Monitoring of *Posidonia oceanica* meadows in Croatian Protected Areas

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MedPAN South – Croatia Pilot Project "Strengthening of the Marine Protected Areas Network in Croatia / *Jačanje mreže morskih zaštićenih područja u Hrvatskoj*"

Data interpretation, report preparation and field collection:
Ivan Guala
c/o Fondazione IMC - International Marine Centre - Onlus
Loc. Sa Mardini 09170 Torregrande (Oristano), Italy
i.guala@imc-it.org

Field data collection:
Milena Šijan, Zrinka Jakl, Mosor Prvan, Margita Radman, Vanja Matas, Nela Sinjkević, Hrvoje Čižmek, Ivana Zubak, Stjepan Budimir, Mišo Pavičić, Sandra Bratinčević, Vanja Matas, Meri Bilan, Ivana Bušelić, Anamarija Vrbatović, Matea Špika
Association for Nature, Environment and Sustainable Development Sunce
Obala HNP 7/III, 21 000 Split, Croatia
info@sunce-st.org

Ante Žuljević, Vedran Nikolić, Nika Stagličić
Institute of Oceanography and Fisheries, Šetalište I. Meštrovića 63, 21000 Split, Croatia

Nikolina Baković, Milena Ramov
Public Institution Nature Park Telašćica
Ulica Danijela Grbin bb, 23 281 Sali, Croatia

Giuseppe Di Carlo
WWF MedPO
Via Po, 25/c, 00198 Roma, Italy
gdicarlo@wwfmedpo.org

Osvin Pečar
National Park Mljet
Pristanište 2, 20 226 Goveđari, Croatia

Martina Markov Podvinski
National Park Kornati
Butina 2, 22 243 Murter, Croatia

Diving Klub Špinut, Lučica 7, 21 000 Split, Croatia

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Brijuni, Telašćica, Lastovo Islands: Zrinka Jakl
Mljet: Osvin Pečar

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FOREWARD

Project MedPAN South has been developed with the objective to speed up the process of establishing an efficient management of marine protected areas in the Mediterranean. The project aims to increase the effectiveness of conservation of important coastal and marine biodiversity of the Mediterranean by improving the management of existing marine protected areas and promoting establishment of new ones. Projects is organised through 5 pilot projects in Algeria, Tunisia, Libya, Turkey and Croatia and regional capacity building and communication activities on the topic of marine protected areas.

The main objectives of the pilot project in Croatia are:

✓ support public institutions for management of Croatian marine protected areas, involved in the project, in the "step by step" development of management plans;

✓ strengthen the capacity of MPA management public institutions in matters related to the management of marine protected areas;

✓ improve networking of Croatian MPAs and other relevant institutions and their integration in MedPAN and AdriaPAN networks, in order to encourage the exchange of information, good practices and solutions to problems of MPAs management.

During the project thematic workshops for the gradual development of management plan have been organised as well as trainings and exchange programmes related to the specifics of marine protected areas. Technical support to MPAs in the implementation of each phase of management plan development has been ensured (experts, studies, etc.).

Direct project beneficiaries and partners are public institutions for management of Croatian marine nature and national parks - Brijuni, Telašćica, Kornati, Lastovo Islands and Mljet. Indirect beneficiaries are coastal county public institutions for management of protected areas.

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Project contact:

Zrinka Jakl – Croatia Pilot Project Coordinator
Association for Nature, Environment
and Sustainable Development Sunce
Obala HNP 7/III, 21 000 Split, Croatia
phone: +385 21 360 779
fax: +385 21 317 254
zrinka.jakl@sunce-st.org
www.sunce-st.org

Giuseppe Di Carlo – Regional Project Coordinator
WWF Mediterranean Programme Office
phone: +39 06 8449 7443 (direct line)
gdicarlo@wwfmedpo.org
www.panda.org/mediterranean

1. INTRODUCTION

In the Mediterranean the endemic seagrass *Posidonia oceanica* (L.) Delile colonizes sandy and hard bottoms from the surface up to more than 40 meters deep. *Posidonia oceanica* builds specific systems, called meadows, which are considered among the most representative and important Mediterranean coastal ecosystems for complexity, persistence and extension (Buia *et al.*, 2004).

Posidonia oceanica meadows play a number of key functions for littoral ecosystems: they produce and export large amount of organic *matte*r and oxygen, form complex ecosystems and support high level of biodiversity and trophic interactions, represent areas of refuge and nursery for fish and invertebrates also of commercial importance, reduce sedimentation and stabilizes the seabed and reduce coastal erosion (Boudouresque *et al.*, 2006). For these reasons, *Posidonia oceanica* meadows are protected by the Habitat Directive 92/43/EU (Annex I, *Posidonion oceanicae*, code 1120) and are included in the reference list of priority habitats of the SPA/BIO Protocol of Barcelona Convention (Association with *Posidonia oceanica*, code III.5.1) (Anonymous, 1999; Relini and Giaccone, 2009),

Moreover, *Posidonia oceanica* is considered a good biological indicator to determine the quality of coastal waters and, in general, the ecological status of Mediterranean marine environment according to the Water Framework Directive 2000/60/EU; (Romero *et al.*, 2007; Gobert *et al.*, 2009; Lopez y Royo *et al.*, 2010). *Posidonia oceanica* is a long-lived and slow growing species; its relevance as ecological indicator is due to the plant or meadow ability to respond strongly with changes of its structural and functional characteristics to environmental alterations (Boudouresque *et al.*, 2006; Lopez y Royo *et al.*, 2010). The sensitivity of *Posidonia oceanica* to environmental changes may be expressed at different levels of organization - cell, tissue, organism, population and / or ecosystem - depending on the nature and the magnitude of the perturbation. Therefore, the assessment of conservation status of the meadows can be achieved through the use of different synthetic descriptors (Buia *et al*. 2004; Montefalcone 2009).

The distribution of the meadows and their structural and functional features are affected by biotic and abiotic factors that work at different spatial and temporal scales. Among the most relevant natural factors, depth and substrate type are crucial for the characteristics of the meadows (Pergent *et al.*, 1995). In addition, *Posidonia oceanica* is sensitive to changes of the marine environment caused by anthropogenic disturbances. In general, pollution, over-sedimentation, eutrophication and increased water turbidity, are the main factors of nuisance

(Cancemi *et al*., 2003; Boudouresque *et al*., 2006). The regression of *Posidonia oceanica* meadows, documented for several areas of the Mediterranean, can be strongly imputed to human impacts (Boudouresque *et al*., 2006; Di Carlo *et al*., 2011): marine works, beach nourishment, dredging, dumping at sea of construction materials, dispersion of pollutants from urban and industrial wastewater, changes in fluvial and sedimentary flows, have direct or indirect effects on the meadows (AA.VV., 2008; Boudouresque *et al*., 2006). The mechanical impacts resulting from anchoring (Figure 1), placement of submarine cables and pipelines and the use of invasive fishing tools (i.e. trawling) are the main factors that threatens the structure of the meadows at a smaller spatial scale (Boudouresque *et al*., 2006).

Figure 1. Evidence of mechanical damage from anchors.

Marine reserves, whose primary objective is the protection of the environment, have also an important economic role. They contributes to the expansion of tourism activities because of the growing interest in marine ecosystems and species of animals and plants, subject to protection (Badalamenti *et al*., 2000). The possibility of observing pristine environments or habitats in good condition or abundant and diverse marine flora and fauna, is strongly attractive for tourists (Cattaneo Vietti and Tunesi, 2007). However, the increase in tourist

activities such as boating or diving may have detrimental effects on coastal ecosystems (Milazzo *et al*. 2002; Cattaneo Vietti and Tunesi, 2007).

Recreational boating is considered one of the main sources of disturbance to benthic communities (Cattaneo Vietti and Tunesi, 2007), mainly due to the mechanical effects of anchors (Agnesi *et al*., 2006). The anchoring affects especially *Posidonia oceanica* shallow meadows in highly frequented bays and mechanical damages vary depending on the type of anchor and anchoring phase (Francour *et al*., 1999, Milazzo *et al*., 2004). However, several other factors may influence the magnitude of the impact: the number and the characteristics of the boats in a certain area, the duration of the tourist season and that of every single berth, the weather conditions, the behaviour of the boaters, and the characteristics of the seabed such as depth and substrate type. Hence, the effects of pressure of pleasure boating, more specifically the impact of anchors should be assessed in relation to different local situations.

1.1. AIMS

In this study, standardized protocols have been developed for monitoring *Posidonia oceanica* meadows in four Croatian marine protected areas (MPA). The monitoring was mainly aimed to highlight any conditions of disturbance in locations highly frequented by recreational boaters. Other purposes of the study were (i) provide a first baseline to ensure that habitat changes are monitored appropriately, (ii) to identify additional potential sources of threat and (ii) to outline measures for the management of the meadows.

The monitoring protocol has been designed to get informative results with actions replicable and, contextually, achievable in a short time and with few resources. Therefore, speditive detection techniques, not involving the removal of biological material, have been proposed. In fact, non-destructive investigations are functional for reducing time and costs related to sampling and analysis of laboratory samples; moreover they are particularly suitable for studies on protected areas because they do not alter the populations under protection (Sale, 1980).

