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From mechanical to chemical impact of anchoring in seagrasses: The premises of anthropogenic patch generation in *Posidonia oceanica* meadows

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ABSTRACT

Intensive anchoring of leisure boats in seagrass meadows leads to mechanical damages. This anthropogenic impact creates bare mat patches that are not easily recolonized by the plant. Several tools are used to study human impacts on the structure of seagrass meadows but they are not able to assess the indirect and long term implication of mechanical destruction. We chose to investigate the possible changes in the substrate chemistry given contrasted boat impacts. Our observations show that hydrogen sulfide concentrations remain high at 15 and 20 m depth (42.6 µM and 18.8 µM) several months after the highest period of anchoring during the summer. Moreover, our multidisciplinary study reveals that anchoring impacts of large boats at 15 and 20 m depth can potentially change the seascape structure. By taking into account both structural and chemical assessments, different managing strategies must be applied for coastal areas under anthropogenic pressures.

Keywords: Anchoring; Conservation; Seagrass; Seascape; Patch

1. Introduction

Over the last decades, marine ecosystems all around the world have been facing impacts of human activities at various extents (Halpern et al. 2008; Jorda et al. 2012). This statement is particularly observed in the Mediterranean Sea at the level of the coastal habitat formed by seagrass meadows (Grech et al. 2012; Giakoumi et al. 2013). Seagrasses play a major ecological and economical role at the level of the global ocean, covering an area reaching up to 500,000 km^2 (Costanza et al. 1997; Short et al. 2007; Cullen-Unsworth and Unsworth 2013). Thus, they constitute a nursery (Beck et al. 2001), a large carbon sink (Fourqurean et al. 2012), as well as a protection against coastal erosion by attenuating waves and currents (Ondiviela et al. 2014). Among Mediterranean seagrasses, *Posidonia oceanica* (L.) Delile is the most studied due to its major ecological and economical role (Ruiz et al. 2009; Vassallo et al. 2013). The meadows it forms are observed from the surface to 40 m depth and are subject to the impact of human activities like coastal development, eutrophication, trawling, fish farms and anchoring (Boudouresque et al. 2009; Giakoumi et al. 2015b).

Along the French Mediterranean coasts, the main substrate affected by boat anchoring appear to be *Posidonia oceanica* (Holon et al. 2015). Anchoring inside *P. oceanica* meadows seems to have various degrees of impact according to its density, frequency, the type of anchor and the depth as well as the size of boats (Boudouresque et al. 2012). Thus, repeated anchoring of cruise ships, at depths greater than 15 m, causes large-scale degradations of the meadows (Ganteaume et al. 2005b; Abadie et al. 2015). In the same way small units, less than 10 m long, can have an important impact at a local scale (Francour et al. 1999; Milazzo et al. 2004; Ceccherelli et al. 2007).

At the present day, studies mainly targeted the degradation of small boats at shallow depths i.e. less than 10 m. Few works treat the effects of larger pleasure ships anchoring which can measure more than 80 m long and have an important impact in confined areas (Abadie 2012). In order to assess their impact, several parameters are classically measured: the meadow density, the mat structure and the bottom cover (Boudouresque et al. 1995; Francour et al. 1999; Pergent-Martini et al. 2005). However, some of these metrics seem not relevant enough to assess the damages observed on P. *oceanica* meadows. More specifically, classical indicators can indicate a good state of conservation of the meadow with no anthropogenic impact when tracks of bare mat (Fig. 1a) are clearly observed (Milazzo et al. 2004; Ganteaume et al. 2005a).

Intensive anchoring can lead to modifications of substrate qualities, passing from meadows to large bare mat areas in which anchoring tracks are visible (Fig. 1b). This phenomenon also induces a change in sediments nature going from carbonate sediments possibly oxygenized by the living plant to fine particles filling crevices inside decomposing organic tissues forming an anoxic bare mat (Mateo and Romero 1997). Such evolution of the substrate qualities can lead to the hydrogen sulfide (H_2S) intrusion in healthy meadows of the area, limiting the plant development (Holmer et al. 2003; Marbà et al. 2006). Thus, it has been observed that in carbonate sediments H_2S concentrations higher than 10 µM can cause a limitation of P. *oceanica* growth (Calleja et al. 2007).

This study aims to trigger a new way to approach the study of the anchoring impact on seagrass meadows by (1) testing the relevance of the classical structural tools (e.g. meadow density and cover, mat compactness); (2) exploring the relevance of chemical properties of the sediment as a new tool; and by extension; (3) assessing the impact of large leisure ships in a confined area; and lastly, (4) investigating the possible consequences for management and conservation of the areas concerned correlated with anchoring pressure.

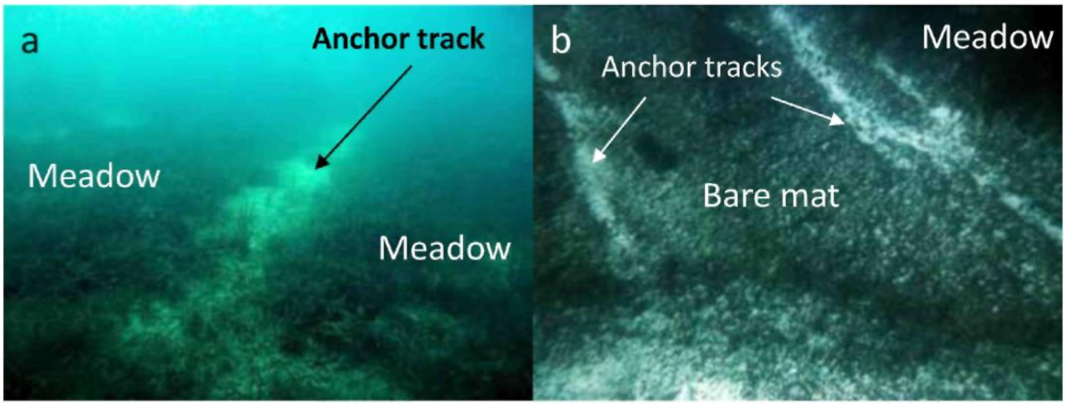


Fig. 1. a) Anchoring track inside a *P. oceanica* meadow at 30 m depth in CalviBay (Corsica, France); b) Bare mat *of Posidonia oceanica* generated by intensive anchoring at 18 m depth in Calvi Bay (Corsica, France) with furrows dug by large ships (photos: Arnaud Abadie).

2. Material and methods

This study was conducted in Alga Bay (8°43'52" E; 42°34'20" N), an area of 1 km^2 of intensive anchoring in Calvi Bay (Corsica, France), colonized by a *P. oceanica* meadow covering 0.78 km^2 (Fig. 2). This site encompasses a particular structure called "return river", a large sand patch where no seagrass meadow can grow, possibly due to strong bottom currents deriving from the surface ones reflected by the coast as described by Boudouresque and Meinesz (1982).

