy is generally similar. The roots of *Posidonia* spp. are thick, soft and extensively branched, but root hairs are rare.

Anatomy: The mature root has an epidermis overlying cortical parenchymatous tissue that encloses gas lacunae and a central stele (Fig. 2). The root epidermis of *Posidonia* spp. is lignified (Larkum *et al.*, 1989).

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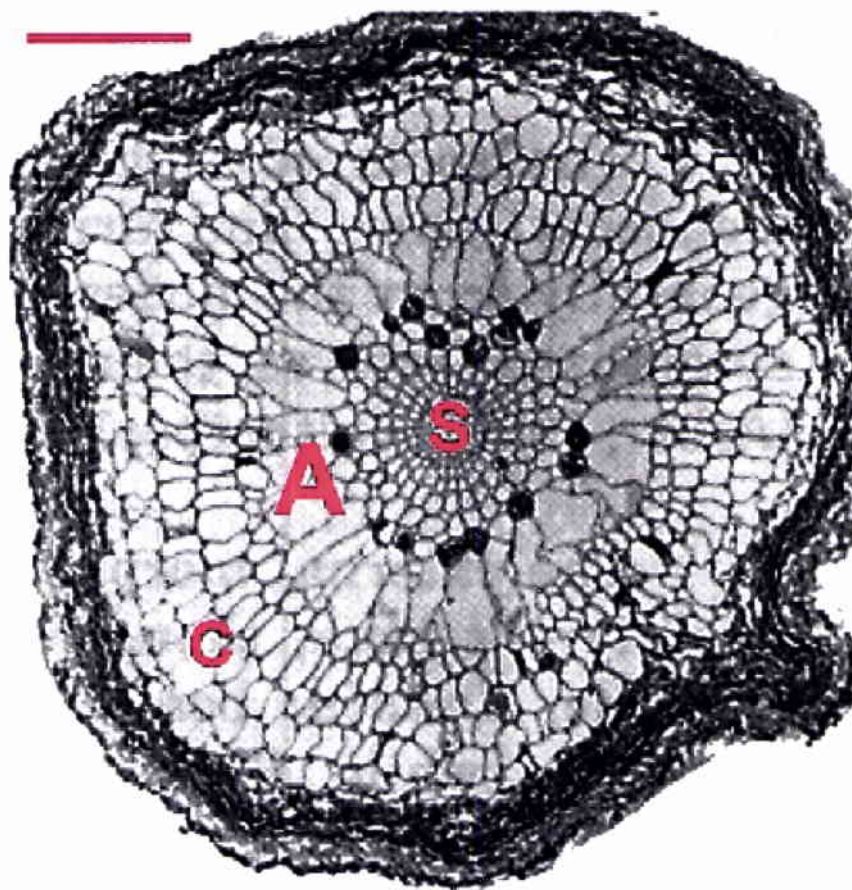


Figure 2 Transverse section of a mature root of *Posidonia ostenfeldii* showing the epidermis overlying cortical parenchymatous tissue (C) that encloses gas lacunae (A) and a central stele (S). Scale = 330 μ m. (From Larkum et al., 1989).

A.2.2.2. Rhizomes

A seagrass rhizome is usually herbaceous, cylindrical to laterally compressed, and monopodially or irregularly branched. Rhizomes are almost always buried under sediment and are usually covered with the persistent fibrous remains of old leaf sheaths, as in *Posidonia* spp. Rhizomes of *Posidonia oceanica* have both horizontal (4-5 cm a year) and vertical growth. The rate of vertical growth varies according to the sedimentation rate to prevent burial, 1 cm per annum is a reasonable supposition.

Anatomy: A transverse section of a rhizome shows a central stele surrounded by a conspicuous endodermis that in *Posidonia* spp. becomes suberized. A cylindrical cortex surrounds the endodermis and the central stele. The cortex may contain vascular and/or fibre bundles, the distribution and number of which appear to be constant within genera (Fig. 3). There may be a distinct exodermis with a thickened wall and a suberized middle

lamella (e.g. *Posidonia* spp.). The epidermis is normally distinct and covered by a cuticle and may contain tannin cells (Larkum *et al.*, 1989).