The descriptors more suitable for assessing the status of the structure of *Posidonia oceanica* meadows in relation to physical and mechanical disturbances are the shoot density and the percentage cover (Marcos-Diego *et al*., 2000). Meadow density is the number of leaf shoots per m^2 (Pergent *et al*., 1995); the cover is expressed as percentage of seabed covered by

live plants with respect to that not covered and made up of sand, rocks and dead *matte* (Buia *et al*. 2004).

2. MATERIAL AND METHODS

The monitoring of structural variables (i.e. shoot density and percentage cover) was designed to assess the conservation status of *Posidonia oceanica* as well as to identify changes in seagrass meadows over time in four Croatian MPAs.

2.1. STUDY AREAS

Investigated MPAs were National Park Brijuni (44°55'N; 13°44'E), Nature Park Telašćica (43°53'N; 15°12'E), Nature Park Lastovo Islands (42°45'N; 16°50'E) and National Park Mljet (42°47'N; 17°22'E) (Figure 2). Sampling protocol has also been developed for National Park Kornati and field sampling is planned for 2012.
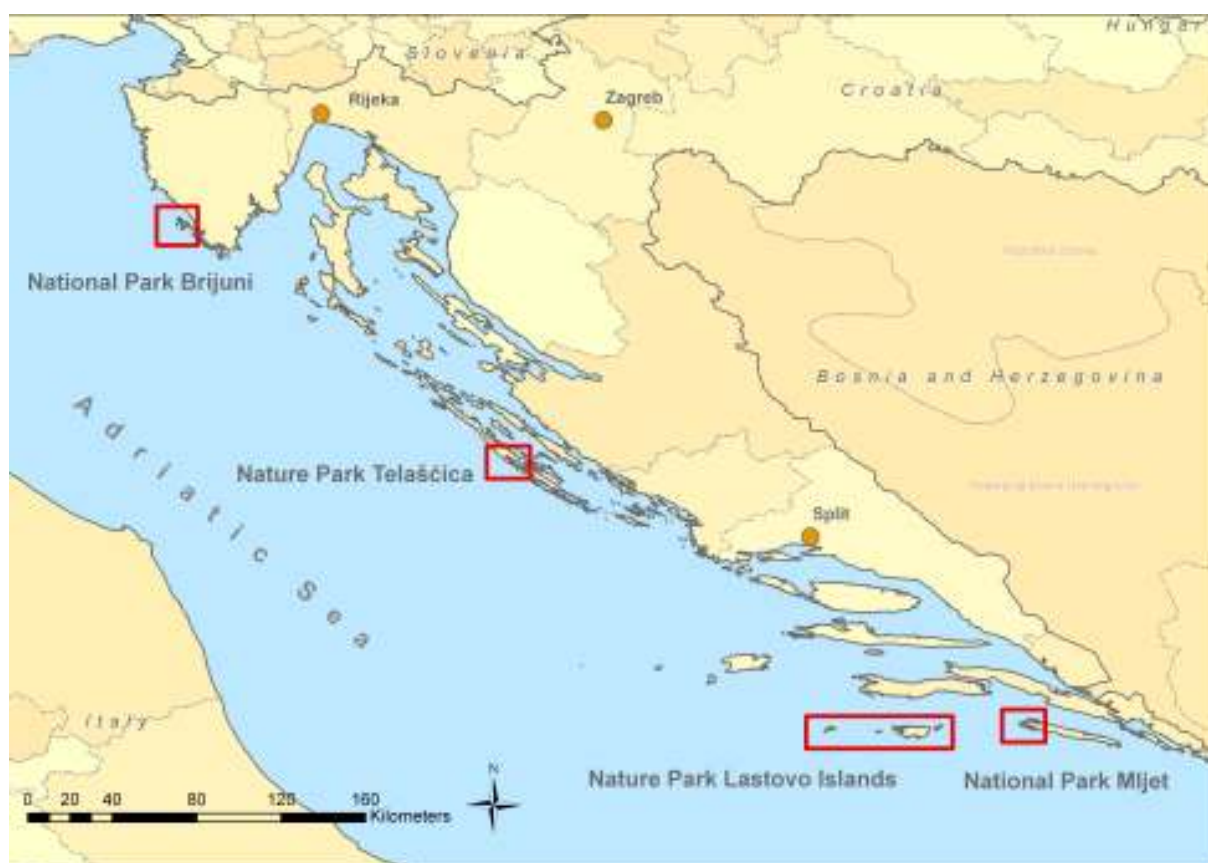

Figure 2. Map of the Croatian coastline with the indication of the studied MPAs; from NW to SE: Brijuni, Telašćica, Lastovo, Mljet.

A part from Brijuni, where boat anchoring is forbidden (Dujmović, personal communication), the investigated MPAs are well known to boaters destinations, both at national and international level. Hence, the main detrimental effects for *Posidonia oceanica* meadows come from anchoring of recreational boats although other type of stressors (e.g. wastewater pollution and trawl fishing activities) can occur locally.

2.2. SAMPLING PROTOCOL AND SELECTION OF VARIABLES

Since detailed cartographic data are lacking, sampling designs were planned according to the knowledge of local MPA operators. In particular, sampling locations were selected where *Posidonia oceanica* meadows colonize shallow water (depth range 5-16 m) and gentle slopes (i.e. horizontal or sub-horizontal bottoms); in fact these are the preferential conditions to recreational boaters for anchoring. On the base an empirical assessment of boat frequentation, meadows were defined as "impacted" (Figure 3) or "reference" when frequentation was high or negligible , respectively.

Figure 3. Sailing boats anchored in the Skrivena Luka bay (Nature Park Lastovo Islands).

The value of the structural variables differs depending on the spatial scale in relation to the environmental heterogeneity of *Posidonia oceanica* meadow (Balestri *et al*., 2003). Therefore, when possible, sampling designs that incorporate different hierarchical spatial scales were planned in order to more adequately represent the complexity of the investigated systems and to avoid the risk of incorrect generalizations (Balestri *et al*. , 2003, see also Benedetti-Cecchi, 2004). Thus, at each location, a number of sites variable according to the

meadow size, depth and slope, was selected in the shallow portion of the meadows where anchoring is more frequent.

Because of different characteristics of the MPAs, in terms of surface, occurrence of impacted/reference location, meadow features (depth, nature of substratum, slope), four different designs were planned in the four MPAs. The most particular case is that of Brjiuni where no comparison between impacted and reference locations could be done due to the presence of a small, single, meadow.

As mentioned above, since mechanical impacts (Figure 4) mostly affect the meadow structure (Francour *et al.*, 1999; Milazzo *et al.*, 2004; Boudouresque *et al.*, 2006), structural descriptors (i.e. shoot density and percentage cover) are considered as the best variables to describe changes due to this kind of impact. The procedures used to assess these variables are described below.

Figure 4. Impact of anchoring on a *Posidonia oceanica* meadow.

2.2.1. Shoot density

The number of leaf shoots per m^2 is one of the most used descriptors to assess the status of *Posidonia oceanica* (Pergent-Martini *et al.*, 2005) and provides information on the changes that the meadows are subject when measured on a pluriannual time scale (Buia *et al.*, 2004).

The values of density are detected by counting the number of leaf shoots (counting twice those in division) within the sampling unit. An area of 1.600 cm^2 is considered the optimal sampling unit for estimating the density of *Posidonia oceanica* (Panayotidis *et al.*, 1981). So, at each site, replicated quadrats (40 x 40 cm) are launched randomly at a distance of at least 1 meter from the other. Subsequently, the values of the single count are reported to the m^2 and averaged.

Based on the number of shoots per m^2 the meadows may be categorized according to the depth (Pergent *et al.*, 1995), which is one of the factors that most influence the density. Pergent *et al.* (1995) identify four classes, which are a function of the theoretical average density calculated for each depth (Table 1) and that reflect the ecological conditions of the meadow (Buia *et al.*, 2004).

Table 1. Classification of *Posidonia oceanica* meadows according to Pergent *et al.* (1995) and ecological significance (from Buia *et al.*, 2004 modified).

Pergent *et al.* (1995)	abnormal density (AD)		low subnormal density (LSD)		normal density (ND)		high subnormal density (HSD)	
Buia *et al.* (2004)	very disturbed beds		disturbed beds		beds in equilibrium			
depth (m)								
1	↔		822	↔	934	↔	1158	↔
2	↔		646	↔	758	↔	982	↔
3	↔		543	↔	655	↔	879	↔
4	↔		470	↔	582	↔	806	↔
5	↔		413	↔	525	↔	749	↔
6	↔		367	↔	479	↔	703	↔
7	↔		327	↔	439	↔	663	↔
8	↔		294	↔	406	↔	630	↔
9	↔		264	↔	376	↔	600	↔
10	↔		237	↔	349	↔	573	↔
11	↔		213	↔	325	↔	549	↔
12	↔		191	↔	303	↔	527	↔
13	↔		170	↔	282	↔	506	↔
14	↔		151	↔	263	↔	487	↔
15	↔		134	↔	246	↔	470	↔
16	↔		117	↔	229	↔	453	↔
17	↔		102	↔	214	↔	438	↔
18	↔		88	↔	200	↔	424	↔
19	↔		74	↔	186	↔	410	↔
20	↔		61	↔	173	↔	397	↔
21	↔		48	↔	160	↔	384	↔

22		↔	37	↔	149	↔	373	↔
23		↔	25	↔	137	↔	361	↔
24		↔	14	↔	126	↔	350	↔
25		↔	4	↔	116	↔	340	↔
26				↔	106	↔	330	↔
27				↔	96	↔	320	↔
28				↔	87	↔	311	↔
29				↔	78	↔	302	↔
30				↔	70	↔	294	↔
31				↔	61	↔	285	↔
32				↔	53	↔	277	↔
33				↔	46	↔	270	↔
34				↔	38	↔	262	↔
35				↔	31	↔	255	↔
36				↔	23	↔	247	↔
37				↔	16	↔	240	↔
38				↔	10	↔	234	↔
39				↔	3	↔	227	↔
40						↔	221	↔

2.2.2. Percentage cover

The coverage is the surface of seabed, expressed as a percentage, covered with live plants of *Posidonia oceanica* compared to that non-covered and consisting of sand, rock or dead *matte* (Buia *et al*., 2004). This variable provides information on both the macrostructure and the health of the meadows (Pergent-Martini *et al*., 2005; Montefalcone, 2009).