Six stations on two different sites in Calvi Bay were studied at three different depths, i.e. 10 m, 15 m and 20 m. Three stations were chosen as control in a continuous meadow with no traces of impact from human activities near the research facility of STARESO (C10, C15 and C20). Three stations were sampled at Alga Bay in areas of intensive anchoring (A10, A15 and A20) where it can generate anthropogenic patches.

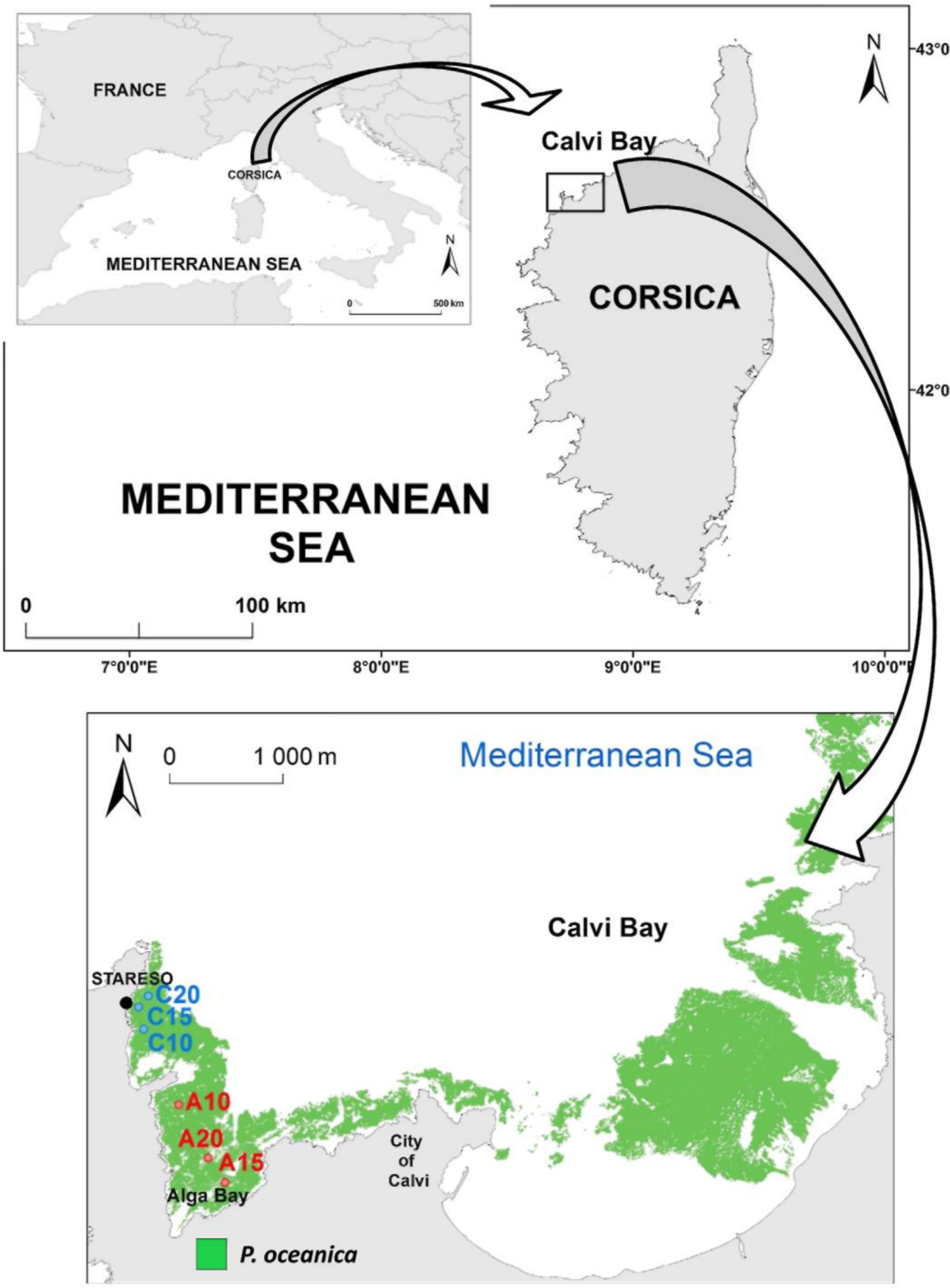


Fig. 2. Map of the study site. Green polygons in Calvi represent the mapping *of Posidonia oceamica* meadows realized with data of 2010 (Abadie 2012).

2.1. Anchoring pressure assessment

A boat counting in Alga Bay was daily performed in the afternoon from May to October 2014 (the touristic period in Corsica) where anchoring frequency is the higher. Ships sizes were classified in three categories according to their length: <10 m; 10-20 m; and >20 m. In parallel, the substrate of anchoring (meadow, rock or sand) was assessed. Moreover, the spatial distribution of boats anchored in the area was investigated by using AIS positioning system of leisure boats (www.marinetraffic.com) from 2012 to 2014 as well as direct catches obtained between 2012 and 2014. These observations were inserted in the G1S software ArcGis® 10 coupled with a map of P. *oceanica* meadows in the area from previous studies (Michel et al. 2012; Jousseaume et al. 2013; Richir et al. 2015).

2.2. Meadow structure

The impact of anchoring on the *P. oceanica* meadows was assessed using six metrics commonly used in the study of its impact on seagrass meadows, i.e. the density, the proportion of orthotropic/plagiotropic rhizomes, the mat compactness, and the rhizomes baring. Ten replicates of the meadow density were randomly counted using quadrats of 25 cm x 40 cm and classified according to the grid of UNEP-MAP-RAC/ SPA (2011). Assessment of the proportion of orthotropic/plagiotropic rhizomes was performed during density measures and interpreted thanks to the classes made by Charbonnel et al. (2000) (Table 1). Mat compactness was investigated given the method and classification of Francour et al. (1999) using a 1 m long rod and a 5 kg weight, repeating ten times the measure for each station (Table 1). Twenty replicates per station of the rhizomes baring, i.e. the distance between rhizome and substrate, were measured and classified according the protocol of Boudouresque et al. (1980). Meadow cover was measured using a 30 cm x 30 cm quadrat hold at arm-length 3 m above the meadow (Gravez et al. 1995). Thirty replicates were performed for this measure and results were interpreted given the scale of Charbonnel et al. (2000) (Table 1). This measure was standardized by keeping the same observer for all measures and placing a depth gauge on the quadrat in order to avoid a distance variation from the vegetation. Finally, 20 longest standing leaves per station (corresponding to the canopy height) were measured in September after the touristic period).

2.3. Conservation Index (CI)

The Conservation Index (CI) was used as a reflection of damages in *P. oceanica* meadows visually observed by scuba diving. Triplicate transects were made to calculate the CI for each station according to the process of Moreno et al. (2001) :

$CI = L/(L + D)$

where L (%) corresponds to the proportion of living *P. oceanica* and D (%) the percentage of bare mat. Four intervals were calculated to assess the meadow's state of conservation on each station:

1. $CI<(x_{mean}-1/2s)$
2. CI from $(x_{mean}-1/2s)$ to x_{mean}
3. CI from x_{mean} to $(x_{mean}+1/2s)$
4. $CI>(x_{mean}+1/2s)$

where mean (x_{mean}) and standard deviation (s) were calculated from all CI values of the study.

