Variations in *Posidonia oceanica* meadow structure along the coast of the Dugi Otok Island (eastern Adriatic Sea)

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Although the anthropogenic impact on Posidonia oceanica *meadows in the Mediterranean Sea has been studied over the last few decades, the data about the status of this endemic magnoliophyte are scarcer from the Adriatic Sea. Samples of* P. oceanica *meadows were collected in June and July 2004 using SCUBA diving at 8 sites in the area of the Dugi Otok Island, all at depths of 10 m. The meadow's shoot density was measured, and shoots were collected to be examined and compared through number of leaves per shoot, leaf surface per shoot, leaf area index, number and biomass of taxa of epiphytic flora. Significant differences in* P. oceanica *meadow structure were found among investigated sites, especially between sites in the vicinity of the fish farm and the other sites. Meadow density decreased at sites in the Dugi Otok channel and the main impact factors seem to be the input of organic matter, originating from the fish cages and sewage input. These human activities are a source of nutrient pollution and stimulate blooms of phytoplankton and higher algae. The sea grass meadow vitality seems to be more negatively affected in the channel. The highest values of shoot density were recorded at investigated sites VR and ME at the outer part of the Dugi Otok Island. The shoot density was very low at site FU, situated in the channel. Biomass of the epiphytic algae exhibited the highest values at the site FU, while at the other investigated sites the values were significantly lower. Concerning the epiphytic macroflora, a total of 55 taxa of epiphytic macroalgae were identified and the class Rhodophyta dominated in all samples. The present study shows the high differences in morphological and structural characteristics of* Posidonia oceanica *meadows among various sites with and without anthropogenic influence.*

INTRODUCTION

The Mediterranean endemic magnoliophyte *Posidonia oceanica* (L.) Delile plays the major ecological role in coastal ecosystems, contributing significantly to the productivity of shallow coastal areas (Bellan-Santini *et al.*, 1994; Pergent *et al.*, 1994; Costanza *et al.*, 1997). Sea grass *P. oceanica* makes up dense meadows from the surface to about 40 m depth and is one of the most productive Mediterranean ecosystems (Ott, 1980; Proccacini *et al.*, 2003). Sea grass meadows produce large quantities of organic matter (leaves and epiphytes), which constitute the basis of the food web both within and outside the ecosystem. This production is also exported to other ecosystems, as the main source of food (Pergent *et al.*, 1994; Walker *et al.*, 2001). Meadows of the endemic *P. oceanica* are also characterized as 'key' ecosystems for the Mediterranean basin (Boudouresque *et al.*, 1990; Hemminga & Duarte, 2000; Larkum *et al.*, 2006).

Posidonia oceanica is legally protected in most Mediterranean countries, but despite these protection measures, significant regression of these meadows has been shown in several sectors of the Mediterranean coastal zone (Platini, 2000; Boudouresque,

2003; Pergent-Martini *et al.*, 2006). In the last 40 years *P. oceanica* seems to have suffered a significant decline in most areas, which can be attributed to a wide array of causes, mostly related to anthropogenic activities such as industrial and urban sewage discharges (Pergent-Martini & Pergent, 1995, 1996; Pergent-Martini *et al.*, 2006), fish farming (Delgado *et al.*, 1997; Ruiz *et al.*, 2001; Pergent-Martini *et al.*, 2006), trawl fishing (Ruiz *et al.*, 1999), illegal fishing techniques and coastal works (Ruiz *et al.*, 1993; Guidetti & Fabiano, 2000). Recent monitoring of the sea grass *Posidonia oceanica* in the Croatian part of the Adriatic Sea showed significant decline in meadows density in the area influenced by human activity. The recent increase in fish farming in the Adriatic Sea has imposed additional anthropogenic pressure on *P. oceanica* meadows.

For *P. oceanica* meadows, Pergent *et al.* (1995) proposed an evaluation scale according to meadow density in relation to depth and to other environmental factors such as turbidity and anthropogenic disturbance. The authors proposed 'beds in equilibrium', in which the meadow density is within normal limits (normal density) or exceptionally high (higher sub-normal density). This can be distinguished from 'disturbed beds' and 'very disturbed beds' in which the density is reduced or limited by several factors and is therefore low (lower sub-normal density) or abnormal (abnormal density). Epiphytes on *P. oceanica* leaves are also more sensitive to environmental changes than the plant host (Panayotidis, 1980). Delgado *et al.* (1999) recorded an increase in the

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biomass of *P. oceanica* epiphytes sampled close to the sites of nutrient and organic matter discharge.

The impact of fish farming facilities on *P. oceanica* meadows was assessed from studies of intensive facilities carried out over the last few years (Pergent-Martini *et al.*, 2006). The disturbances caused by these fish farms were measured by means of both abiotic (light and sedimentation) and biotic variables (meadow density, leaf biometry and epiphytes). Fish farming induces high organic and nutrient loading into the surrounding water that cause significant degradation of water quality and are likely to be responsible for changes such as the degradation of sea grass meadows (Holmer *et al.*, 2003; Pergent-Martini *et al.*, 2006). The results showed significant degradation of these sea grasses or even their disappearance and the sediment showed a strong increase in organic matter that could lead to anoxia phenomena. Other factors can also negatively affect the plant, for example an epiphytic bloom that shades the sea grass leaves or grazing pressure increase caused by fish and sea urchins.

The main problem about *P. oceanica* in the Adriatic Sea is that the meadows are insufficiently studied and there is no precise data about their distribution and condition. Limited information is available on leaf features and epiphytic macroflora in *P. oceanica* meadows from the Croatian coast and Croatian islands. The lack of old historical references and the high costs of new surveys prevent any regular monitoring programme in the Adriatic Sea.

Measuring meadow density (number of shoots per m^2) as a direct method is probably the most straightforward. Shoot density depends on structure and functionality of a meadow as well as on its ability to adapt to substrate variability (Scardi *et al.*, 2006). It also plays an important role when estimates of quantitative properties of *P. oceanica* beds are to be calculated.