Percentage cover can be assessed using the Line Intercept Transect (LIT) technique (Bianchi *et al*., 2004; Montefalcone *et al*., 2007). The LIT is a centimetre-marked line laid on the bottom along which the occurrence of live *Posidonia oceanica* and the nature of the substrate (sand, rock, dead *matte*) are recorded. Four LITs, each of 10 m length and randomly positioned, were carried out in each site; for each LIT, the intercept to the nearest centimetre corresponding to the point where the key attributes changed under the line divers was recorded (Figure 5).

In each LIT, the length of each key attribute (Lx) is the distance occurring between two recorded intercepts, and it is calculated by subtraction (Figure 5). Their percent cover (R%) along a transect of 10 m length, was calculated by the following formula

$$R\% = \sum(L_x/10*100)$$

Thus, percentage cover data provides information of the amount of different substrata and live *Posidonia oceanica* covering the sea bed (Figure 5).
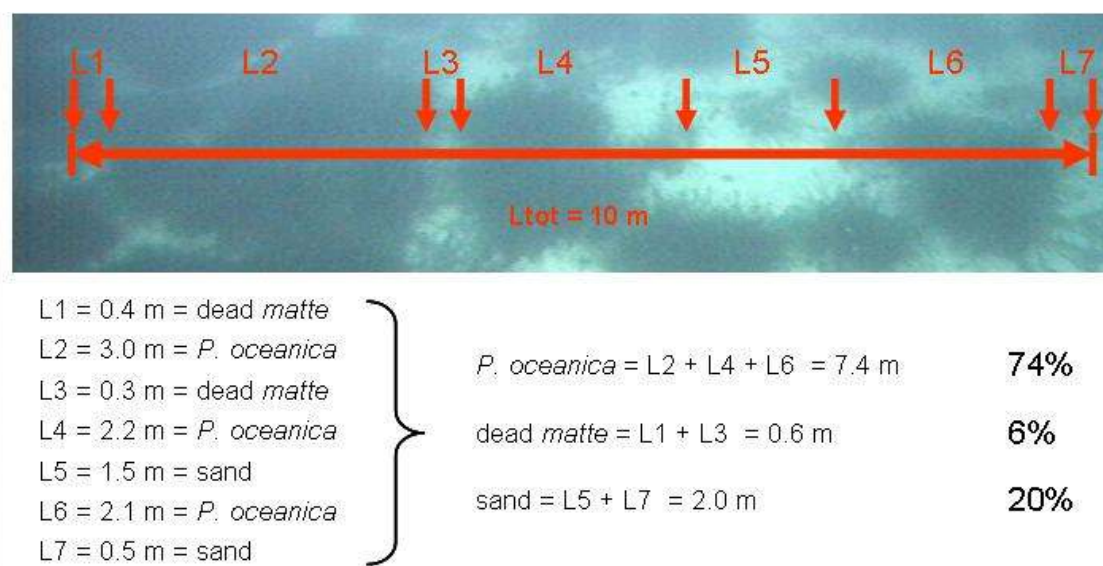
Figure 5. Explanation of the LIT method for the assessment of percentage cover.

Moreover, percentage cover allows to calculate, for each LIT, the conservation index (CI). The CI (Moreno *et al*., 2001; Montefalcone *et al*., 2006) is an environmental index, useful to assess the state of health of the meadows, related to the proportional abundance of dead *matte* relative to live *Posidonia oceanica* and is expressed by the formula

$$CI = P/(P + D),$$

where P is the percentage cover of live *Posidonia oceanica* and D is the percentage cover of dead *matte*.

The conservation status of *Posidonia oceanica* meadows have to be evaluated locally, on the basis of the temporal evolution of the values of CI measured on multi-year time scale (but see also Moreno *et al*., 2001, Montefalcone *et al*., 2006 and Montefalcone, 2009 for information on the rating systems of the conservation status so far adopted at local and regional level).

2.3. SAMPLING ACTIVITIES

2.3.1. National Park Brijuni

The area of Pojer is home to a small meadow of *Posidonia oceanica*, which covers approximately 5 hectares, to 8-12 meters deep (Figure 6 and Annex I)

The meadow is one of the most north recorded *Posidonia oceanica* meadows in the Croatian side of the Adriatic Sea, however exact data on *Posidonia oceanica* meadow distribution in this part of Adriatic is unavailable. Meadow on Brijuni is not known to be subject to severe anthropogenic disturbances at local scale except seasonal and small range wastewaters pollution from nearby village (Dujmović, personal communication). In the absence of obvious severe nuisance factors and by virtue of the small size of the meadow, the monitoring has been planned with the aim to identify changes in the structural variables of the meadow over time.
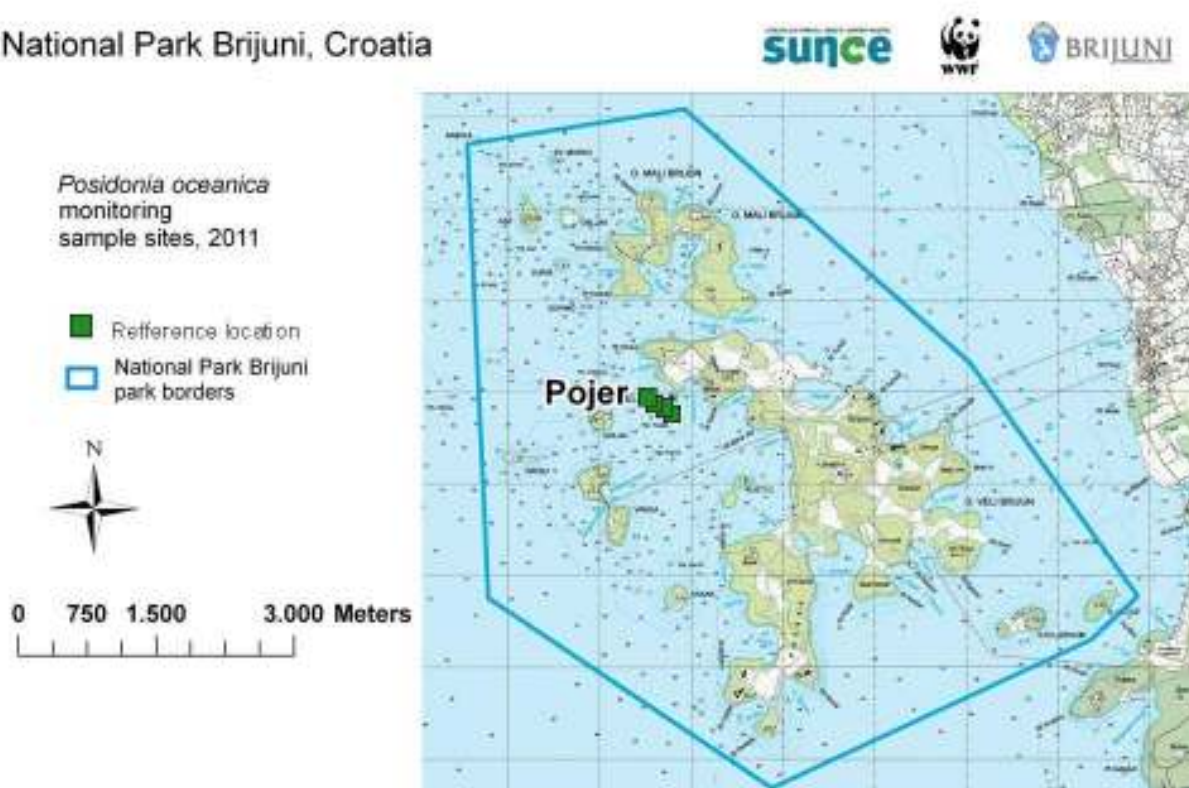
Figura 6. Map of National Park Brijuni with sampling location (Pojer).

Field activities were carried out September 10th, 2011 with the technical support of the Public Institution National Park Brijuni. Four sites were randomly selected within the meadow of Pojer, at 10 m deep and approximately 100 m apart. The geographic position of each site was recorded using a GPS with a nominal precision of 10 m. Detailed map of the sampling sites is reported in the Annex I.

In order to assess the meadow density 8 to 10 replicated counts of leaf shoots were carried out at each site. For percentage cover, 5 LITs were positioned in the most western site (site 1) and 4 LITs in sites 2 and 3; only one transect was positioned in the most easterly site (site

4) because of a limited presence of *Posidonia oceanica* in this portion of the meadow (see Annex I for detailed map of sampling sites).