2.4. Sediment chemistry and nutrients

Sediment chemistry was studied by sampling pore water in the control (C) and anchoring (Am) meadow, as well as in anchoring bare mat patches' sediments (Ap) given the method of Gobert et al. (2006). Collection was performed in September after the warmest period of the year and in November when seawater temperature starts to decrease.

Thus, the concentration of several essential components was studied in the substrate: dissolved dioxygen (O_2), free hydrogen sulfides (H_2S) and nutrients.

The pore water sampling for O_2 measurements was made within the oxygenic layer at a maximum depth of 1 cm in the sediments. O_2 concentration was obtained using a iodine titration with thiosulfate according to the method of Winkler (1888) with an automatized system for small sampling volumes (Carpenter 1965; Strickland and Parsons 1972) adapted by R. Biondo, (Laboratory of Oceanology-

University of Liège).

The sample collection for H_2S and nutrient analysis was performed in triplicates inside the layer encompassing the plant living parts at 10 cm depth in the substrate. H_2S concentration was measured with a silver/ sulfide ISM-146 FTH 25-XS electrode, coupled with a Sulfide *Anti*-Oxydant Buffer (SAOB) solution given the protocol of Brooks (2001). For detailed protocols of the measure of O_2 and H_2S, see Appendix S1 and S2 in supplementary material. Ammonium ($NH4^+$) and Nitrite (NO_2^-)/ Nitrate (NO_3^-) concentrations were measured by using a SKALAR auto-analyzer following the method of Aminot and Kérouel (2007) adapted for oligotrophic samples (Laboratory of Oceanology-University of Liège).

Table 1: Meadow structure parameter interpretation according to their value.

Parameter	Unit	Range	Interpretation	Classification reference
Meadow density	Shoots·m^{-2}	Depends of depth	High	UNEP-MAP-RAC/SPA 2011
			Good	
			Normal	
			Moderate	
			Bad	
Proportion of orthotrophic/plagiotropic rhizomes	%	<30%	Stable meadow	Charbonnel et al. 2000
		30 to 70%	Slight trend to progress	
		>70%	Net trend to progress	
Mat compactness	cm	<50cm	Strong compactness	Francour et al. 1999
		50 to 100 cm	Medium compactness	
		>100 cm	Weak compactness	
Rhizomes baring	cm	<5cm	Low baring	Boudouresque et al. 1980
		5 to 15 cm	Medium baring	
		>15cm	High baring	
Meadow cover	%	>80%	Very high covering	Charbonnel et al. 2000
		60-80%	High covering	
		40-60%	Medium covering	
		20-40%	Low covering	
		<20%	Very low covering	

2.5. Statistical analyses

Statistical analyses were performed under the R 3.0.2 software using the FactoMineR package. Normality of structural parameters values was checked using a Shapiro-Wilk test. Stations were then statically tested two by two (control vs anchoring) for each depth (10 m, 15 m and 20 m) with an unpaired t-test (after checking their homoscedasticity with a Fisher test) for Gaussian data, and with a Mann Whitney test for non-parametric ones. *T*-tests were followed by a Tukey post-hoc test and Mann Whitney tests by a Dunns test.

Relations between the structural (i.e. meadow density, mat compactness, rhizomes baring, meadow cover, plagiotropic/orthotropic rhizomes proportion, Conservation Index and canopy height) and chemical (i.e. O_2, H_2S and NH_4^+ concentrations) parameters were investigated with a Pearson matrix of correlation.

Finally, a cluster analysis was performed using the Ward method of aggregation and Euclidean distances between individuals for testing dissimilarity. Clusters were thus defined by minimizing the loss of inertia (i.e. the Euclidean distance) between several individuals (i.e. stations) when grouping them. Prior to the analyses, data were standardized to take into account the difference of units. First, structural parameters alone were considered. Then only chemical features were used. Finally, both structural and chemical parameters were computed in order to study the impact of chemical parameters on the evaluation of the meadows' state of conservation.

3. Results

3.1. Ships: spatial distribution and frequenting

The spatial ship distribution in Alga Bay follows a bathymetrical zo-nation according to data from 2012 to 2014 (Fig. 3). Small boats (length < 10 m) appear to anchor at shallow depths (< 5 m, Fig. 3). The majority of ships measuring between 10 and 20 m prefers to anchor outside the meadow in the main part of the return river at depths shallower than 15 m, when bigger ships choose to lay their anchors in the meadow from 10 to 30 m depth (Fig. 3).

Anchoring substrates vary widely according to ship sizes in Alga Bay (Fig. 3). Sand appears to be the preferred anchoring substrate for small boats (53%, Fig. 3) while those of medium and large size chose *P. oceanica* meadows (respectively 55% and 84%, Fig. 3). In general, ships anchor equally in sand (45%) and meadow (47%), few anchoring on rock (8%, Fig. 3).

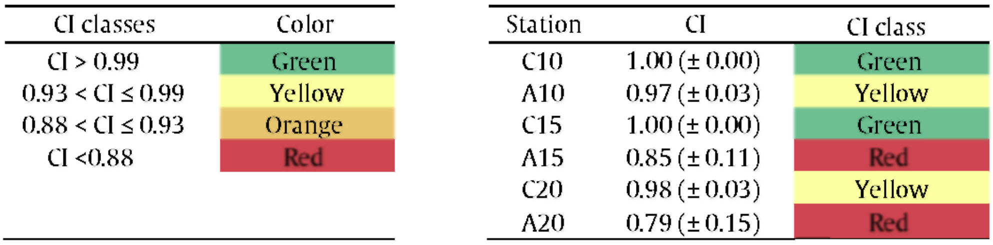
A total of 1768 ships anchoring in Alga Bay were observed from May to October 2014, encompassing 43% (754 ships) of small boats (length < 10 m), 53% (935 ships) of medium size (length 10-20 m) and 7% (79 ships) of large size (length > 20 m) (Fig. 4). Period of most intense anchoring occurs from mid-July to mid-August, reaching a frequenting peak of 74 ships the 7th of August (Fig. 4).