A.2.2.3. Leaves

The bright green leaves of *Posidonia oceanica* grow from the orthotropic rhizomes and are ribbon-like with rounded apex. Each shoot contains a mean number of 6-7 leaves organized in a fan-like structure, with the youngest leaves in the middle of the shoot and the oldest surrounding them. Leaves grow from a basal meristem. This adaptation allows growth of the leaf even when the apex, which becomes the oldest part, undergoes degenerative phenomena. According to Giraud (1979) we can distinguish:

- *Young leaves*: up to 5 cm long without a base
- *Intermediate leaves*: more than 5 cm long without a base
- *Adult leaves*: more than 5 cm long with a base

Even though the plant forms new leaves throughout the year, greater production of young leaves occurs between September and December. In this period the mean length of the shoots becomes shorter due to the loss of the older leaves that, reaching the maximum length during spring-summer months, are detached from the rhizomes during autumn sea-storms, so that the meadows appear taller in late spring and shorter in winter.

Anatomy: Cuticle. There are no stomata in seagrasses, but a thin cuticle covers the leaf surface. The cuticle of seagrass leaves offers low resistance to ion movement and carbon diffusion in the absence of a functional stomatal system. The appearance of the cuticle under electron microscopy differs among seagrass species: *Posidonia* spp. has a distinctive porous texture (Fig. 4). This structure may serve as an ion-water exchange column regulating nutrient flow between seagrasses, epiphytes and surrounding water (Larkum *et al.*, 1989).

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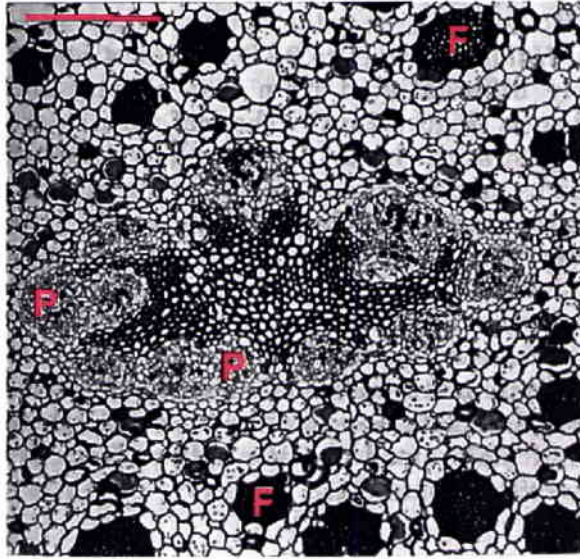


Figure 3 *Posidonia australis*. The rhizome internode has central xylem tissue surrounded by phloem tissue (**P**) in the stele. Cortical tissue has many fibre bundles (**F**) and parenchyma cells. Scale = 50 μm . (From Larkum et al., 1989).