The aim of the present study was to obtain evidence on the relationships between the regression of *P. oceanica* meadows and environmental disturbances caused by anthropogenic pressure, which proliferated in the last decades due to the tourism and aquaculture development along most of the Mediterranean coast. The objective of the present study was to collect data on widely used *P. oceanica* meadow structural attributes (number of leaves per shoot, leaf surface per shoot, leaf area index, number of epiphytic taxa and their coverage), which is the first first attempt to investigate the status of the *P. oceanica* meadows in Croatia. Furthermore, since nearshore fish-farming activities are carried out in the vicinity of the *P. oceanica* meadows in the Dugi Otok channel, the other objectives were to determine if the nutrient enrichment of the environment in the Dugi Otok channel represents a source of direct disturbance to the growth and structure of the meadows, and to compare these results to oligotrophic waters, such as those of the outer coast of the Dugi Otok Island.

MATERIALS AND METHODS

Study area

Posidonia oceanica shoots were sampled in June and July 2004 at eight meadows near Dugi Otok Island in the central part of the Adriatic Sea. The research was carried out at the outer part of Dugi Otok Island at sites Veli Rat (VR), Sakarun (SK), Mežanj (ME) and Brbinjšćica (BR); in the inner part of Dugi Otok Island at site Savar (SA); near the Iž Island at sites Iž (IZ) and Mrtovnjak (MR) and in the area of Fulija Islet (FU) (Figure 1). The northernmost meadow was located at the site Veli rat (VR) (44°09′11″N 14°49′13″E) and it extended from 1 to 32 m of the depth, whereas the southernmost one was located at the site Iž (IZ) (44°00′42″N 15°08′21″E) and it extended from 6 to 35 m of the depth. The sites VR and ME were situated towards the open sea, the same as the sites SK and BR, but these meadows were growing in large bays. The other four sites (SA, FU, IZ and MR) were situated in a channel with strong sea bottom currents. The *Posidonia* meadow near the Fulija Islet was under the influence of a tuna farm (at a distance of approximately 300 m). This tuna farm, together with the tuna farm near the Kudica Islet (3 km north from the Fulija Islet), produce up to 1500 tons of tuna fish per year. At the study site SA the *Posidonia* meadow was under the influence of local small village sewage whereas the other investigated sites were not affected by human activities.

Sampling methods

Sampling of *Posidonia oceanica* shoots was made by means of SCUBA diving. The density of the sea grass bed (number of shoots per m^2) was estimated at each site using 50 × 50 cm quadrates, with minimum 10 replicates per site. The quadrates were randomly chosen along the meadow, all at the same depth of 10 m. Meadows were assigned to one of the categories proposed by Pergent *et al.* (1995). For estimation of the biometric features of the leaves, ten shoots were collected randomly from each investigated site during the summer period and preserved in seawater–formalin (5%) solution. The leaf width from the orthotropic shoots was measured using calipers. The samples were also examined for number of leaves (adult and intermediate), leaf surface per shoot (cm^2 per shoot) and leaf area index (m^2 per m^2) (Pergent *et al.*, 2003). The number of epiphytic algae taxa (Rhodophyceae, Fucophyceae and Chlorophyceae) and their coverage (expressed as percentage of leaf surface) (Piazzi *et al.*, 2004) were also determined. The epiphytic algae from the rhizome and the leaf base were also investigated.

From the same collected shoots epiphytic algae were scraped with a scalpel from the entire adult and intermediate leaves (5 leaves per shoot, minimum 10 replicates), isolated from epiphytic fauna under a stereomicroscope, dried for 48 h at 75°C and the total dry weight per shoot was determined (Cancemi *et al.*, 2003).

Onset Computers data loggers were used for measurements of light intensity at all of the study sites at 10 m depth. Sea temperature was measured with Onset Computers temperature data loggers at 10 m depth at two sites; the Fulija Islet (FU) in the channel and at Mežanj (ME) towards the open sea in the period of one year (from 1.1.2004 to 1.1.2005).

Data of the investigated parameters were only available at one sampling time, therefore a one-way ANOVA was used in order to assess differences between investigated sites followed by Tukey's honestly significant difference test. The Statistica 7.0 for Windows software was used. The similarity in macroalgal composition among sampling sites was analysed by calculating the Bray–Curtis similarity and showed by cluster analysis and multidimensional scaling (MDS) using PRIMER 5.0.
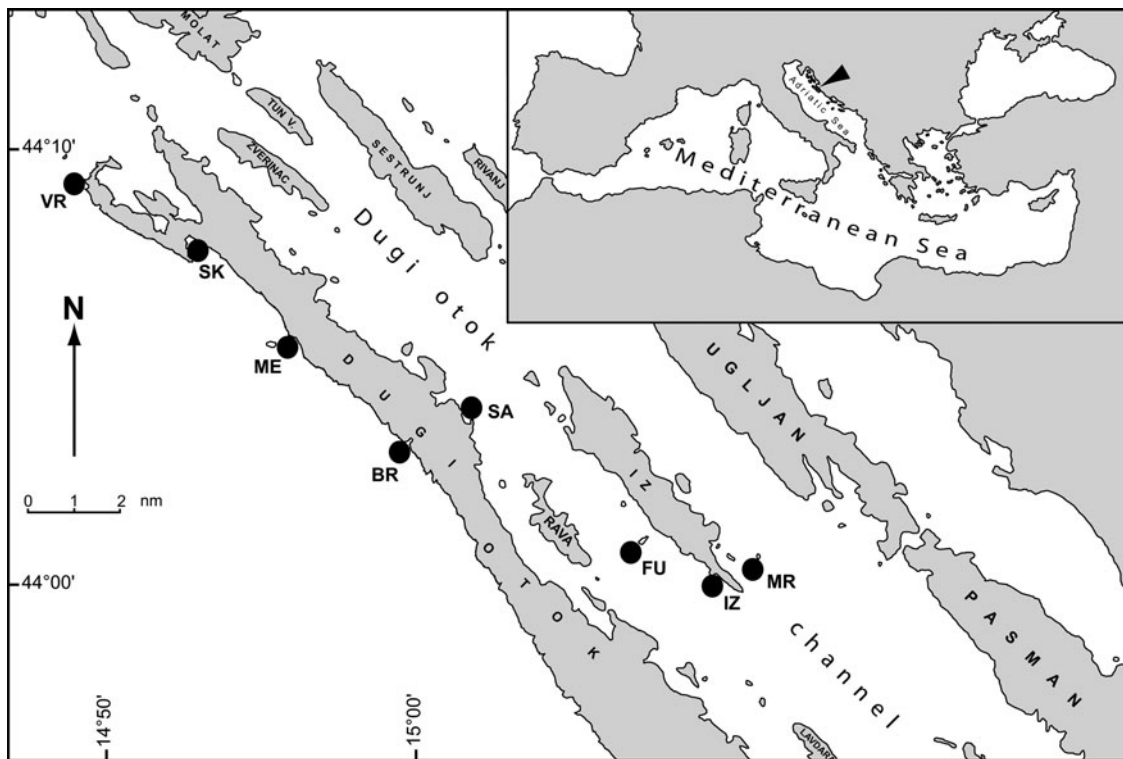
Fig. 1. Map of the Dugi Otok Island and the positions of the sampling sites (in abbreviations).