2.3.2. Nature Park Telašćica

Field activity was carried out from June 15th to 19th 2011 at five locations in the Telašćica Nature Park (Figure 7). Four location (Čuška Dumboka, Kobiljak, Sestrica, Lučica) are supposed to be subjected to high pressure of boat anchoring, one (Garmenjak) has been considered as reference because it is not known as preferential boaters destination.
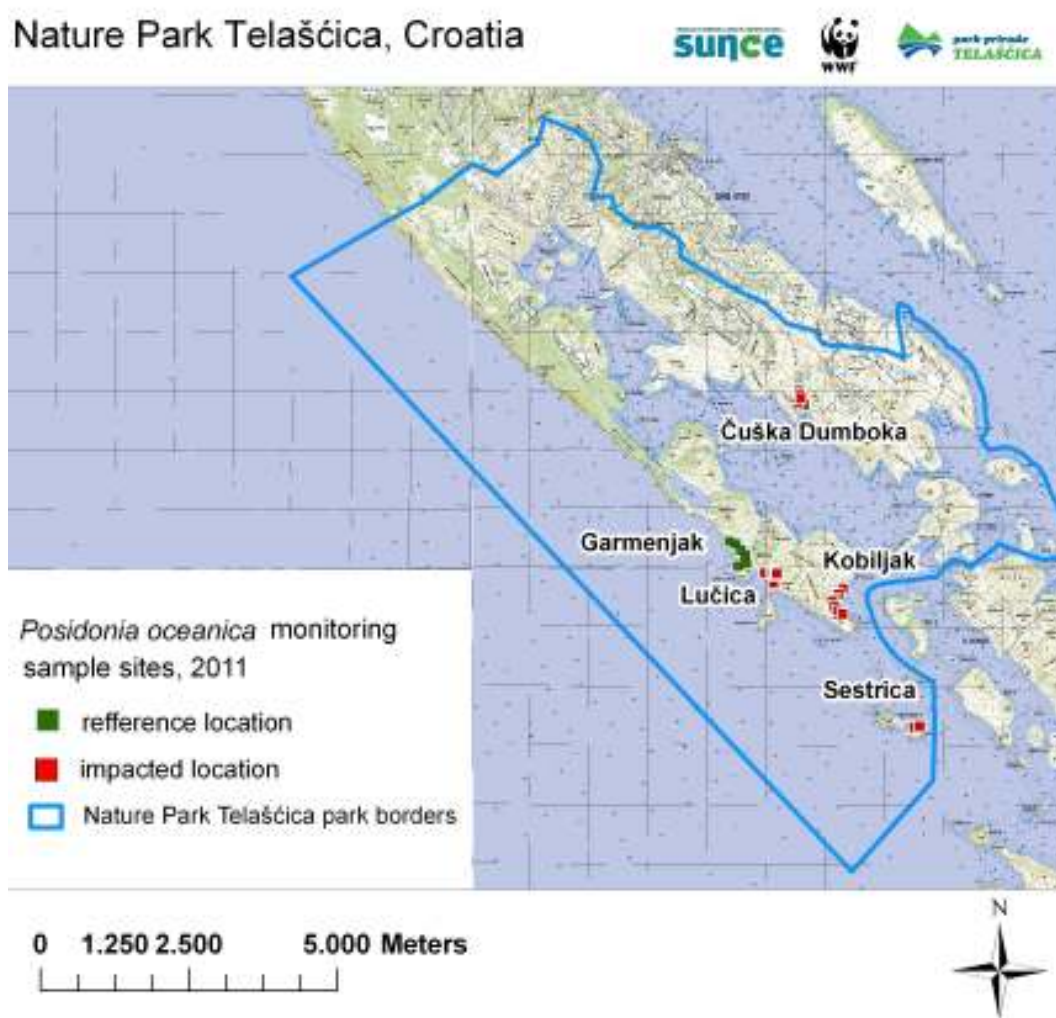
Figura 7. Map of Nature Telašćica Park with sampling locations; red = impacted locations (Čuška Dumboka, Kobiljak, Sestrica, Lučica); green = reference location (Garmenjak).

In each location a number of sites (varying from 4 to 9 according to the meadow size) was selected in the shallow portion of the meadows (from 8 to 14 m in depth). The geographic position of each site was recorded using a GPS with a nominal precision of 10 m. Detailed

maps of each location are reported in the Annex II. At each sites, leaf shoots were counted in 8 to 10 replicated quadrats; in two sampling sites (sites 2 and 4 in Kobiljak) 15 and 20 replicates were carried out because of training of students and local MPA operators. Percentage cover was assessed by means of 4 LITs for each sampling sites.

2.3.3. Nature Park Lastovo Islands

Monitoring was been carried out from May 29th to June 3rd 2011 at six locations in the Nature Park Lastovo Islands (Figure 8). Four location (Zaklopatica, Pasadur, Makarac, Skrivena Luka) are subjected to high pressure of boat anchoring, two (Kopište, Davjenica) have been considered as reference because they are not known as preferential boaters destinations. An additional impacted location (Saplun) was investigated the July 9th, 2011.
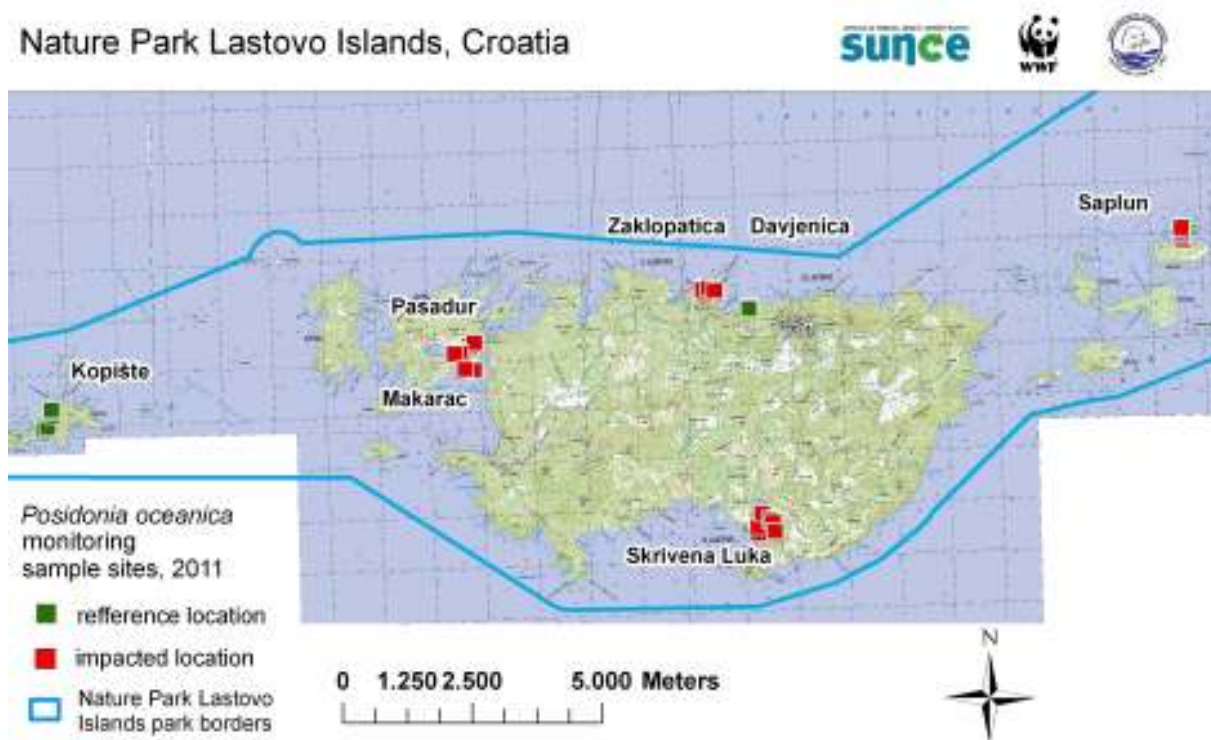
Figure 8. Map of Nature Park Lastovo Islands with sampling locations; red = impacted locations (Zaklopatica, Pasadur, Makarac, Skrivena Luka, Saplun); green = reference locations (Kopište, Davjenica).

At each location a number of sites (varying from 2 to 8 according to the meadow size) were selected from 5 to 16 m in depth. The geographic position of each site was recorded using a GPS with a nominal precision of 10 m. Detailed maps of each location are reported in the Annex III.

For each site, replicated counts of leaf shoots (4 to 12 according to the meadow distribution at each site) were carried out for assessing the meadow density and 4 LITs were positioned for percentage cover.

2.3.4. National Park Mljet

Four locations were selected in the Mljet National Park (Figure 9), two were considered impacted (Lokva and Polače), the other (Srednja and Međuporat) as reference. Sampling was carried out on August 17th 2011.

The number of sites varied from 2 to 6 according to the meadow size, depth range from 6 to 16 m. The geographic position of each site was recorded using a GPS with a nominal precision of 10 m. Detailed maps of each location are reported in the Annex IV.

At each sites, 8 replicated quadrats were used to count the number of leaf shoots and 4 transects for assessing the percentage cover of the meadow.