3.2. Meadow structure and conservation

Meadow densities are classified as "normal" according to their depths for two control (C) stations (C10 and C20) and all anchoring (A) stations A10, A15 and A20 (Fig. 5a). The control station at 15 m depth appears as "good" (437 ± 112 shoots.m^{-1}). Mean values of meadow density obtained at 15 m depth are significantly different (t-test: $t = 892$; $p = 0.0097$; df = 18). The same statement is made concerning the proportion of orthotropic/plagiotropic rhizomes which correspond to a stable meadow for all stations (Fig. 5e) except for the station A20 which is considered to have a "slight trend to progress" (70/30 ± 25%). Mat compactness is characterized as "strong" for all stations sampled (Fig. 5b) with the exception of A20 with a "medium" mat compactness revealed by a relatively high penetration length of the rode (51 ± 9 cm). A significant difference (*t*-test: $t = 6.172$; $p < 0.0001$; df = 18) of the compactness was observed at 20 m between the control station and the anchoring one. Rhizomes baring is "medium" for all stations except for C10 (3.6 ± 1.9 cm) and A20 (2.0 ± 1.1 cm) where it is "weak" (Fig. 5c), these differences at 10 m (*t*-test: $t = 4.493$; $p = 0.0001$; df = 28) and 20 m (Mann Whitney test: $p = 0.0002$; U = 24) being confirmed by the statistical analysis. Meadow cover varies from "very high" (C10, C15, and A10) to "high" (C20, A15 and A20) (Fig. 5d), thus highlighting a difference of the mean meadow cover between the control station and the anchoring one at 15 m depth (Mann Whitney test: $p < 0.0001$; U = 182.5). At last, canopy height shows no significant differences between control and anchoring stations, its value decreasing from June to September at all depths (Fig. 5f).

The state of conservation of each control station, expressed by the Conservation index (CI), is the higher for C10 and C15 (1 ± 0.00 for each station), and decreases (0.98 ± 0.03) for C20 (Table 2). At Alga Bay, the anchoring site, the station at 10 m depth (A10) appears to have a state of conservation (CI = 0.97 ± 0.03) similar to C20 while the deeper station stations (A15 and A20) have a lowest one (with a CI of respectively 0.85 ± 0.11 and 0.79 ± 0.15; Table 2).

Table 2 : Interpretation scale of the Conservation Index (CI) and its mean value (± SE). C: control; A: anchoring. 10, 15 and 20: depth.



CI classes	Color
CI > 0.99	Green
0.93 < CI ≤ 0.99	Yellow
0.88 < CI ≤ 0.93	Orange
CI <0.88	Red

Station	CI	CI class
C10	1.00 (± 0.00)	Green
A10	0.97 (± 0.03)	Yellow
C15	1.00 (± 0.00)	Green
A15	0.85 (± 0.11)	Red
C20	0.98 (± 0.03)	Yellow
A20	0.79 (± 0.15)	Red

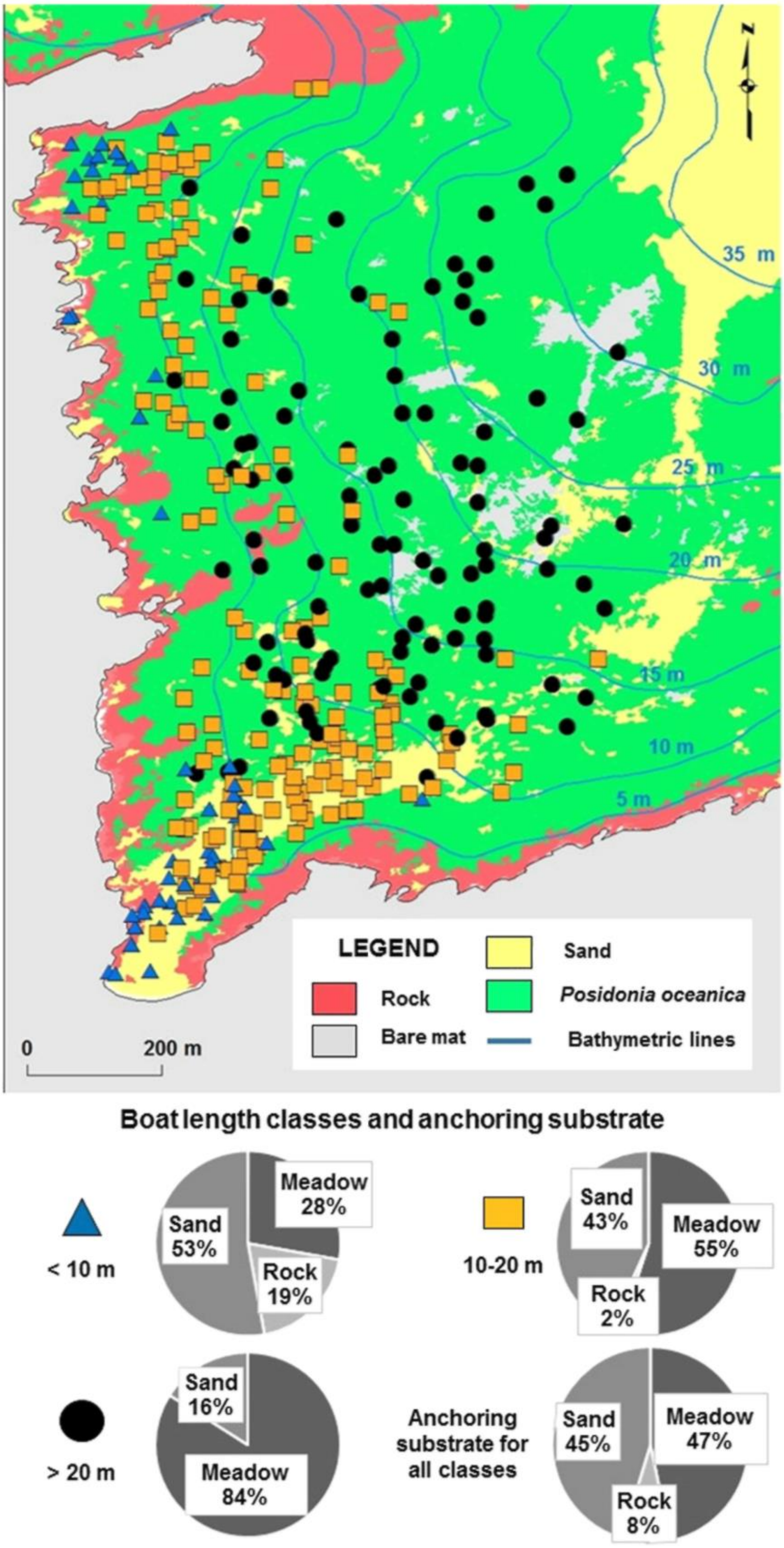


Fig. 3. Ship positioning using AIS and direct catches from 2012 to 2014 at Alga Bay coupled with a map of marine habitats and proportion of anchoring on the three different substrates in 2014 for ships with a length lower than 10 m; between 10 and 20 m, upper than 20 m and for all classes.