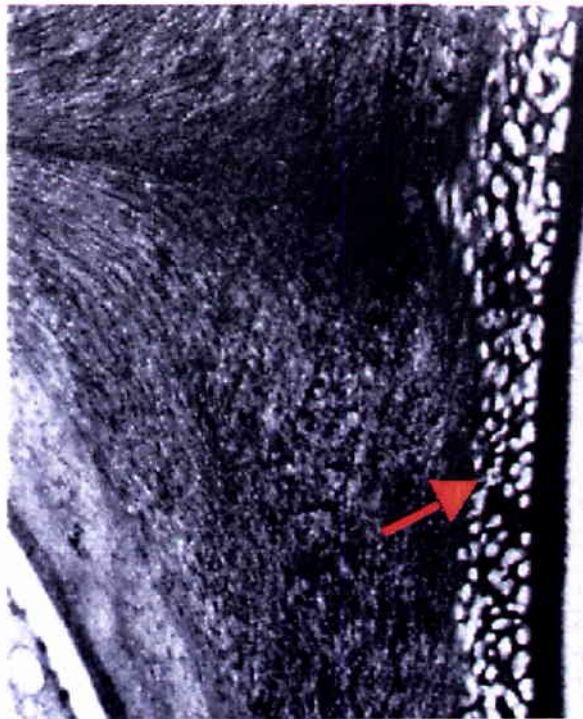


Figure 4 An electron microscope image of a portion of *Posidonia oceanica* leaf showing the porous texture beneath the cuticle (arrow). $\times 22,000$. (From Colombo et al., 1983).

Epidermis. The epidermis of the leaf blade is rich in chloroplasts. In *Posidonia* and in other genera, the epidermis is uniformly chlorenchymatous and contains tannin (Fig. 5). The wall of epidermal cells, particularly the outer tangential wall, are thickened but never lignified. Cell walls of the seagrass epidermis contain pectin, protein, cellulose and other polysaccharide components (Colombo *et al.*, 1983 for *Posidonia oceanica*). The epidermis of *Posidonia* is distinct from that of other seagrasses, as wall ingrowth and convoluted plasmalemma are absent. The vacuoles of *Posidonia* spp. and other genera of seagrasses contain polyphenolic substances (tannin). A well-developed system of plasmodesmata, arranged in primary pit fields, connects adjacent epidermal and mesophyll cells in *Posidonia oceanica* (Colombo *et al.*, 1983) (Fig. 6).

Leaf fibre cells. Fibre cells are present in the leaves of certain genera of seagrasses but absent from others. Their occurrences and distribution are sometimes useful taxonomic characters (e.g. in *Posidonia* spp.) (Larkum *et al.*, 1989). They are normally abundant near the leaf margins, in *Posidonia oceanica* they are present in the hypodermal layers just beneath the epidermal cells (Fig. 7). A fibre strand usually consists of several fibre cells with thickened but not lignified cell walls (Fig. 8). Thus, together with epidermal cells, the fibre cells could provide tensile strength while retaining a high degree of flexibility, allowing the strip-like leaf blades to withstand vigorous wave action.

Mesophyll and Gas Lacunae. The mesophyll of seagrasses is composed of homogeneous, thin-walled, but highly vacuolated parenchyma cells (Fig. 5). The thin peripheral cytoplasm contains few chloroplasts, which contain small starch grains. The mesophyll cells surround gas lacunae of varying size. In *Zostera* spp. gas lacunae are prominent and regularly arranged while in *Posidonia* spp. gas lacunae are present but less prominent (Figs. 9 and 10). In *Posidonia oceanica* the gas spaces are small which is a diagnostic feature for the species. There are regular septa interrupting the lacunae along the leaf. Each septum (diaphragm) consists of a group of small parenchyma cells with minute intercellular spaces, which are about 0.5 – 1.0 µm in diameter in *Halophila ovalis* (Roberts *et al.*, 1984). These septa provide a physical barrier to flooding but allow gas continuity to be maintained within the lacunar system. Gas lacunae have been considered to be important in seagrass photosynthesis. It has been shown that they contain nitrogen, oxygen, and carbon dioxide, and it has been estimated that 50-67% of photorespired carbon dioxide in *Talassia testudinum* leaves is recycled (Larkum *et al.*, 1989).

Leaf Vascular System. Vascular systems in seagrasses are similar in structure and composition to those of vascular land plants; all have sieve elements, xylem elements and vascular parenchyma cells. A distinct layer of sheath encloses each longitudinal vascular bundle of the leaf (Fig. 11). In *Posidonia* spp. the wall of the bundle sheath cells is thin and lignified. Sieve elements may be normal (e.g. in *Posidonia* spp.) or nacreous-walled but both elements have similar cytoplasmic properties. Each sieve element has a distinct plasmalemma and contains monocotyledonous plastids with protein crystalloids, but lacks a nucleus and ribosomes at maturity. The number and size of xylem elements in seagrasses, as in other aquatic plants, are much reduced in comparison with those in vascular land plants. In *Posidonia* spp. the xylem consists of several elements with highly lignolyzed walls and little lignification (Larkum *et al.*, 1989).