Figure 9. Map of National Park Mljet with sampling locations; red = impacted locations (Lokva and Polače); green = reference locations (Srednja and Mezuporat).

2.4. DATA PROCESSING

Shoot density, percentage cover of live *Posidonia oceanica*, dead *matte* and different substrate types (sand/mud and rocks), as well as Conservation Index, were calculated for each replicate. Data were then averaged for each MPA, distinguishing between impacted and reference conditions, in order to provide a common view of the general status of *Posidonia oceanica* meadows in the four investigated MPA.

Data were also processed for each single MPA distinguishing between impacted and reference locations and among sites. For Brijuni data analysis has been limited to calculate shoot densities and covering indexes for the meadow of Pojer since any other conditions are lacking. For the other MPAs, univariate analysis of variance (ANOVA) was also carried out on both variables, shoot density and percentage cover, for assessing differences between different level of pressure (impacted *vs.* reference) and among locations. Cochran's test was performed to check *a priori* the assumption of homogeneity of variances and data were transformed when necessary; if transformations did not produce homogeneous variances, ANOVA was, nevertheless, done because the wide sample size and high number of degree of freedom in the residual (Benedetti-Cecchi, 2004). Student-Newman-Keuls test (SNK test) was used for *post hoc* multiple comparisons of means.

Moreover, a multivariate analysis was performed to assess the similarity among MPAs, pressure conditions (impacted *vs.* reference) and locations within each MPA. Variables were: CI, percentage covers of live *Posidonia oceanica*, *Cymodocea nodosa*, dead *matte*, and different substrate types, mean and standard deviation of shoot density at each site, initial, mean and final depth of each transect. Each variable was square-root transformed, in order to reduce too high variances in replicate samples, and normalised (Clarke and Warwick, 2001). Euclidean distance was used as the measure of dissimilarity of samples from each other (Clarke and Warwick, 2001). Finally, non-metric Multi Dimensional Scaling (nMDS) was carried out on averaged data of locations.

3. RESULTS

Overall, 17 locations (11 impacted and 6 reference) and 91 sites (67 impacted and 24 reference) were investigated in the four MPAs; 746 counts and 360 transects (LITs) were carried out in the whole sampling campaign.

The mean density assessed at the scale of MPA confirms that *Posidonia oceanica* meadows show sign of regression in all areas, and that only the reference meadows of Telašćica and Lastovo appear to be in equilibrium at the depths where sampling was done (Figure 10).
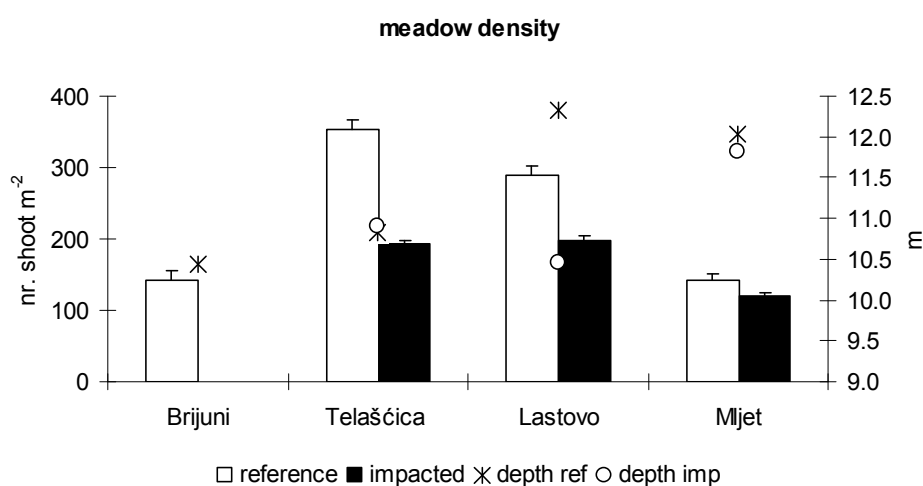

Figura 10. Mean values (+se) of the shoot density and depth at each MPA. Black & white bars are impacted and reference locations, respectively.

On the contrary, percentage cover of live *Posidonia oceanica* is always higher in reference meadows than those impacted, while the latter are characterized by higher values of dead *matte* (Figure 11). Hence, the values of CI match with the *a priori* assumption that impacted locations are subjected to higher level of disturbance than reference (Figure 12). In most of the meadows, occurrence of sandy/muddy and rocky bottoms without live shoots of *Posidonia oceanica* appears negligible (Figure 11). It is worth to point out that a few shoots of *Cymodocea nodosa* were recorded in Telašćica; nonetheless their occurrence was not considered and reported in the graphs because of the insignificance of their cover (150 cm along one single transect).

Substrate type - impacted

Substrate type - reference

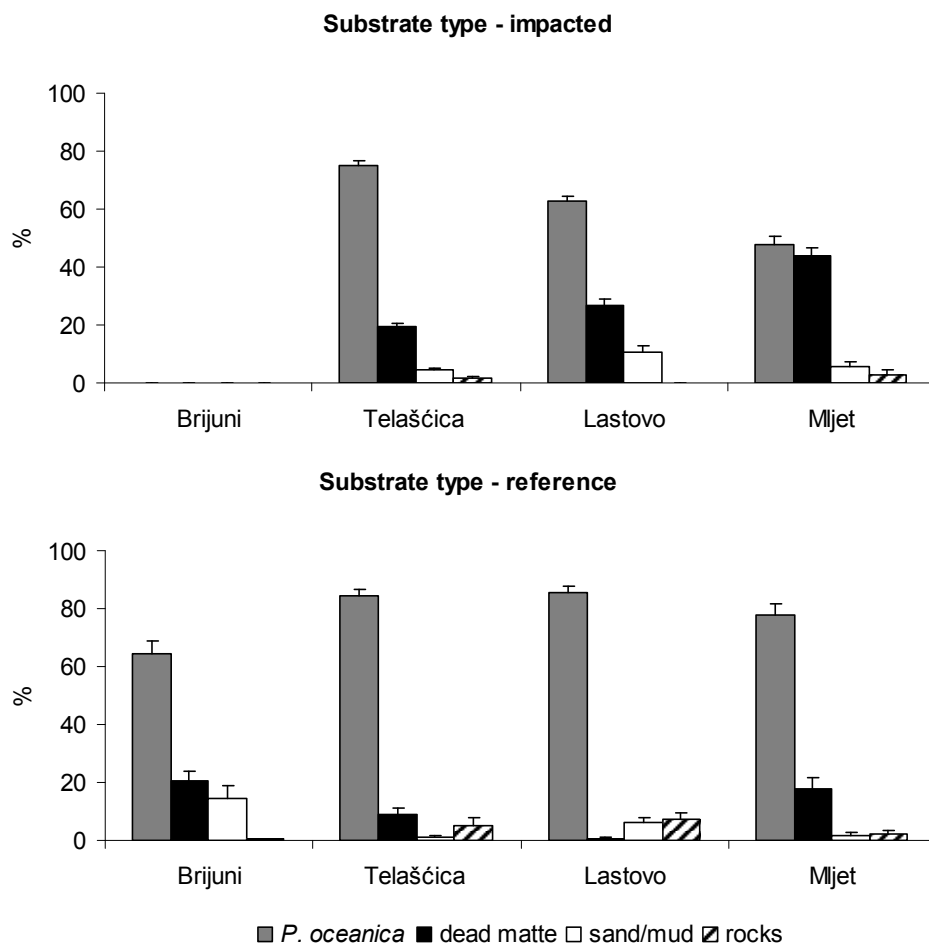■ *P. oceanica* ■ dead matte □ sand/mud ▨ rocks

Figura 11. Mean values (+se) of percentage cover of different substrate and live *Posidonia oceanica* in impacted (above) and reference (below) conditions.
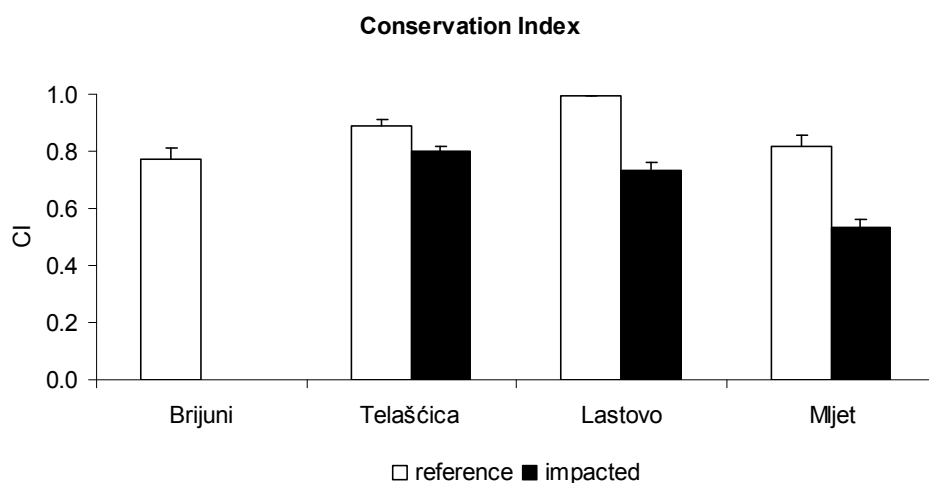
Conservation Index

□ reference ■ impacted

Figura 12. Mean values (+se) of CI at each MPA. Black & white bars are impacted and reference locations, respectively.