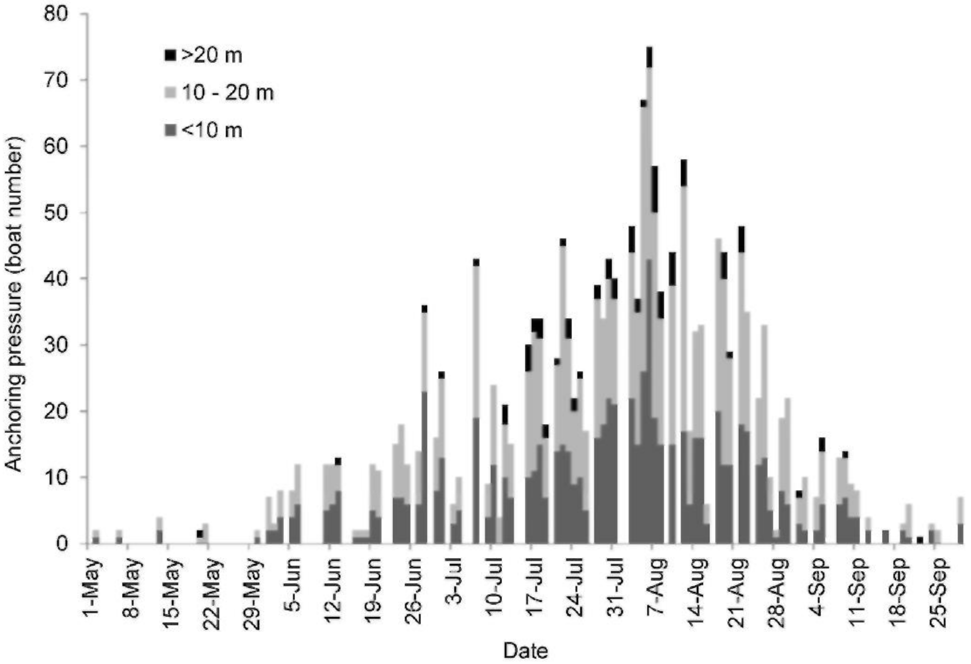


Fig. 4. Daily boat counting and size classes from June to September 2014 in Alga Bay.

3.3. Patch and meadow chemistry

In September, lowest concentrations of O_2 were found inside the patch at -10 m (Ap10: 102.7 µM) while a decrease with the depth in control meadows is observed (Table 3). The same pattern is observed in November with concentrations similar in both control (C) and anchoring (Am) meadows. Low concentrations in O_2 are related to high concentration in H_2S inside the patches at the three depths in September (20.5 µM at -10 m; 9.9 µM at 15 m; 12.8 µM at 20 m). High sulfide concentrations are also found in control meadows in September, except at 15 m (Table 3). In November, H_2S concentrations are relatively low at -10 m for all stations when they remain high at 15 and 20 m (except for C15).

NO_2^- and NO_3^- show very low concentrations in both September and November. NH_4^+ shows in September an increase of its concentration along with the depth (Table 3), as well as higher values inside anchoring patches (except for Ap15). The same pattern is observed in November with the exception that lower values are found in anchoring patches at 10 m instead of 15 m (Table 3).

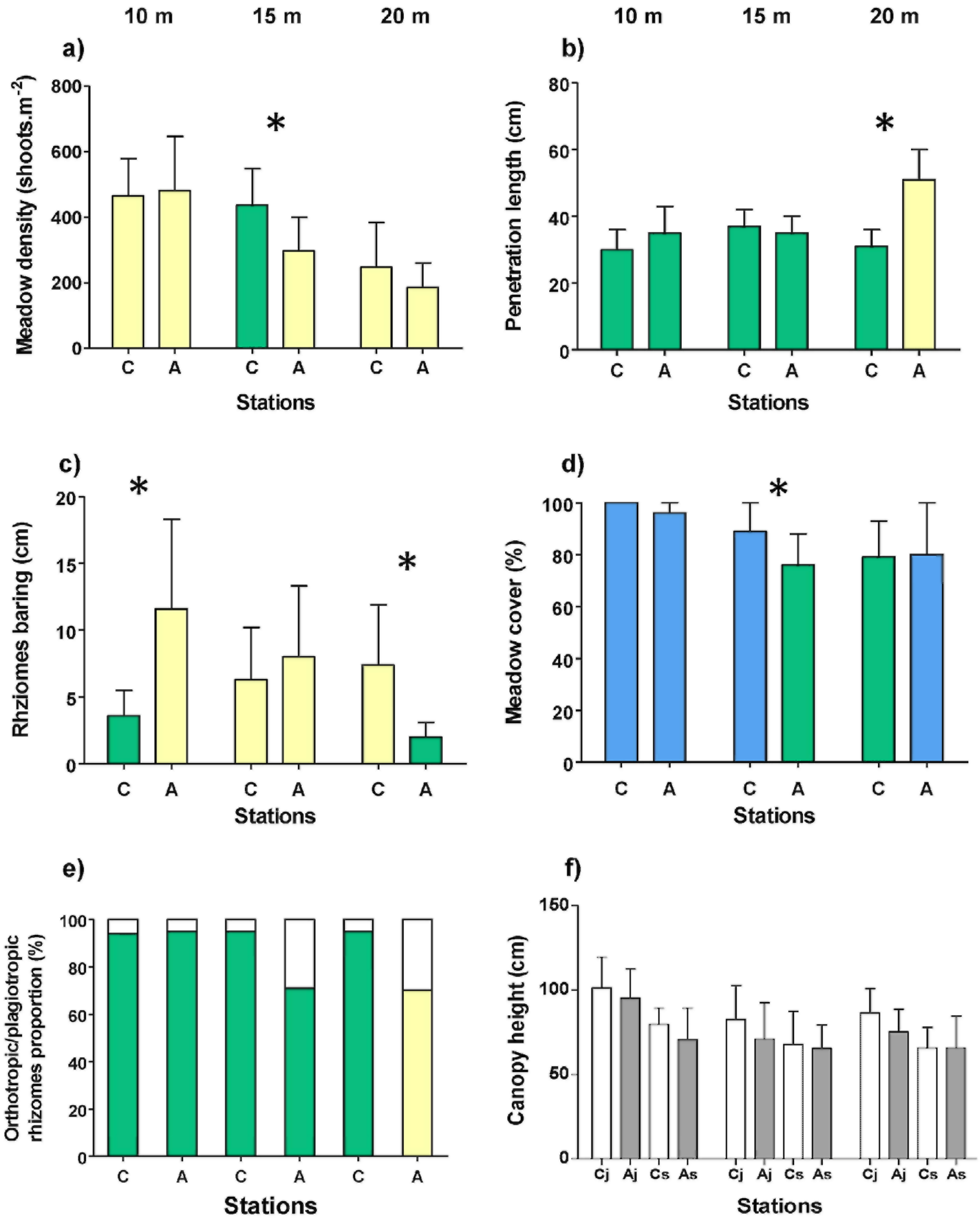


Fig. 5. Mean value (± SE) at 10,15 and 20 m depth of a) meadow density (green: good; yellow: normal) ; b) penetration length (green: high compactness; yellow: medium compactness); c) rhizomes baring (green: weak; yellow: medium); d) meadow cover (blue: very high covering; green: high covering); e) orthotropic/plagiotropic rhizomes proportion, colored bar: orthotropic, white bar: plagiotropic (green: stable meadow; yellow: slight trend to progress); f) mean canopy height" above a pair of bars indicates a significant difference between the two mean values. C = control; A = anchoring; j = June; s = September.