RESULTS

Sea temperature showed seasonal variability and ranged from 10.3°C to 23.1°C with no differences among sampling sites (Figure 2). At the sampling site Fulija Islet the temperature was on average 1°C higher than at the sampling site Mežanj. Maximum sea temperature was recorded in August, while minimum temperature values were measured in February and March, when the water column was entirely mixed.

Significant differences in the light intensity between the sites were observed in winter and summer (one-way ANOVA, $P < 0.01$) (Figure 3). The highest light intensity value was measured in winter (3.48 log Lum/sqm) and summer (3.19 log Lum/sqm) at sampling site ME. The lowest light intensity values were recorded in winter (2.43 log Lum/sqm) and in summer (2.06 log Lum/sqm) at the site FU.
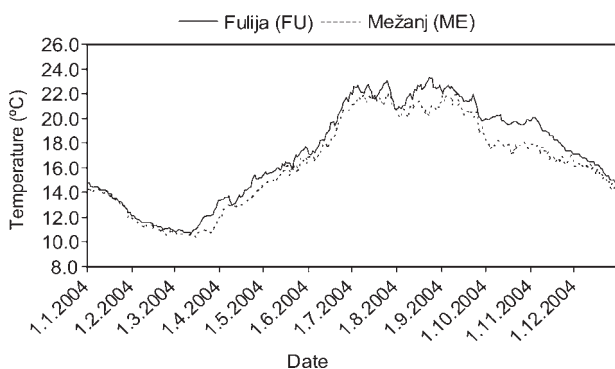

Fig. 2. Sea temperature at the sites Fulija (FU) and Mežanj (ME) at 10 m depth.

Significant differences between all investigated parameters of *Posidonia oceanica* meadows were detected among meadows at channel sites and meadows at open sea sites (Table 1).

The highest density of *P. oceanica* meadows, measured at 10 m depth, was found at sites VR (707.7 ± 72.4 shoots/m²) and ME (689.6 ± 45.9 shoots/m²) (Figure 4A). The lowest values were recorded at the site FU (37.8 ± 2.2 shoots/m²) in the vicinity of the fish farm. Significant differences were found in sea grass shoot density between sites at the outer part of the Dugi Otok Island (VR, SK, ME and BR) and sites in the channel (one-way ANOVA, $P < 0.001$) (Tables 1 & 2). Significant decline in sea grass density provides evidence of damage to the sea grass meadow. The *Posidonia* bed density values at the site FU are significantly beneath the values that are normally recorded at the 10 m depth (Tukey test $P < 0.05$). According to the classification scale established by Pergent *et al.* (1995), these values are far beneath the 'abnormal density' of very disturbed beds. The lower values of bed density at the site FU could be associated with local hydrodynamic conditions (current velocities ranged from 6 to 25 cm s^{-1}) that affect the deposition of organic matter from tuna cages. Sites SA, IZ and MR are classified as disturbed beds or beds tending towards regression. All these sites are from the Dugi Otok channel with strong sea bottom currents. Sites SK and BR belong to the outer part of the Dugi Otok Island, but are situated in large bays and these meadows are classified as beds in equilibrium, tending towards normal bed density. At sites VR and ME meadows are classified as dense to very dense beds or higher subnormal density.

The highest values of leaf length were measured at sites VR (92.9 ± 6.9 cm), SK (83.9 ± 8.1 cm) and ME (83.6 ± 6.1 cm),
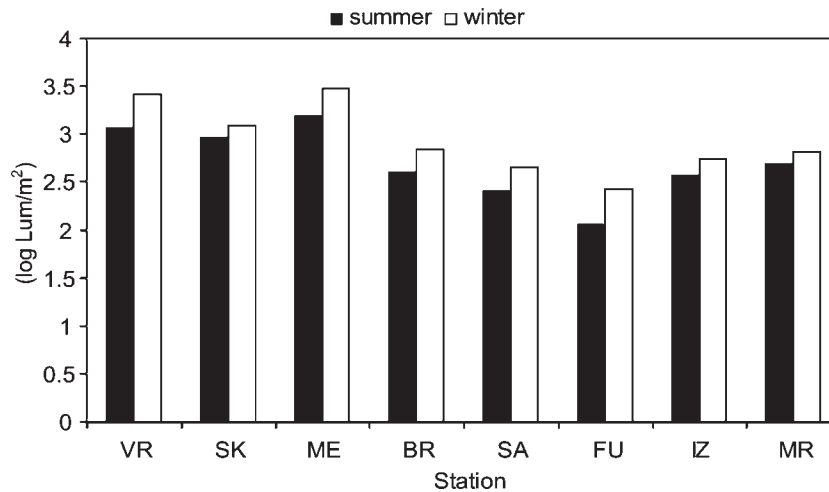
Fig. 3. The highest values of the light intensity in summer and winter at study sites (at 10 m depth) in log Lum/m².

while the lowest value was measured at the site FU (43.6 ± 5.3 cm) (Figure 4B). Significant differences were found in the leaf length between sites at the outer part of the Dugi Otok Island and sites in the channel (one-way ANOVA, $P < 0.001$).