Below, results are reported separately for each MPA.

Since only one location was investigated in Brijuni, details at the scale of sites are shown for meadow density, whereas, for the other MPA, the scale of location is reported.

3.1. NATIONAL PARK BRIJUNI

A total of 32 quadrats and 14 LITs were sampled in the meadow of Pojer.

The shoot density was 143 ± 12 (mean ± standard error); the meadow belongs to the class AD according to Pergent *et al*. (1995). This value reflects conditions of very high disturbance according to Buia *et al*. (2004). Moreover, a clear gradient in meadow density is evident from site 1 to site 4: the number of shoots decrease eastward, independently to the depth that is almost invariable. (Figure 13).
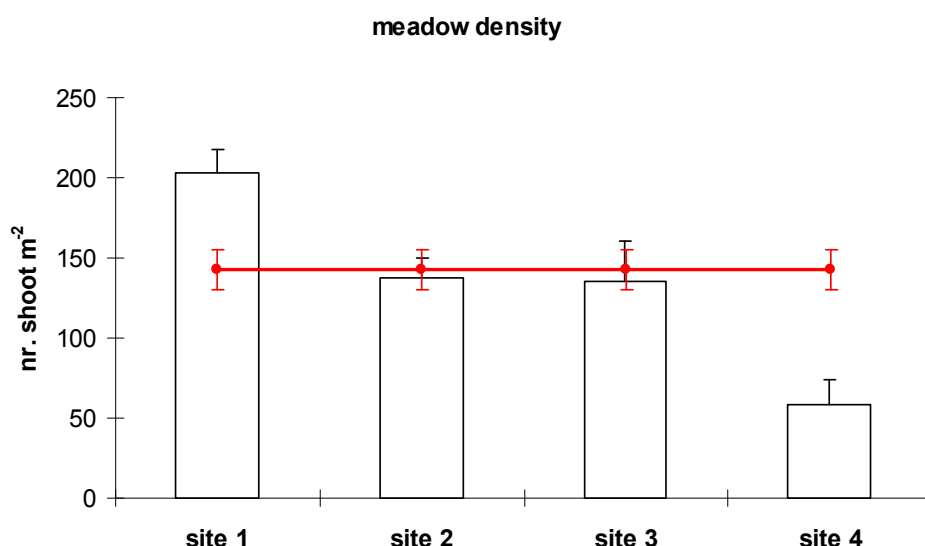
meadow density

Figura 13. Mean values (+se) of the shoot density at each site. The red line is the mean (± se)density of the meadow.

Live *Posidonia oceanica* covers the 65% of the bottom and the percentage cover of dead *matte* is 21% (Figure 14); these values correspond to a mean CI equal to 0.77. Sand and mud together cover the 14%, while the rocky substrate is negligible (Figure 14).
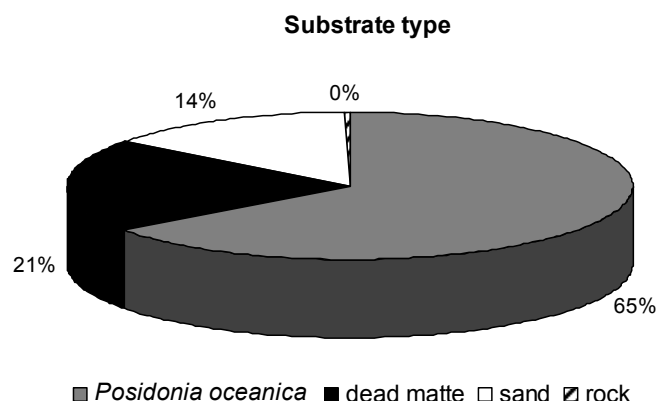
Substrate type

Figura 14. Percentage cover of different substrata and live *Posidonia oceanica*.

3.2. NATURE PARK TELAŠĆICA

A total of 33 sites, 303 quadrats and 130 LITs were sampled in the five locations selected at Telašćica Nature Park (Table 2).

Table 2. Summary of sampling effort.

pressure	locations	nr. of sites	nr. of quadrats	nr. of LITs	mean depth
Impacted	Čuška Dumboka	9	78	36	11
Impacted	Kobiljak	7	80	27	11
Impacted	Sestrica	4	35	16	10
Impacted	Lučica	7	58	28	10
Reference	Garmenjak	6	52	23	11
Total number	**6**	**33**	**303**	**130**	

The mean shoot density ranges from 173 ± 7 (mean ± standard error) to 354 ± 13 shoots m^{-2} (Figure 15). All impacted locations belong to the class AD while the reference meadow in Garmenjak is ND according to Pergent *et al.* (1995). These values reflect conditions of very high disturbance in the impacted locations, while the reference meadow is "in equilibrium", according to Buia *et al.* (2004). Analysis of variance shows significant differences (p<0.001) between impacted (193 ± 4) and references locations (354 ± 13) and among locations (Table 3). Čuška Dumboka, Kobiljak and Lučica have similar densities that are significant lower than Sestrica; Garmenjak has values that are significantly higher than all the impacted locations.
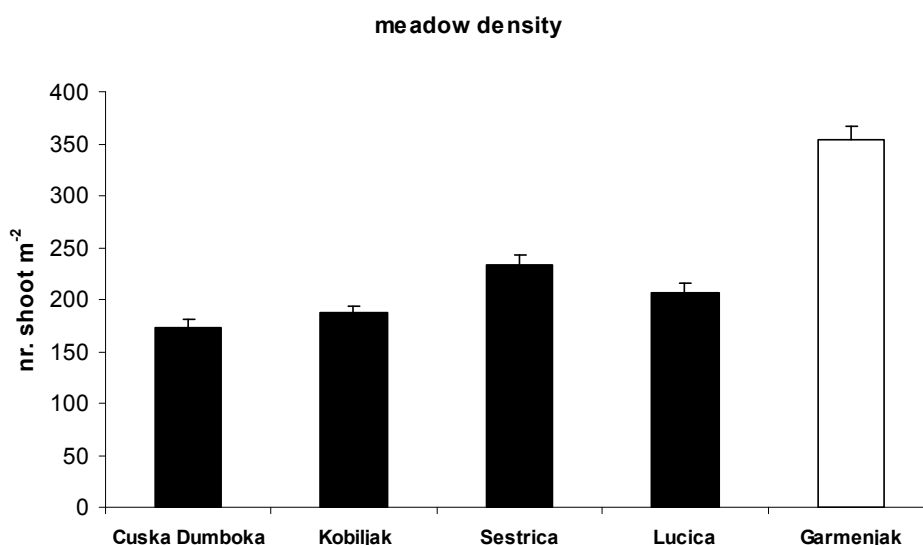
meadow density

Figura 15. Mean values (+se) of the shoot density at each location. Black & white bars are impacted and reference locations, respectively.

The percentage cover of live *Posidonia oceanica* ranges from 69% (Čuška Dumboka) to 89% (Sestrica) in the impacted locations; it covers the 85% of the seabed of the reference location (Figure 16). The percentage cover of dead *matte* shows the highest values in Kobiljak and Čuška Dumboka (27 and 24%, respectively) while Lučica and Sestrica have similar values to Garmenjak (9%). Sands cover the 10% of seabed in Lučica; they are negligible in the other locations. Also, the rocky bottom is not very representative with the highest values of cover (5%) in Garmenjak (Figure 16). A small spot of *Cymodocea nodosa* was recorded in Skrivena Luka; its occurrence is not reported in the graphs because of its paucity.

Substrate type

■ *Posidonia oceanica*　■ dead matte　□ sand　▨ rock
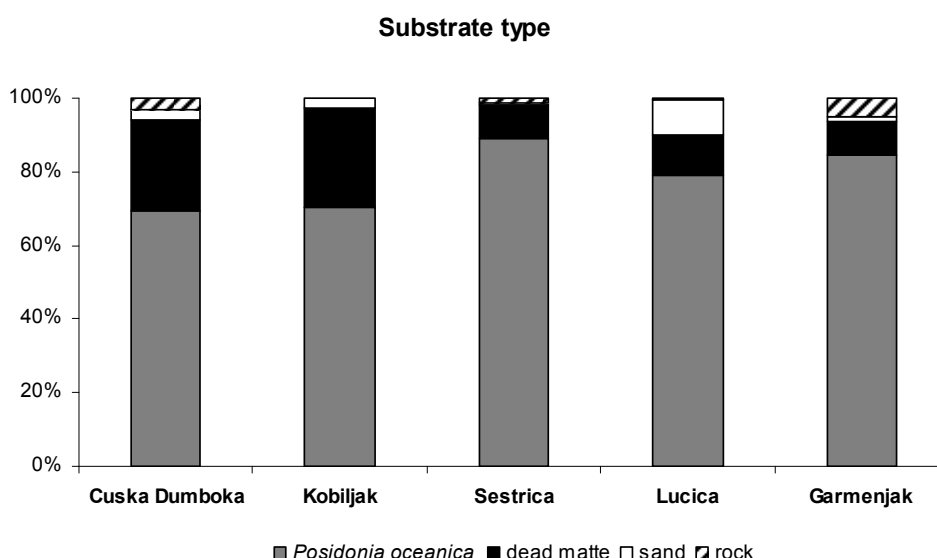
Figura 16. Percentage cover of different substrata and live *Posidonia oceanica*.