Table 3 : Concentration of dissolved oxygen (O_2) within the first centimeter of substrate and mean concentration of free hydrogen sulfides (H_2S), nitrite (NO_2^-), nitrate (NO_3^-) and ammonium (NH_4^+) in the first ten centimeters of substrates (± SE) in September (Sep.) and November (Nov.) at control (C), anchoring meadow (Am) and anchoring patch (Ap) stations.

	O_2(µM)		H_2S (µM)		NO_2^- (µM)		NO_3^- (µM)		NH_4^+ (µM)	
Station	Sep.	Nov.	Sep.	Nov.	Sep.	Nov.	Sep.	Nov.	Sep.	Nov.
C10	206.0	226.8	39.1 (±37.7)	4.0 (±1.1)	0.06 (±0.00)	0.20 (±0.12)	0.22 (±0.09)	1.92 (±1.54)	4.68 (±2.44)	4.40 (±1.56)
Am10	232.7	213.4	12.0 (±12.4)	4.2 (±0.8)	0.08 (±0.03)	0.06 (±0.00)	0.22 (±0.09)	0.30 (±0.21)	4.74 (±1.78)	4.44 (±0.87)
Ap10	102.7	143.6	20.5 (±30.2)	9.3 (±4.7)	0.06 (±0.00)	0.06 (±0.00)	0.14 (±0.03)	0.24 (±0.00)	10.18 (±8.13)	2.76 (±0.22)
C15	195.1	199.6	0.4 (±0.5)	8.0 (±5.3)	0.06 (±0.00)	0.08 (±0.03)	0.42 (±0.31)	0.38 (±0.18)	10.34 (±6.21)	6.32 (±3.81)
Am15	145.2	189.3	8.1 (±8.4)	33.4 (±44.7)	0.12 (±0.00)	0.08 (±0.03)	0.76 (±0.70)	0.42 (±0.33)	10.76 (±5.38)	12.38 (±8.65)
Ap15	116.9	122.0	9.9 (±7.2)	42.6 (±56.2)	0.08 (±0.03)	0.06 (±0.00)	0.16 (±0.03)	0.22 (±0.12)	6.70 (±3.47)	16.82 (±7.69)
C20	191.8	210.8	16.5 (±21.9)	20.9 (±19.4)	0.08 (±0.03)	0.08 (±0.03)	0.30 (±0.16)	0.34 (±0.19)	14.38 (±7.99)	7.44 (±3.31)
Am20	177.7	211.5	0.9 (±0.8)	13.2 (±16.1)	0.08 (±0.03)	0.08 (±0.03)	0.40 (±0.34)	0.26 (±0.09)	13.82 (±9.51)	7.70 (±5.77)
Ap20	155.5	183.6	12.8 (±21.0)	18.8 (±8.3)	0.06 (±0.00)	0.10 (±0.07)	0.16 (±0.07)	0.50 (±0.45)	20.74 (±26.40)	13.14 (±15.02)

Table 4 : Pearson's matrix of correlation comparing both structural and chemical parameters of the control and anchoring meadow at a depth of 10,15 and 20 m. Density: meadow density; Compact: mat compactness; Rhiz. Bar.: rhizome baring; Cover: meadow cover; Ortho. prop.: Orthotropic rhizomes proportion; CI: conservation index; ch: canopy height; o2: oxygen; h2 s: hydrogen sulfide; nh4: ammonium; j: June; s: September; n: November.

Variables	Density	Compact	Rhiz. Bar.	Cover	Ortho. prop.	CI	ch_j	ch_s	o2_s	o2_n	h2s_s	h2s_n	nh4_s	nh4_n
Density	1	-0.556	0.463	0.876	0.703	0.740	0.720	0.696	0.660	0.282	0.410	-0.635	-0.900	-0.618
Compact.		1	-0.478	-0.369	-0.669	-0.787	-0.570	-0.462	-0.259	-0.146	-0.667	-0.004	0.452	0.180
Rhiz. Bar.			1	0.159	0.367	0.354	0.204	-0.096	0.360	-0.251	-0.063	0.052	-0.405	-0.041
Cover				1	0.681	0.652	0.896	0.881	0.812	0.689	0.588	-0.872	-0.889	-0.864
Ortho. prop.					1	0.972	0.807	0.492	0.794	0.464	0.430	-0.626	-0.470	-0.760
CI						1	0.763	0.530	0.658	0.364	0.496	-0.509	-0.492	-0.639
ch_j							1	0.848	0.852	0.826	0.767	-0.761	-0.744	-0.875
ch_s								1	0.533	0.752	0.857	-0.648	-0.814	-0.668
o2_s									1	0.687	0.329	-0.871	-0.612	-0.941
o2_n										1	0.710	-0.731	-0.438	-0.818
h2s_s											1	-0.289	-0.569	-0.427
h2s_n												1	0.594	0.961
nh4_s													1	0.574
nh4_n														1

3.4. Computation of structural and chemical parameters

Among the structural parameters, rhizome baring (Rhiz. Bar.) appears to have weak correlation with all the other one (Table 4). Mat compactness (Compact.) appears to be less correlated with meadow cover (Cover) and the canopy height in June (ch_j), this last parameter having also few links with the proportion of orthotropic/plagiotropic rhizomes (Ortho. Prop.; Table 4). Between chemical parameters, H_2S concentrations in November (h2s_n) show a strong correlation with O_2 (o2_n) and NH_4^+ (nh4_n) ones at the same period (Table 4). This link is not found in September where only H_2S and NH_4^+ concentrations are correlated. Through the two different periods, oxygen in September (o2_s) is strongly correlated with the hydrogen sulfide in November (h2s_n) and ammonium (nh4_n). Looking at both structural and chemical parameters, all chemical parameters appear correlated with meadow cover (Cover) and canopy height (ch_j and ch_s) and to a lesser extent with meadow density (Density; Table 4). In contrary, both mat compactness (Compact.) and rhizome baring (Rhiz. Bar.) are not linked. These observations are more contrasted concerning the proportion of orthotropic/plagiotropic rhizomes (Ortho. Prop.) and the Conservation Index (CI) where correlations are only found in November for H_2S and NH_4.

The cluster analysis computing the structural parameters alone shows three classes linking anchoring stations at 15 and 20 depths (A15 and A20), when the two control meadows corresponding (C15 and C20) are grouped with the anchoring station at 10 m (A10), leaving the control one (C10) within a single class (Fig. 6a). When using only chemical features the cluster result changes, aggregating the stations A10 and C10 together and grouping C15, C20 and A20, leaving A15 alone (Fig. 6b). Adding the chemical parameters to the structural ones, three classes, encompassing each two stations, are found linking A15 and A20 but aggregating C10 with A10 and C15 with C20 (Fig. 6c).

Few variables being too highly correlated, i.e. with a correlation greater than 0.900 (Table 4), they are not overrepresented in the clustering analysis.