The highest mean number of leaves (adult and intermediate) per shoot was recorded at sites ME (8.7 ± 0.9) and BR (8.1 ± 1.2) (Figure 4C). The lowest values were recorded at sites IZ (5.7 ± 0.7) and FU (5.7 ± 1.4).

The highest values of the leaf width were measured at sites ME (9.2 ± 0.2 mm) and SK (8.9 ± 0.3 mm) and lower values were measured at sites FU (7.3 ± 0.4 mm) and BR (7.5 ± 0.3 mm) (Figure 4D). Significant difference was found in the mean number of leaves and the leaf width between investigated sites (one-way ANOVA, $P < 0.001$).

Leaf area index decreased as the meadow density decreased, and the maximum value was observed at sites ME and VR, where the densest meadows occurred (Figure 5A). High values of leaf surface per shoot (cm² per shoot) were recorded at the sites ME and VR (Figure 5B). Biomass of the epiphytic algae exhibited the highest values in the vicinity of the tuna farm (site FU; 198.8 ± 74.4 mgdw/shoot), with significant differences between that and the other investigated sites (one-way ANOVA, $P < 0.001$). Considerably high biomass was also recorded at the site SA (74.1 ± 16.3 mgdw/shoot) (Figure 5C). At the other

investigated sites, the biomasses of the epiphytic algae were not exceeding more than 50 mgdw/shoot. Significant differences in biomasses of the epiphytic algae between these sites were not found ($P > 0.05$). The lowest value was measured at the site ME (12.9 ± 4.8 mgdw/shoot). Maximum values of the epiphyte coverage of leaf surface were measured at the site FU (more than 35%) (Figure 5D).

Fifty-five macroalgal species were found on leaves from the investigated sites (9 Chlorophyceae, 15 Pheophyceae and 31 Rhodophyceae) (Table 3). Macroalgal assemblages on leaves were dominated by the encrusting Corallinaceae *Hydrolithon farinosum* (Lamouroux) Penrose and Chamberlain, *Jania rubens* Lamouroux, *Pneophyllum fragile* Kützing and *Haliptilon attenuata* Garbary & Johansen and by the Pheophyceae *Halopteris filicina* (Grateloup) Kützing and *Myronema orbiculare* J. Agardh. The highest number of macroalgal species was found at the site FU (29 species) and the lowest number at the site SA (15 species).

The most abundant species, on the rhizomes and the leaf base, were the Rhodophyceae *Peyssonnelia rubra* (Greville) J. Agardh, *Polysiphonia denudata* (Dillwyn) Kützing and *Womersleyella setacea* (Hollenberg) R.E. Norris.

The similarity of the macroalgae species growing as epiphytes, based on the presence or absence of taxa, was relatively high for sites from the Dugi Otok channel and the open sea. The cluster analysis and multidimensional scaling showed that two distinct groups exist (Figure 6). One group contains samples collected from the channel (A) and the other consists of open sea samples (B). In group B, the highest similarity was found between sites VR and ME, two sites with highest values of morphological characteristics of investigated *P. oceanica* meadows. The Bray–Curtis similarity was the highest between sites VR and ME (75.68%), sites IZ and FU (70.83%) and sites BR and SK (68.29%). The lowest values of similarity were found between sites FU and ME (20.41 %) and sites IZ and ME (20.51%).

DISCUSSION

The results presented in this paper demonstrate the differences in morphological and structural characteristics

Table 1. Summary of the results of the one-way ANOVAs testing the investigated parameters of *Posidonia oceanica* meadows between meadows at channel sites and meadows at open sea sites.

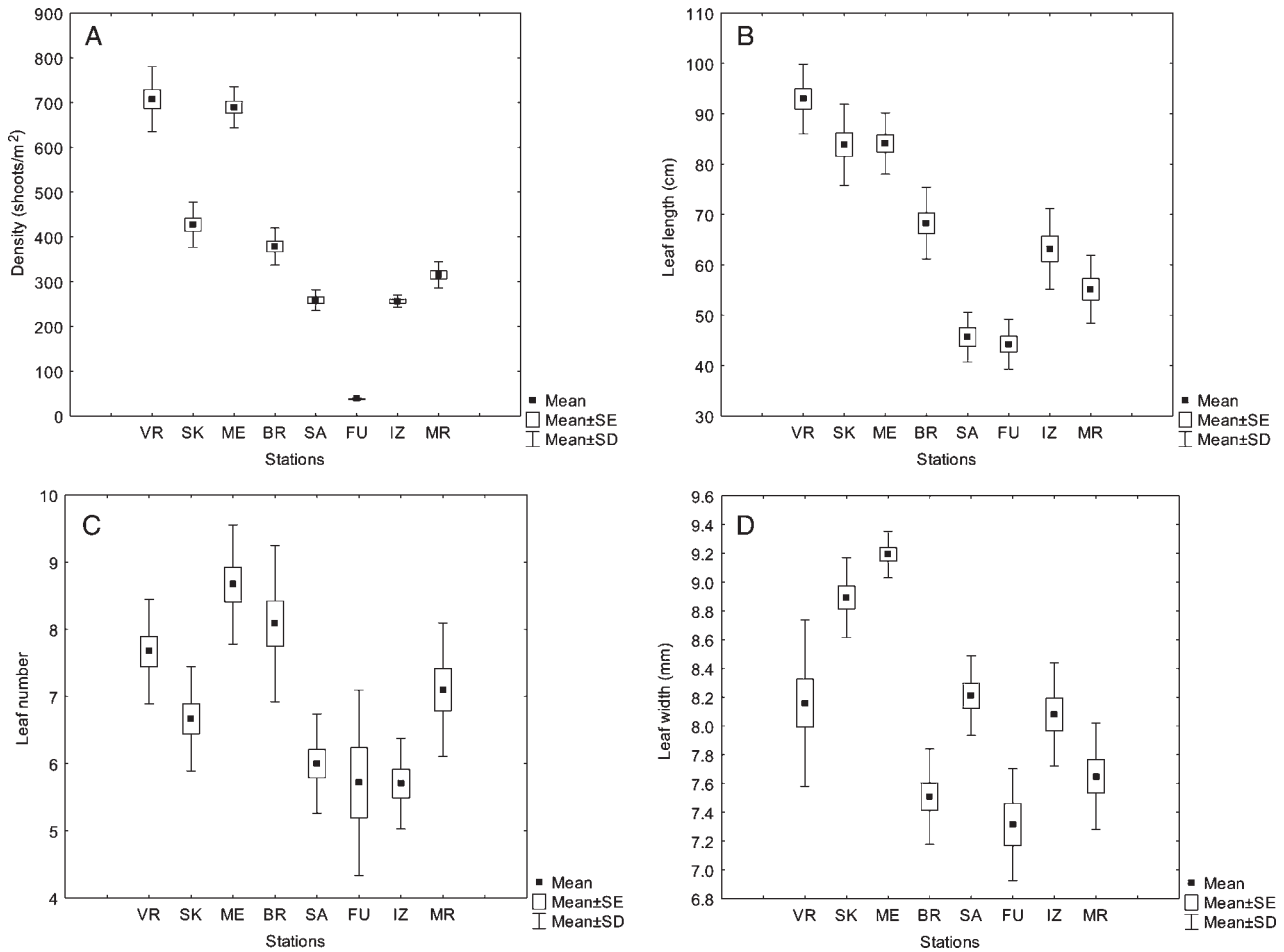|	df	MS	F-ratio	P value
Density (shoots/m²)	1	8923.1	105.59	<0.001
Leaf length (cm)	1	1869.3	159.81	<0.001
Leaf number	1	56.26	45.31	0.0037
Leaf width (mm)	1	7.11	16.95	0.0022
Leaf area index (m²/m²)	1	133.96	103.91	<0.001
Leaf surface (cm²/shoots)	1	2560.37	26.51	<0.001
Biomass of epiphytic algae (mg dw/shoot)	1	6930.4	33.16	<0.001
Epiphyte coverage of leaf surface (%)	1	939.93	23.46	0.0014