The Conservation Index of the five meadows ranges from 0.72 to 0.91 (Figure 4). Overall, the reference location has significantly higher values than impacted ones (0.89 ± 0.02 vs. 0.80 ± 0.02, p < 0.01); nonetheless the analysis of variance reveals that the CI in Sestrica and Lučica do not differ from Garmenjak (Figure 17 and Table 4) while Čuška Dumboka and Kobiljak have values significantly lower (p<0.001).
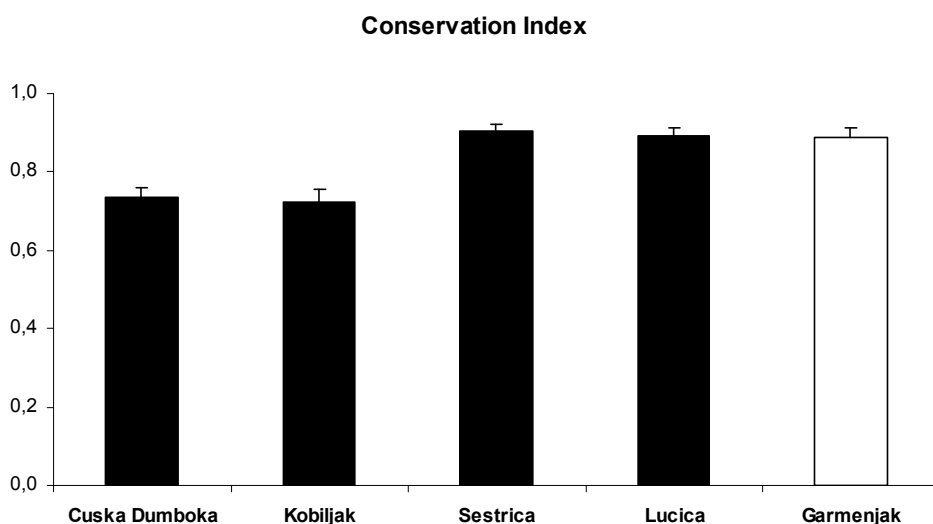
Conservation Index

Figura 17. Mean values (+se) of Conservation Index at each location. Black & white bars are impacted and reference locations, respectively.

Table 3. Results of 1-way ANOVAs on *Posidonia oceanica* shoot density: (a) test for pressure (Im = Impacted; Re = Reference) and (b) locations (CD = Čuška Dumboka; Ko = Kobiljac; Se = Sestrica; Lu = Lučica; Ga = Garmenjak).

(a)	shoot density				
Source of variation	SS	df	MS	F	p
Pressure	1054,93	1	1054,93	160,388	0,00
Residual	1979,79	301	6,58		
Chocran's C-test	C = 0.523, p ns				
trasformation	2nd root transformation				
SNK test	Im < Re				

(b)	shoot density				
Source of variation	SS	df	MS	F	p
Location	1193,39	4	298,35	48,285	0,00
Residual	1841,33	298	6,18		
Chocran's C-test	C = 0.241, p ns				
trasformation	2nd root transformation				
SNK test	CD=Ko=Lu < Se < Ga				

Table 4. Results of 1-way ANOVAs on Conservation Index: (a) test for pressure (Im = Impacted; Re = Reference) and (b) locations (CD = Čuška Dumboka; Ko = Kobiljak; Se = Sestrica; Lu = Lučica; Ga = Garmenjak).

(a)	Conservation Index				
Source of variation	SS	df	MS	F	p
Pressure	0,15724	1	0,15724	6,916	0,009589
Residual	2,91002	128	0,02273		
Chocran's C-test	C = 0.687, p<0.01				
trasformation	none				
SNK test	Im < Re				

(b)	Conservation Index				
Source of variation	SS	df	MS	F	p
Location	0,86202	4	0,21550	12,215	0,000000
Residual	2,20524	125	0,01764		
Chocran's C-test	C = 0.374, p<0.01				
trasformation	none				
SNK test	CD=Ko < Lu=Se=Ga				

3.3. NATURE PARK LASTOVO ISLANDS

On the whole, 7 locations, 36 sites, 267 quadrats and 144 LITs were sampled at Lastovo Nature Park (Table 5).

Table 5. Summary of sampling effort.

pressure	locations	nr. of sites	nr. of quadrats	nr. of LITs	mean depth
Impacted	Zaklopatica	6	33	24	11
Impacted	Pasadur	6	50	24	12
Impacted	Makarac	3	28	12	12
Impacted	Skrivena Luka	8	64	32	9
Impacted	Saplun	5	34	20	9
Reference	Kopište	6	48	24	13
Reference	Davjenica	2	10	8	11
Total number	**6**	**36**	**267**	**144**	

The mean shoot density ranges from 144 ± 6 (Pasadur) to 296 ± 27 shoots m^{-2} (Davjenica) (Figure 18). All impacted locations belong to the class AD (that reflects conditions of very high disturbance) a part from Saplun that is LSD (disturbed bed). Only the reference meadow in Kopište is ND, that means meadow "in equilibrium"; on the contrary, although the values of density recorded in Davjenica are the highest, they indicate LSD for that depth (-11 m), that means disturbed meadow. Analysis of variance shows significant differences (p<0.001)

between impacted (198 ± 6 shoots m^{-2}) and references locations (289 ± 14 shoots m^{-2}) and among locations (Table 6).
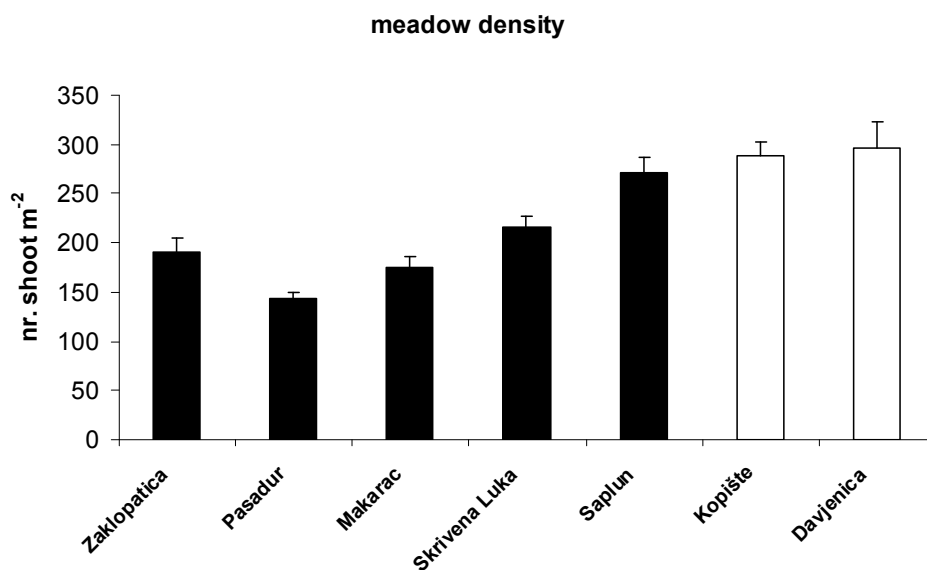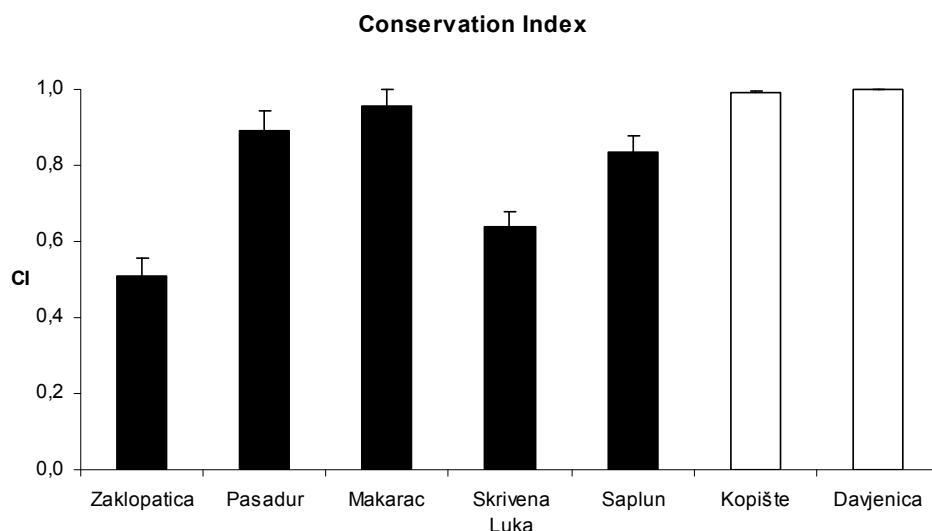
meadow density

Figura 18. Mean values (+se) of the shoot density at each location. Black & white bars are impacted and reference locations, respectively.

The percentage cover of live *Posidonia oceanica* ranges from 50% (Zaklopatica) to 82% (Saplun) in the impacted locations while exceeds the 85% in both reference locations (Figure 19). The covering of dead *matte* is quite variable, from 4% in Makarac to 49% in Zaklopatica, in the impacted locations; it is virtually zero in the reference locations (1% in Kopište and 0% in Davjenica) (Figure 19).