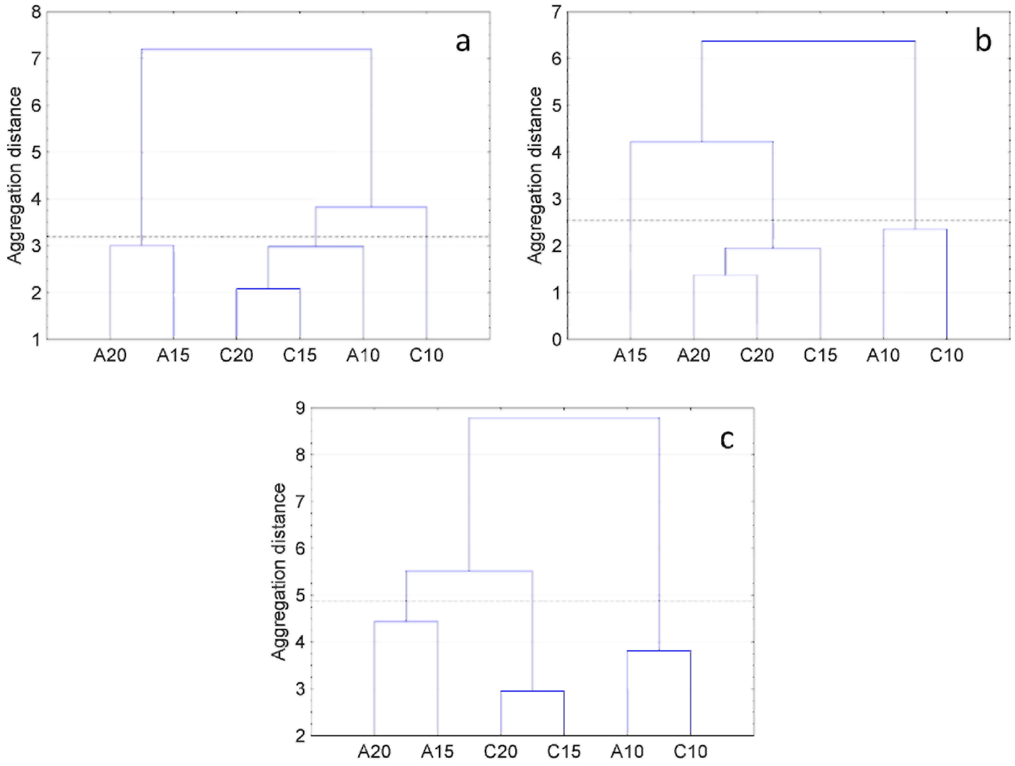


Fig. 6. Cluster analysis of the stations described by a) structural parameters alone; b) chemical parameters alone and c) both structural and chemical parameters of the control (C) and anchoring (A) meadow at a depth of 10,15 and 20 m. The dotted line materializes classes' separation according to their dissimilarity.

4. Discussion

By studying both structural and chemical parameters of two seagrass meadows, one facing intensive anchoring and the other being under no known human pressure, this study highlights the influence of large boats anchoring on the chemistry of *Posidonia oceanica* meadows' substrate and thus, on the seascape structure too.

4.1. An intensive anchoring for a small area?

The first step in a study of anchoring impact on seagrasses should be the analysis and characterization of its frequency according to the size and bathymetry of the area. In the present work, the anchoring pressure at Alga Bay, reaching 0.8 boats$\cdot$ha$^{-1}\cdot$d^{-1} during the peak period, appears moderate compared to previous works in Corsica (Jousseaume et al. 2013) or in Port-Cros, France witnessing up to 8.8 boats$\cdot$ha$^{-1}\cdot$d^{-1} (Ganteaume et al. 2005a). However, anchoring pressure cannot be described by boats density alone but requires in complement the proportion of boats size and their favorite substrate. In this case, although being less numerous than small and medium boats, big ships (length > 20 m) largely favors meadows for anchoring (84%) to sandy and rocky bottoms, leading to more important mechanical damages at higher depths (Ganteaume et al. 2005b). It is mainly due to the fact that these ships need deep water to anchor and that *P. oceanica* meadows are more present at these depths. Conversely, small boats anchor on shallow sites where rocky and sandy substrates are dominant. Thus, taking into account all these aspects, anchoring appears to be intensive in Alga Bay with a high probability of an impact visible on the meadow structure.

4.2. From structural to chemical impact of anchoring

In this study, the analysis of the classical parameters referring to the structure of the *Posidonia oceanica* meadow are not clearly able to depict the direct observation made by scuba diving (i.e. large patches of bare mat crossed by anchoring tracks, Fig. 1). Here rhizomes baring and mat compactness appear not relevant for the study of anchoring, anchors impacting the superficial part of the mat while mat compactness investigate the whole mat thickness. This statement has already been made for mat compactness by Milazzo et al. (2004) and Ganteaume et al. (2005a) for meadow cover and density. Unlike these works, this case encompasses the study of big boats damages and not only the one of small to medium ships. Thus, large ships anchoring will directly pull out whole portions of the meadow and lead to the creation of anthropogenic patches (Fig. 7), revealed by the Conservation Index, the meadow density, and the proportion of orthotropic/plagiotropic rhizomes. In contrast rhizomes will not be partially uprooted and no effect will be witnessed by the rhizomes baring and mat compactness. Thus, large ships' anchoring causes a mechanical destruction similar to the trawling one (Boudouresque et al. 2009; Kiparissis et al. 2011 ; Pergent etal. 2013).

Another limit of the structural parameters is their incapacity to assess the impact of the substrate change (from leafy to bare mat).

Seagrasses are known to be able to release oxygen in the sediments through their roots to create a small oxic zone (Pedersen et al. 1998; Greve et al. 2003), this function being suppressed by the destruction of the canopy by anchoring and thus of the photosynthesis process. These modifications are particularly observable by studying the impact of fish farms, their action leading to large areas of bare mats where an increase of the organic matter in decomposition leads to a decrease of the oxygen available and the intrusion of hydrogen sulfide (Pergent-Martini et al. 2006; Holmer and Frederiksen 2007; Apostolaki et al. 2010). Although no input of organic matter - at least not in the same range as fish farms - are involved in anchoring, the same process is observed here with a decrease of oxygen concentration at the surface of the sediments at all depths and an intrusion of hydrogen sulfide (H_2S) inside anchoring patches. High temperatures enhancing high concentrations of H_2S in *Posidonia oceanica* meadows (Garcia et al. 2012), a decrease along with the temperature should be observed in this case in November. In contrary, an increase in both meadows and patches is observed at the stations where the biggest boats anchor (A15 and A20), no thermocline being observed during the two sampling periods with an homogeneous water column of a temperature of 23.5 °C in September and 20.8 °C in November (Richir et al. 2015).