Fig. 4. Mean values (with standard error and standard deviation) of shoot density (A), leaf length (B), leaf number (C) and leaf width (D) at study sites.

of *Posidonia oceanica* meadows among various sites with and without anthropogenic influence. Eutrophication, as an anthropogenic cause of *P. oceanica* meadows decline, is a serious problem in coastal areas of the Mediterranean Sea. Symptoms of fish farm activities, local sewage discharge and eutrophication of the surrounding areas as a result of these facilities have been documented and confirmed by various authors in the past (Delgado *et al.*, 1997; Marbà & Duarte, 1997; Ruiz *et al.*, 2001; Cancemi *et al.*, 2003; Marbà *et al.*, 2006; Pitta *et al.*, 2005, 2006). It is well known that even minimal inputs of inorganic phosphorus into farm bottom water can drastically affect the planktonic and algae system

(Maldonado *et al.*, 2005). Released nutrients enhance phytoplankton epiphyte and macroalgal growth, which in turn reduces the light available to sea grass. Patches of green macroalgae *Ulva rigida* C. Agardh, recorded at the site FU at depths from 10 to 25 m, together with the genus *Enteromorpha* throughout the world in water contaminated with domestic sewage, like that at the site SA, indicate such effects. It is well-known that continued inputs of dissolved nutrients, from fish farming activities, usually result in drastic environmental changes that affect both planktonic and benthic communities at the local scale (Ruiz *et al.*, 2001; Cancemi *et al.*, 2003).

Table 2. Results of the Tukey HSD test on meadows density (shoots/m²) at the investigated sites. Significant differences ($P < 0.05$) are indicated in bold.

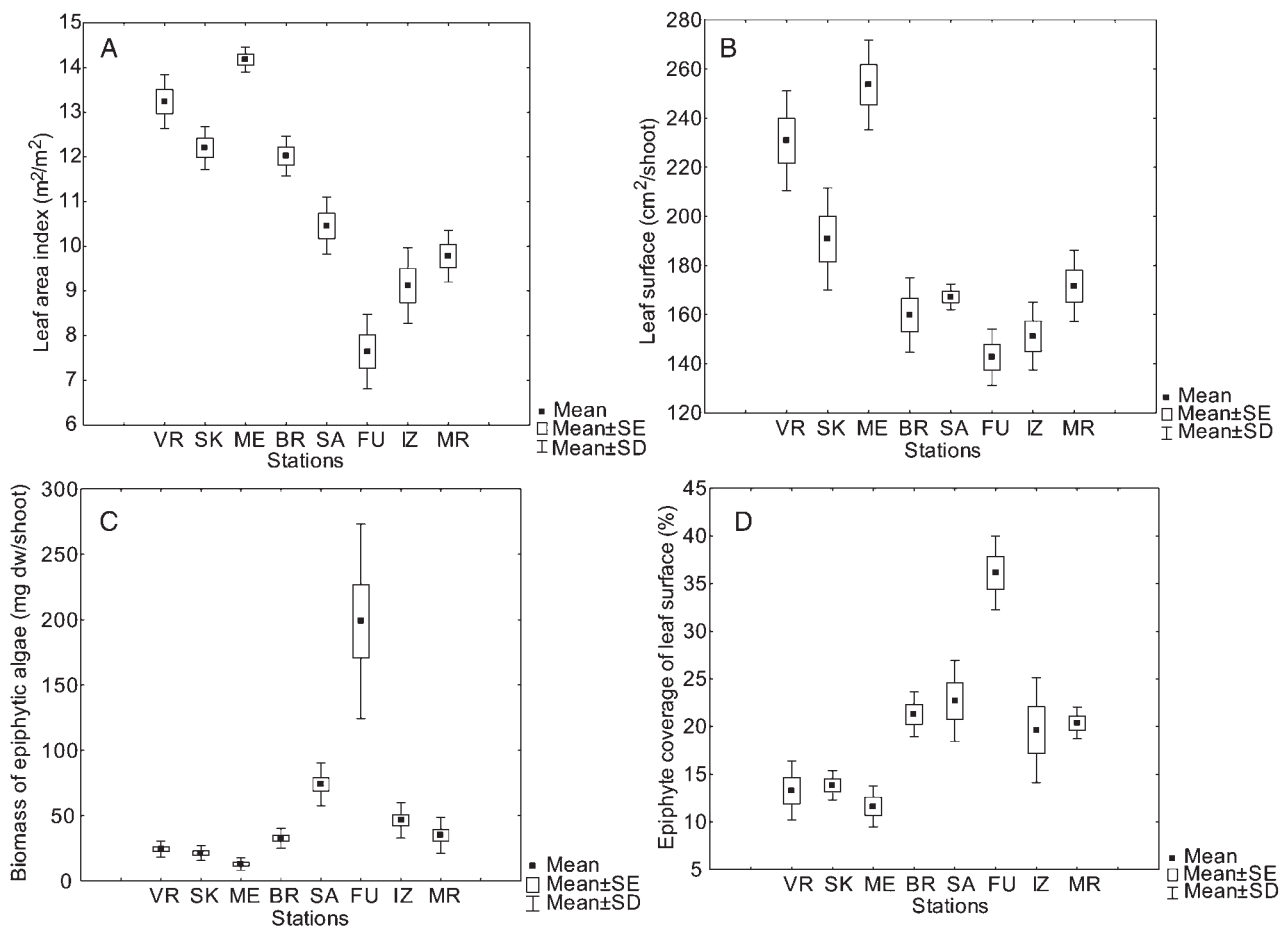|	VR	SK	ME	BR	SA	FU	IZ
Density (shoots/m²)							
VR							
SK	**0.000**						
ME	0.970	**0.000**					
BR	**0.000**	0.126	**0.000**				
SA	**0.000**	**0.000**	**0.000**	**0.000**			
FU	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**		
IZ	**0.000**	**0.000**	**0.000**	**0.000**	1.000	**0.000**	
MR	**0.000**	**0.000**	**0.000**	**0.022**	0.083	**0.000**	0.063