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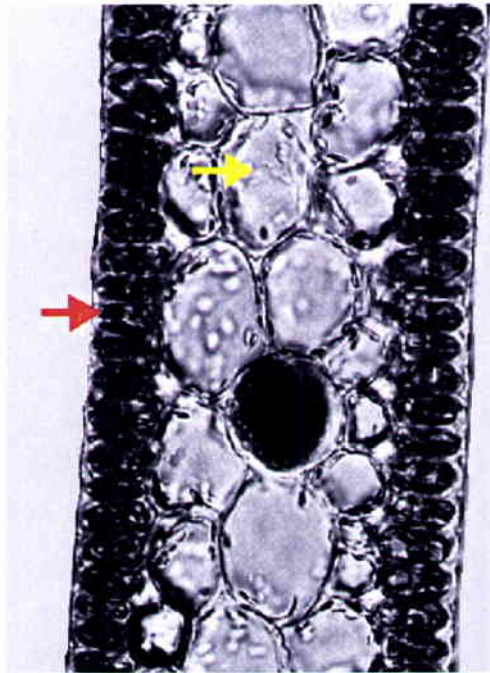


Figure 5 *Posidonia oceanica*. Leaf blade cross section showing the monolayered epidermis (red arrow), the mesophyll with large highly vacuolated parenchyma cells (yellow arrow) and small intercellular spaces. x320. (From Colombo et al., 1983).



Figure 6 The system of plasmodesmata in *Posidonia oceanica* arranged in primary pit fields, which connect adjacent epidermal and mesophyll cells. x23,000. (From Colombo et al., 1983).

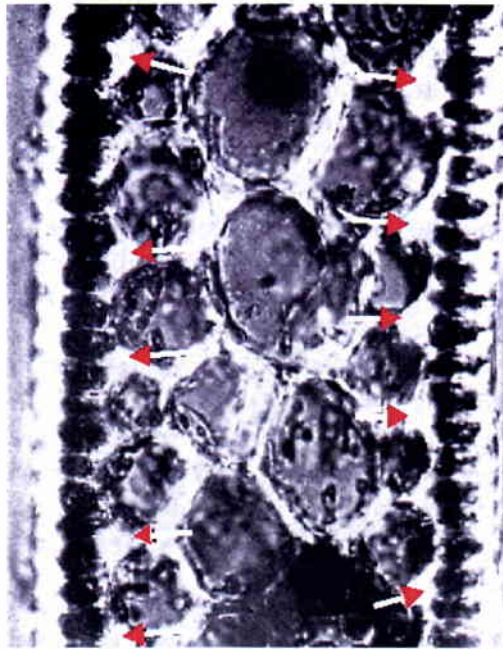


Figure 7 Leaf fibre cells (arrows) present in *Posidonia oceanica* in the hypodermal layers just beneath the epidermal cells.. x400. (From Colombo et al., 1983).

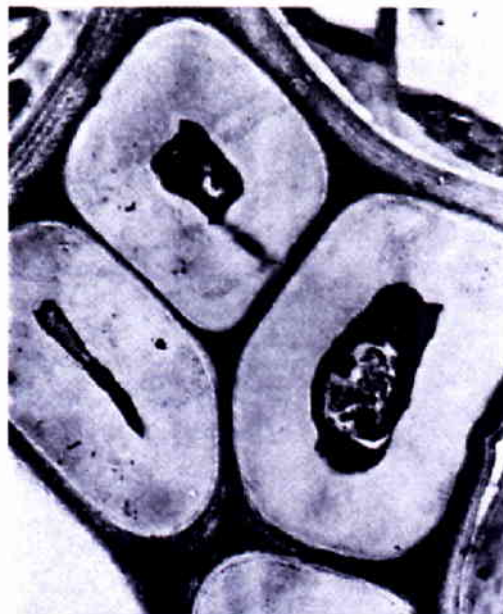


Figure 8 A fibre strand consisting of several fibre cells with thickened but not lignified cell walls.. x9,000. (From Colombo et al., 1983).