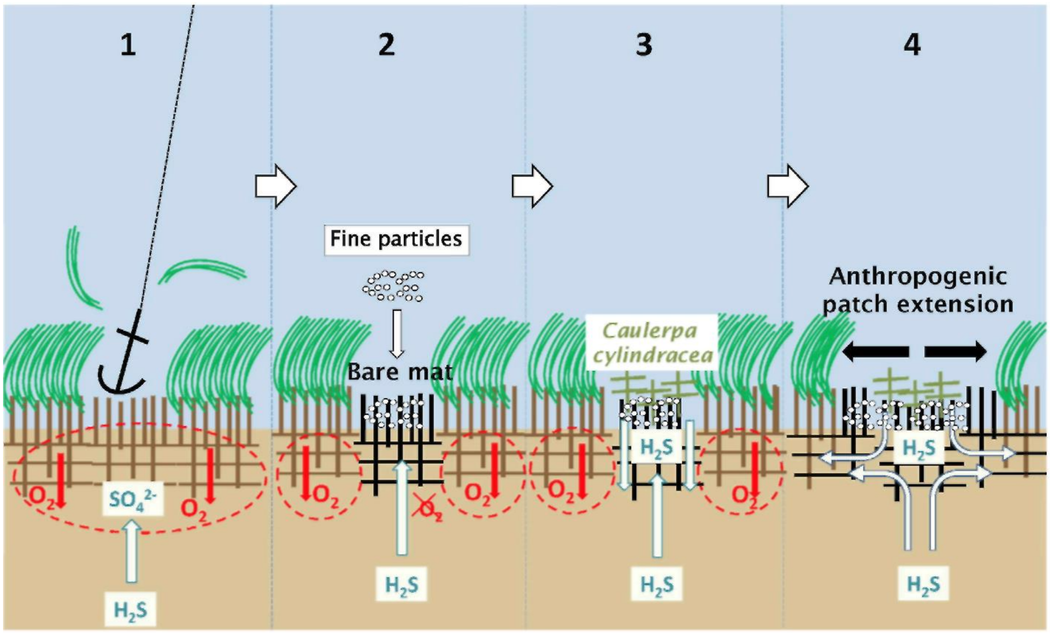


Fig. 7. Hypothesis on the succession of processes from mechanical damages to the expansion of anthropogenic patches; 1) destruction of the canopy by anchoring, 2) fine particles deposit leading to an increase of the organic matter and its degradation; 3) settlement of the alien species *Caulerpa cylindracea;* and 4) expansion of the anchoring patch with intrusion of hydrogen sulfide (H_2S).

The higher concentrations of pore water inorganic nitrate found in Alga Bay at 15 and 20 m depth inside anchoring patches and the surrounding meadow, are another consequence of the change in substrate. Here, the higher concentrations in ammonium ($NH4^+$) could be the result of the particulate organic nitrate's ammonification (Romero et al. 2006). These values constitute a sufficient nutrient enrichment for *P. oceanica* development although recolonization does not occur (Lopez et al. 1998; Gobert et al. 2002).

These unsuitable conditions for a recolonization by the meadow (Marbà et al. 2006), coupled with the continuation of the high anchoring rate in the area, will thus favor the expansion of anthropogenic patches and modify the whole sediment chemistry (Pergent-Martini et al. 2006). In this way, a new arrangement of anthropogenic patches, possibly combined with natural ones, leads to a new seascape (Abadie et al. 2015). The new areas of bare mat thus created are a suitable substrate for the settlement of the alien species *Caulerpa cylindracea* Sonder (Katsanevakis et al. 2010; Kiparissis et al. 2011) which is able to release H_2S inside sediments (Garcias-Bonet et al. 2008) (Fig. 7). The so new-generated small patches may lead to a long-term larger fragmentation of the meadow through aggregation process. Such phenomenon has been studied in *Zostera noltii* Hornemann meadows on the Portuguese coast by Cunha et al. (2005). In a wider viewpoint, vegetation systems, and thus seagrass seascapes, are theoretically subject to aggregation models (Irvine et al. 2016).

4.3. The contribution of chemical parameters in seagrass meadows conservation's assessment

This study highlights the fact that using structural tools alone to assess anchoring in seagrass meadows can lead to a misevaluation of their state of conservation. In the present work, control stations at 15 and 20 m, meaning meadows with no traces of impact from human activities, form a cluster, i.e. they have the same characteristics than the anchoring station at 10 m depth. It reflects no significant anchoring impact on the meadow at this depth. Similarly, chemical features alone aggregate a station visibly impacted by anchoring (A20) with meadows (C15 and C20) under no anthropogenic pressures. When adding chemical parameters to the structural ones, this station is classified in the same group than the control site at the same depth, reveling that no impact is observable. In the same way stations Al5 and A20 are grouped, stating the impact of anchoring at these depths. Considering the long term process induced by a change in sediments chemistry, the measure of several chemical parameters can provide information about the possible recovery of a meadow under an intensive human pressure (Holmer et al. 2008). We suspect that it will be difficult for the meadow in Alga Bay at 15 and 20 m depth to recover given the continuation of anchoring while the meadow at 10 m seems to have the same state of conservation than the control one.

Observation of chemical modifications within seagrass meadows linked with anthropogenic impacts has already been highlighted by Jones and Unsworth (2016) in *Zostera marina* Linnaeus across the British Coast. This study thus reveals an excess of nitrogen within *Z. marina* leaves, associating this observation with the poor water quality and the disturbance of boat-based activities, of which anchoring. Chemical response of the plant to anthropogenic pressures was also stated within *Cymodocea nodosa* (Ucria) Ascherson meadows in Greece by Papathanasiou et al. (2016). These accounts indicate that physiological and chemical changes observed within the meadows are not confined to *P. oceanica* and the Mediterranean Sea. They are also observed in other seagrass species with contrasted morphology and seasonal response to environmental changes, supporting the importance of multi-disciplinary approaches for conservation assessment.

Measurement of chemical features remains however time consuming and requires specific equipment for each element studied. In this way, new simplified protocols should be developed. Nevertheless, like structural parameters who have already proved their utility to assess seagrasses meadow state of conservation (Montefalcone et al. 2006; Gobert et al. 2009; Lopez y Royo et al. 2010), chemical measures are non-destructive, have a good capacity of replication and a great potential to provide a deeper insight (Romero et al. 2007). Moreover, these tools can be integrated in the future conservation indices based on an ecosystemic approach in the framework of the European Marine Strategy Framework Directive (MSFD) (Personnic et al. 2014). They also can be used in the development of descriptors specific to anchoring, built for an easy comprehension by stakeholders and managers.

5. Conclusion

Mechanical damages of intensive anchoring in *P. oceanica* meadows induce a change in the substrate nature leading to the generation of bare mat areas (anthropogenic patches) at 15 and 20 m depth where the bigger ships are observed. Modifications in chemical processes, and more precisely the intrusion of hydrogen sulfide, decrease the possibility of a recolonization by the meadow leading to the expansion of patches. The development of non-destructive chemical indicators easy to perform, coupled with structural tools, will provide precious information for assisting decisions in conservation issues about mechanical impacts in seagrass meadows which, in some cases, are stoppable when effectively assessed (Giakoumi et al. 2015a).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at

http://dx. doi.org/10.1016/j.marpolbul.2016.06.022.

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