Fig. 5. Mean values (with standard error and standard deviation) of leaf area index (A), leaf surface (B), biomass of epiphytic algae (C) and epiphyte coverage of leaf surface (D) from the study sites.

Another impact on *Posidonia* meadows at sites FU and SA is accumulation of organic wastes in the seabed which is unfortunately not studied yet, although it could be confirmed by the presence of muddy composition in the sediments. A significant fraction of the organic matter input is incorporated into the sediment and mineralized there, resulting in high organic content in the sediment. The impact of organic loading in the sediment may be long lasting, even if the water quality improves over time. This is proved by the observation that the decline of *Posidonia* meadows continued for years after closure of the fish farm (Delgado *et al.*, 1999). Total degradation of *Posidonia oceanica* meadows was observed in circumference of 120 m around the tuna cages at the site FU, where total sea grass die-off was observed, while the shoot density was significantly reduced within a radius of 500 m from the tuna cages. The effective impact derived from the fish farm is highly variable and depends on local hydrodynamics, amount of fish in the cages, fish feeding strategy and the sinking rate of faecal pellets (Karakassis *et al.*, 1999, 2001).

Meadow density of *P. oceanica* depends on depth and density decreases from shallower to deeper waters with decrease of light intensity (Pergent *et al.*, 1995). Transparency and light intensity could be fairly stable in open water areas, but this is not the case in many coastal environments where water turbidity may fluctuate profoundly as a result of physical,

chemical and biotic factors. The lower values of light intensity (both in winter and summer), measured at 10 m depth were observed at sites FU and SA, both under the influence of high organic inputs. Observed evident changes in the community around the tuna farm cages were increased abundance of epiphytes on sea grass leaves and reduced water-column transparency (decrease in light accessibility) that has impact on photosynthetic performance of *P. oceanica*. The number of epiphytic taxa and the biomass vary significantly and indicate both physical (temperature) and chemical (nutrient concentrations) environmental conditions (Pergent *et al.*, 1995).

The 55 taxa of epiphytic macroalgae identified at the study sites are comparable with the 78 taxa reported by Blundo *et al.* (1999) and with 74 taxa reported by Piazzi *et al.* (2002), and not on the other hand with 19 taxa recorded by Cinelli *et al.* (1984). All these records were from the western Mediterranean. In the epiphytic community, the Rhodophyceae dominated in all samples. The number of identified taxa decreased in channel sites (FU, IZ, SA and MR), reflecting a similar trend for the coverage of *P. oceanica* leaves by epiphytes. The shoot density, leaf length, leaf width and leaf surface were increased at open sea sites (VR, ME, SK and BR), where the epiphytic load was less abundant. Leaf area index decreases at sites with decreased meadow density. The reason for differences in the examined biometric features of *P. oceanica* between the sites at the outer part of Dugi Otok

Table 3. List of macroalgal epiphytes on leaves of *Posidonia oceanica* from sampling sites.