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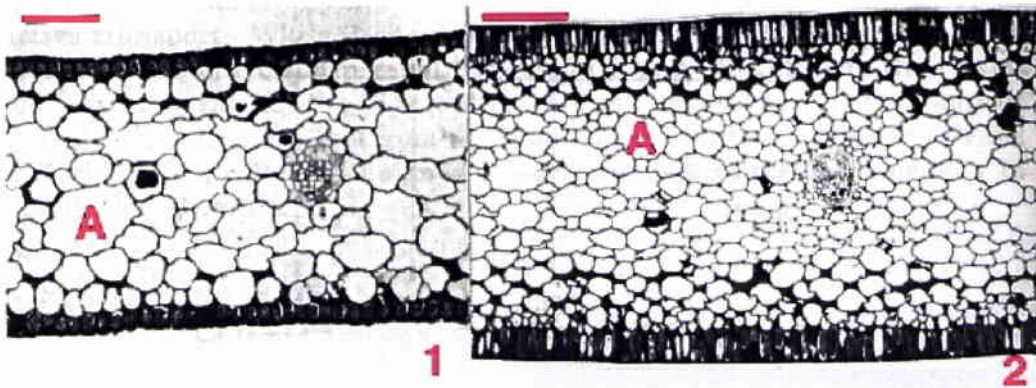


Figure 9 Transverse sections of 1) *Posidonia sinuosa*, scale = 20 μm and 2) *Posidonia coniacea*, scale = 20 μm , showing mesophyll cells surrounding gas lacunae (A) of various sizes. (From Larkum et al., 1989).

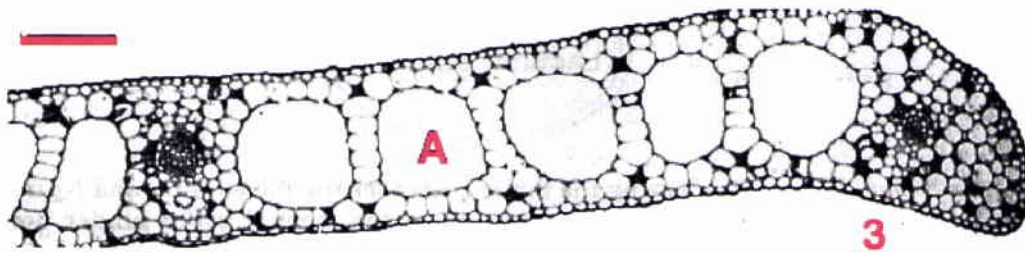


Figure 10 Prominent and regularly arranged gas lacunae (A) in *Zostera muelleri*. Scale = 200 μm . (From Larkum et al., 1989).

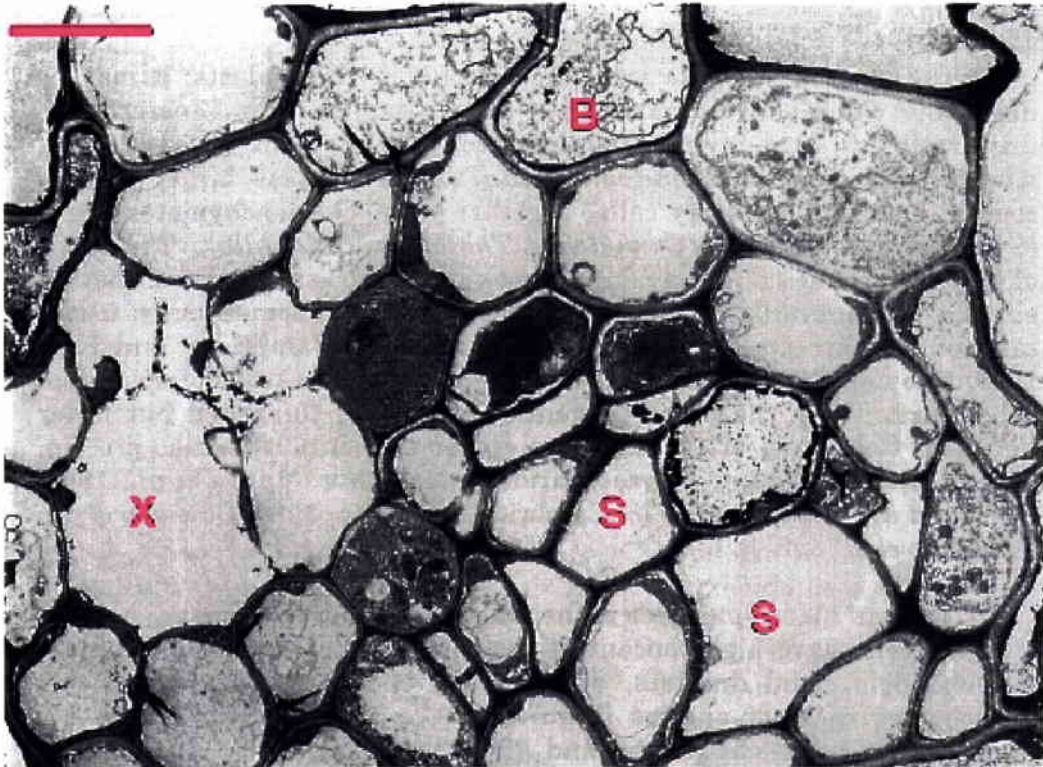


Figure 11 Vascular organization in *Posidonia australis*. Vascular bundle sheath cells (**B**) surround vascular tissue containing thin-walled sieve elements (**S**) and xylem elements (**X**) with little secondary thickening and lignification. Scale = 5 μm . (From Larkum et al., 1989).

A.3. Epiphytes

The long leaf blades and orthotrophic rhizomes of *Posidonia oceanica* offer an excellent substratum for sessile organisms that form a well-structured epiphyte community. This community consists of a pool of algae and sessile animals with life cycles and functional responses fitted closely to the particular nature of the highly dynamic substratum represented by the leaves. It has been demonstrated that the structure of the community changes along an age gradient running from base to tip of each leaf and from the interior to the exterior of the shoot. Encrusting carbonate body epiphytes are largely dominant on *Posidonia* leaves.

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SACLANTCEN SM-372

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Title Characteristics of the Mediterranean seagrass <i>Posidonia oceanica</i> contributing to high-frequency acoustic scattering.		
Abstract <p><i>Posidonia oceanica</i> meadows are the most important ecosystem for the life cycle of coastal Mediterranean benthos with a fundamental role in the primary production of the neritic system and a decisive influence on other vegetation and animal communities. <i>Posidonia oceanica</i> meadows are undergoing a slow but progressive regression, the most common cause of which is enhanced turbidity and the consequent reduction of water transparency. The first step towards the preservation of coastal environments is to define their extent and condition. Echographic surveys allow general maps to be obtained, but accurate seafloor characterization requires knowledge of the characteristics of seabed vegetation which affect acoustics propagation.</p> <p>A preliminary study quantified the gas within the leaves of <i>Posidonia oceanica</i> as a function of the plant life cycle. An order of magnitude of the volume of the different elements considered and their relative importance expressed as percentages is given. The values obtained were considered with reference to the seasonal changes of the phenological variables from which they depend and also to the main structural variable: the density of leaves.</p> <p>A future step based on the present work will be the development of a model of acoustic scattering by <i>Posidonia oceanica</i> derived from a model developed for gassy sediments. The final result would enhance the capability of rapidly acquire information on the extension of meadows, plant density and height. From an operational point of view it could improve the performance of mine-hunting sonars.</p>		
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