Taxa/stations	VR	SK	ME	BR	SA	FU	IZ	MR
CHLOROPHYCEAE								
Bryopsis muscosa Lamouroux					+			+
Chaetomorpha aerea (Dillwyn) Kützing						+		
Chaetomorpha crassa (C. Agardh) Kützing					+	+	+	
Cladophora albida (Hudson) Kützing		+						
Cladophora coelothrix Kützing		+	+			+		
Cladophora sericea (Hudson) Kützing	+		+					
Cladophora vagabunda (Linnaeus) Van den Hock					+	+		
Pseudochlorodesmis furcellata (Zanardini) Börgesen						+	+	
Valonia utricularis (Roth) C. Agardh	+	+		+				
PHEOPHYCEAE								
Asperococcus bullosus Lamouroux					+	+		
Asperococcus compressus Griffiths *ex* Hook						+	+	
Acinetospora crinita (Carmichael *ex* Harvey) Sauvageau					+	+		
Arthrocladia villosa (Hudson) Doby				+				
Asperococcus bullosus Lamouroux					+	+	+	+
Dictyota linearis (C. Agardh) Greville				+				
Feldmannia irregularis (Kützing) Hamel		+						
Halopteris filicina (Grateloup) Kützing	+	+	+	+	+	+	+	+
Halopteris scoparia (Linnaeus) Sauvageau		+			+	+	+	
Myriactula rivulariae (Suhr) J. Feldmann			+					
Myrionema orbiculare J. Agardh	+	+	+		+	+	+	+
Myrionema strangulans Greville	+	+	+					+
Petalonia fascia (Müller) Kuntze						+		
Sphacelaria plumula Zanardini						+	+	
Sphacelaria rigidula Kützing						+		
RHODOPHYCEAE								
Acrosorium venulosum (Zanardini) Kylin		+			+	+	+	+
Antithamnion cruciatum (C. Agardh) Nägeli			+					
Apoglossum ruscifolium (Turner) J. Agardh		+			+			
Asparagopsis armata Harvey					+	+	+	
Botryocladia chiajeana (Meneghini) Kylin						+		
Ceramium ciliatum (Ellis) Ducluzeau						+	+	+
Ceramium circinatum (Kützing) J. Agardh	+		+					
Ceramium codii (Richards) Mazoyer	+		+					+
Ceramium rubrum (Hudson) C. Agardh				+				
Ceramium tenuissimum (Lyngbye) J. Agardh		+						
Crouania attenuata (Bonnemaison) J. Agardh						+	+	+
Dasya hutchinsiae Harvey *in* Hooker		+	+					
Dasya ocellata (Grateloup) Harvey						+		
Erythrotrichia carnea (Dillwyn) J. Agardh	+		+					
Haliptilon attenuata Garbary *et* Johansen	+	+		+	+	+	+	+
Hydrolithon farinosum (Lamouroux) Penrose & Chamberlain	+	+	+	+	+	+	+	+
Jania rubens (Linnaeus) Lamouroux	+	+		+	+	+	+	+
Laurencia obtusa (Hudson) Lamouroux	+		+	+				+
Monosporus pedicellatus (Smith) Solier		+		+				
Peyssonnelia rubra (Greville) J. Agardh	+	+	+	+	+		+	+
Plenosporium borreri (Smith) Nägeli						+		
Pneophyllum fragile Kützing	+	+	+	+		+		+
Polysiphonia adriatica Schiffner		+						
Polysiphonia scopulorum (Harvey) Womersley					+	+		
Polysiphonia violacea (Roth) Greville	+	+	+					
Pterosiphonia pennata (C. Agardh) Falkenberg		+		+			+	
Pterothamnion plumula (Ellis) Nägeli						+	+	
Spyridia filamentosa (Wulfen) Harvey						+	+	+
Stylonema alsidii (Zanardini) Drew	+		+	+				
Stylonema cornu-cervi (Reinsch) Haucl			+					
Womersleyella setacea (Hollenberg) R.E. Norris	+	+	+	+				

Island could lie in the fact that sites VR and ME are situated towards the open sea and the meadows at sites SK and BR are growing in large bays. Several epiphytic macroalgae (mostly Rhodophyceae) from the investigated sites normally inhabit waters deeper than 10 m (like *Womersleyella setacea* (Hollenberg) R.E. Norris), but could be found in shallow areas because of decreased light intensity in lower part of *Posidonia* leaves. The epiphytic load in the meadow near the
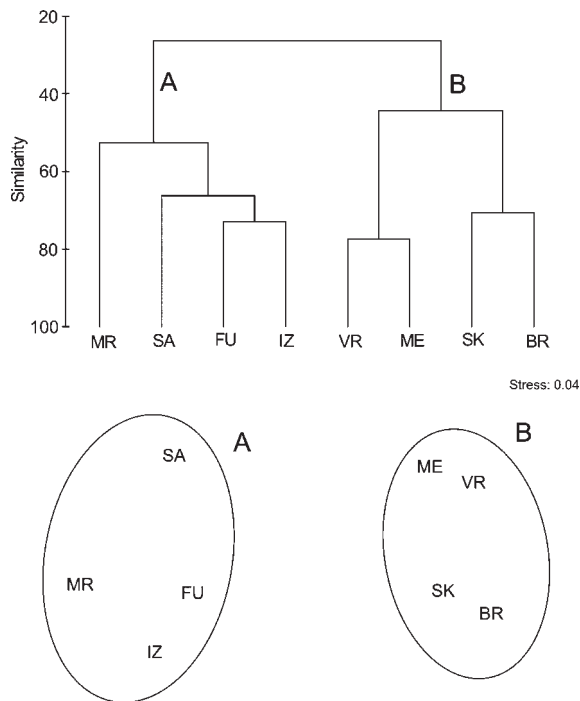
Fig. 6. Results of cluster analysis and multi-dimensional scaling of similarity of the macroalgae species growing as epiphytes on *Posidonia oceanica* leaves at 10 m depth. Channel sites (A) and open sea sites (B).

cages showed its maximum during the period of higher insolation and temperatures in spring and summer. Significantly higher epiphyte biomass was found at the site in the vicinity of the tuna farm (FU) and near local sewage (SA) in comparison to the other investigated sites. These results can be used as the direct evidence of the nutrient enrichment, mostly in NH_4^+, also noted by Cancemi *et al.* (2003). The extensive development of macroalgal epiphytes on the *Posidonia* leaves at the investigated sites limits the amount of light available to the plant. There are also opposite observations where the epiphyte loading was much smaller in the fish farm area than in the reference site, mostly because of high herbivore pressure found close to the fish farm (Delgado *et al.*, 1997; Pergent *et al.*, 1999; Ruiz *et al.*, 2001). This kind of high herbivore activity was not found during our research. Light reduction is one of the most important factors responsible for sea grass decline in the eutrophied waters. The degradation of the *Posidonia* meadows near the Fulija Islet (FU) could be also attributed to a light reduction caused by shading near cages, and dispersion of organic detritus. However, the degradation of the *Posidonia* meadows also occurred because their leaves were covered by benthic mucilaginous aggregates.

Because of the algal bloom during the late spring the meadow was covered with a thick carpet that obstructs the *Posidonia* leaves to photosynthesize (Figure 7). The potential damage on *Posidonia* meadows by accumulation of
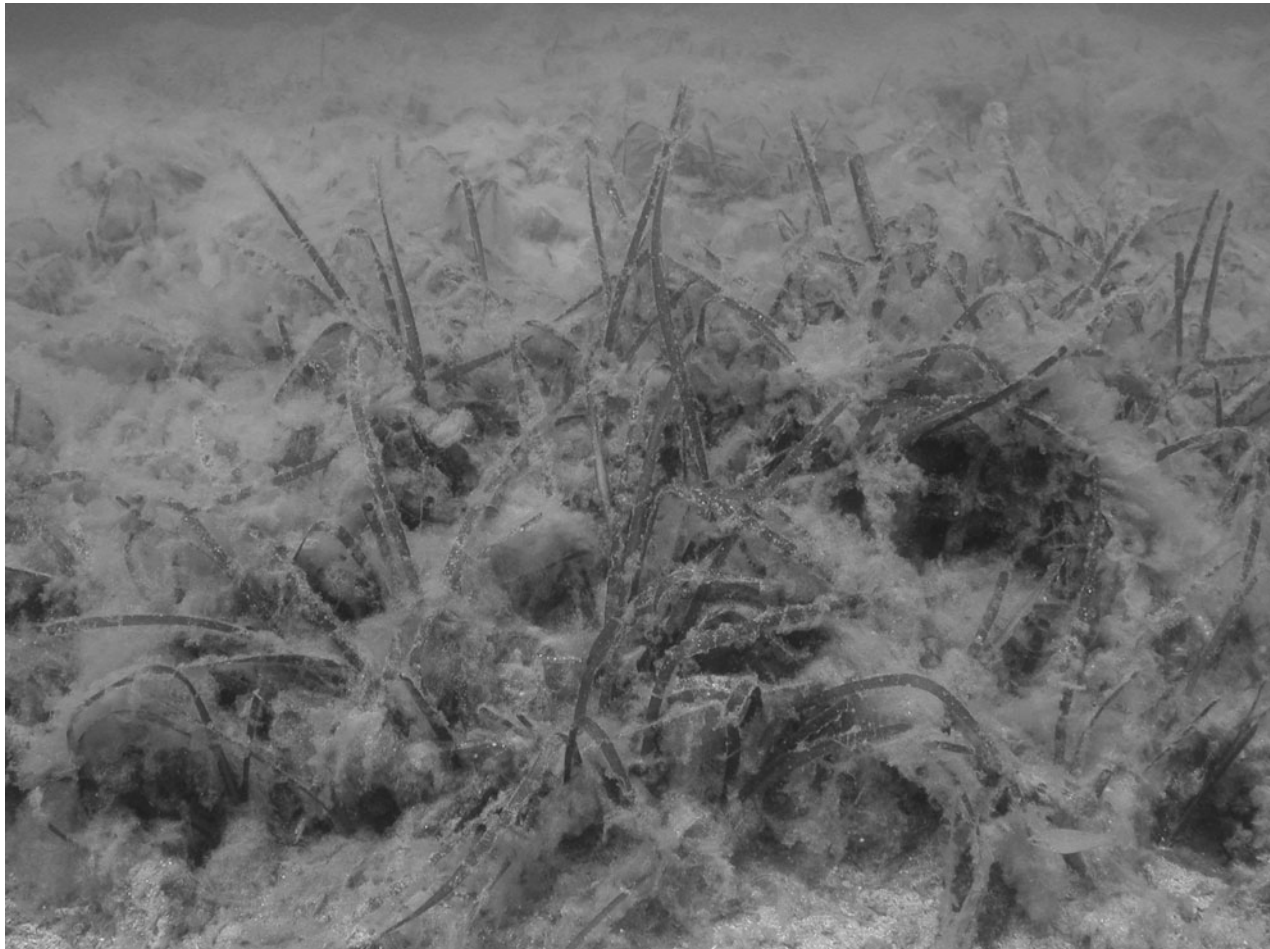

Fig. 7. Heavy bloom of filamentous algae *Acinetospora crinata* (Carmichael *ex* Harvey) Kornmann at the site FU.

these aggregates includes shading, changes in nutrient concentrations of sediment and, at the end, anoxia. The lack of oxygen can favour the activity of anaerobic bacteria and production of toxic substances, such as hydrogen sulphide (Delgado *et al.*, 1999). The mucilage accumulation is probably related to water motion that has been reported to attenuate as a power function of depth in *P. oceanica* beds (Gambi *et al.*, 1989). This process of regression was accompanied by a heavy bloom of pluricellular filamentous algae, principally *Acinetospora crinata* (Carmichael *ex* Harvey) Kornmann. In recent years the occurrence of benthic mucilaginous aggregates has become an increasing problem along the Adriatic coastline and in many other areas of the Mediterranean Sea (Giuliani *et al.*, 2005). The appearance of these benthic aggregates shows a seasonal pattern becoming noticeable in the field as small, yellowish tufts in early spring and continued until the end of summer forming, under favourable environmental conditions, extensive patches at the bottom. The macroscopic development is caused by few associated filamentous species: *Nematochrysopsis marina* (Feldmann) Billard and *Chrysonephos lewisii* (Taylor), two fast growing multicellular benthic chrysophytes, together with a free-living form of the brown alga *Acinetospora crinita*. This kind of mucilaginous cover drastically reduced photosynthesis and it has been reported that mucilaginous carpets could suffocate sea grass beds (Den Hartog, 1994; Delgado *et al.*, 1997; Cancemi *et al.*, 2003; Holmer *et al.*, 2003). The *Posidonia* meadows at the site FU were heavily affected and the accumulation of aggregates did not vary with depth. The covering was massive and took the form of a blanket that appeared to be anchored to the upper portion of the heavily epiphytized leaves. There was no *A. crinata* or other filamentous algae recorded at the other investigated sites in our research.

In general, all four meadows investigated in the Dugi Otok channel showed a generalized state of regression. This regression state reaches its maximum at site FU (Fulija Islet) where the meadow merely survives in an extremely degraded situation caused by aquaculture activity. Based on shoot density measurement, meadows at the outer part of the Dugi Otok Island were in a comparatively better state of health.

The results of this research show significant degradation or even disappearance of the sea grass meadows when fish farming cages are set up above or in the vicinity of a *Posidonia* meadow. In some areas beneath the cages at the site FU only the rhizome mattes remained. Influence of light limitation due to increased turbidity, interplay between nutrients, epiphytes and the plant tolerance to changes in sediment quality deserves further investigation to understand *P. oceanica* meadow decline associated with anthropogenic activities in the Mediterranean Sea.

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