Substrate type

☐ *Posidonia oceanica* ■ dead matte ☐ sand ▨ rock
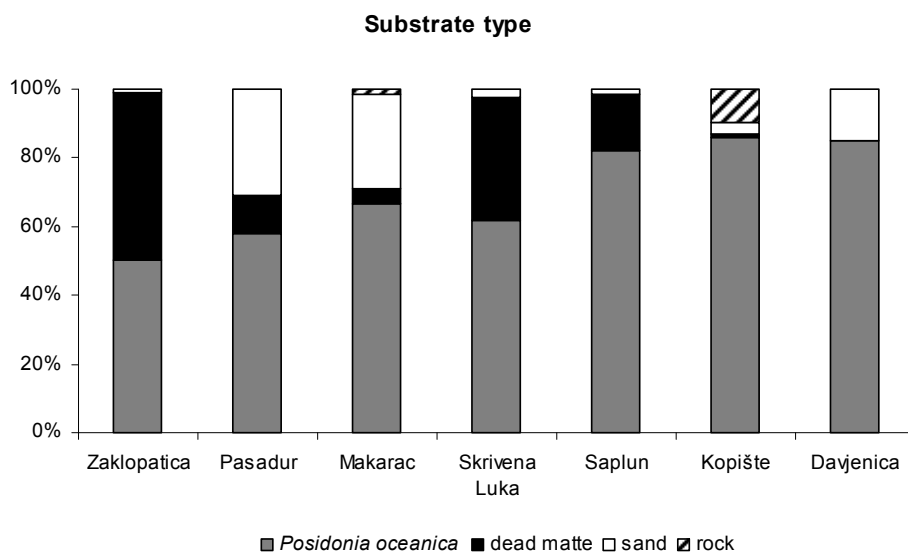
Figura 19. Percentage cover of different substrata and live *Posidonia oceanica*.

The conservation status of the seven meadows is described in figure 20. CI ranges from 0.5 (Zaklopatica) to 1 (Davjenica). Analysis of variance reveals significant differences ($p < 0.001$) in the conservation status between Impacted (CI = 0.73 ± 0.03) and References (CI = 0.99 ± 0.01) and among locations (Table 7). Nevertheless, Pasadur, Makarac and Saplun have similar CI to reference conditions; only the values recorded in Zaklopatica and Skrivena Luka, are significantly lower than other meadows.

Conservation Index

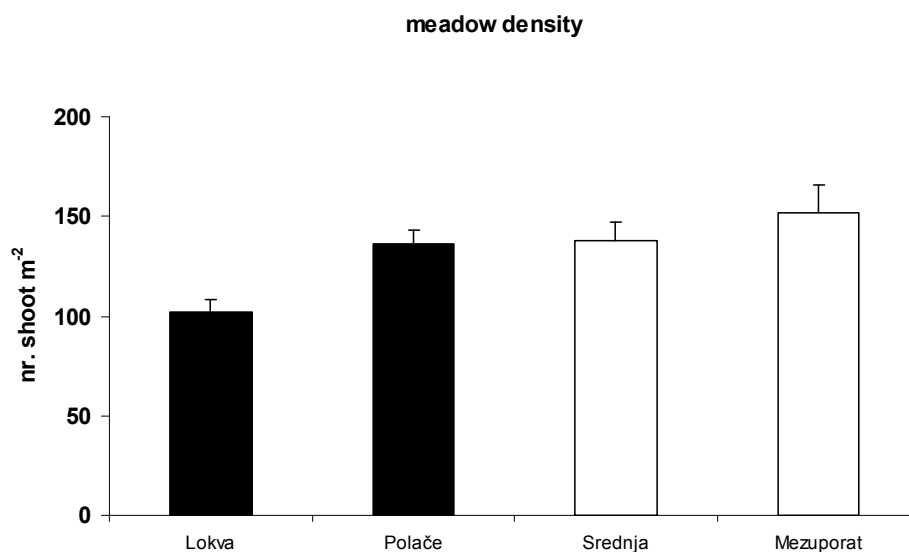Figura 20. Mean values (+se) of Conservation Index at each location. Black & white bars are impacted and reference locations, respectively.

Table 6. Results of 1-way ANOVAs on *Posidonia oceanica* shoot density: (a) test for pressure (Im = Impacted; Re = Reference) and (b) locations (Za = Zaklopatica; Pa = Pasadur; Ma = Makarac; SL = Skrivena Luka; Sa = Saplun; Ko = Kopište; Da = Davjenica).

(a)	shoot density				
Source of variation	SS	df	MS	F	p
Pressure	379883	1	379883	46.712	0.000000
Residual	2155089	265	8132		
Chocran's C-test	C = 0.588, ns				
trasformation	none				
SNK test	Im < Re				

(b)	shoot density				
Source of variation	SS	df	MS	F	p
Location	745571	6	124262	18.055	0.00
Residual	1789401	260	6882		
Chocran's C-test	C = 0.242, p<0.01				
trasformation	none				
SNK test	Za=Pa=Ma=SL < Sa=Ko=Da				

Table 7. Results of 1-way ANOVAs on Conservation Index: (a) test for pressure (Im = Impacted; Re = Reference) and (b) locations (Za = Zaklopatica; Pa = Pasadur; Ma = Makarac; SL = Skrivena Luka; Sa = Saplun; Ko = Kopište; Da = Davjenica).

(a)	Conservation Index				
Source of variation	SS	df	MS	F	p
Pressure	1.65496	1	1.65496	29.581	0.000000
Residual	7.94453	142	0.05595		
Chocran's C-test	C = 0.991, p<0.01				
trasformation	none				
SNK test	Im < Re				

(b)	Conservation Index				
Source of variation	SS	df	MS	F	p
Location	4.54875	6	0.75812	20.564	0.00
Residual	5.05074	137	0.03687		
Chocran's C-test	C = 0.303, p<0.01				
trasformation	none				
SNK test	Za < SL < Pa=Ma=Sa=Ko=Da				

3.4. NATIONAL PARK MLJET

A total of 4 locations and 18 sites were investigated and 144 quadrats and 72 LITs were sampled at Mljet National Park (Table 8).

Table 8. Summary of sampling effort.

pressure	locations	nr. of sites	nr. of quadrats	nr. of LITs	mean depth
Impacted	Lokva	6	33	24	11
Impacted	Polače	6	50	24	13
Reference	Srednja	4	28	12	12
Reference	Mezuporat	2	64	32	12
Total number	**4**	**18**	**144**	**72**	

The mean shoot density ranges from 102 ± 7 (Lokva) to 152 ± 14 shoots m^{-2} (Mezuporat) (Figure 21). Both impacted and reference locations belong to the class AD (that reflects conditions of very high disturbance). The analysis of variance shows significant differences (p<0.05) between impacted (119 ± 5 shoots m^{-2}) and references locations (142 ± 8 shoots m^{-2}) and among locations; nonetheless, only in Lokva the density was significantly lower than the other locations while in Polače values were similar to those recorded in reference locations (Figure 21 and Table 9).
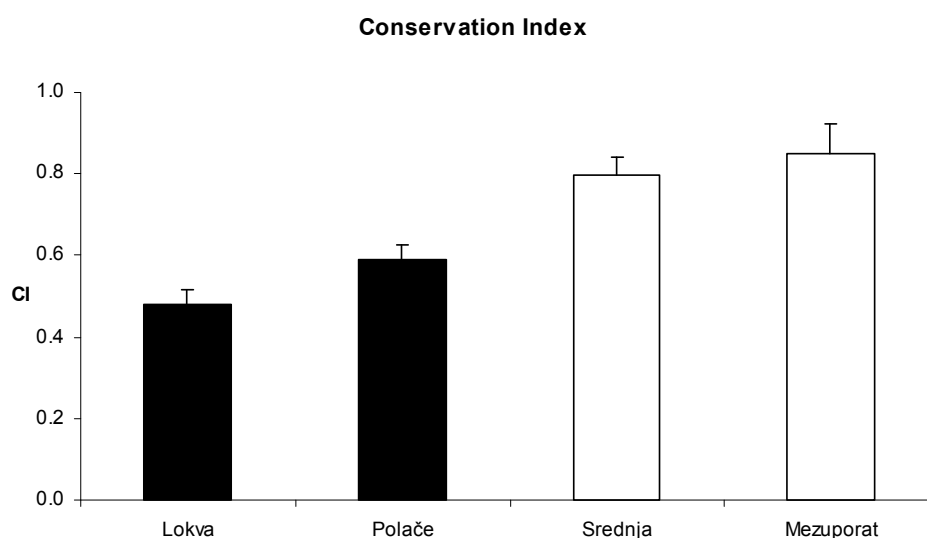
meadow density

Figura 21. Mean values (+se) of the shoot density at each location. Black & white bars are impacted and reference locations, respectively.

The percentage covers of live *Posidonia oceanica* were 44% (Lokva) and 50% (Polače) in the impacted locations and close to 80% in the reference conditions (Figure 22). The covering of dead *matte* in the impacted locations was nearly twice than that recorded in the reference locations (50% and 38% in Lokva and Polače, and 19% and 15% in Srednja and Mezuporat, respectively; Figure 22). Both rocky and sandy bottoms were poorly represented, with the highest coverage values that never exceeded 6% (Figure 22).

Substrate type

□ *Posidonia oceanica* ■ dead matte □ sand ▨ rock
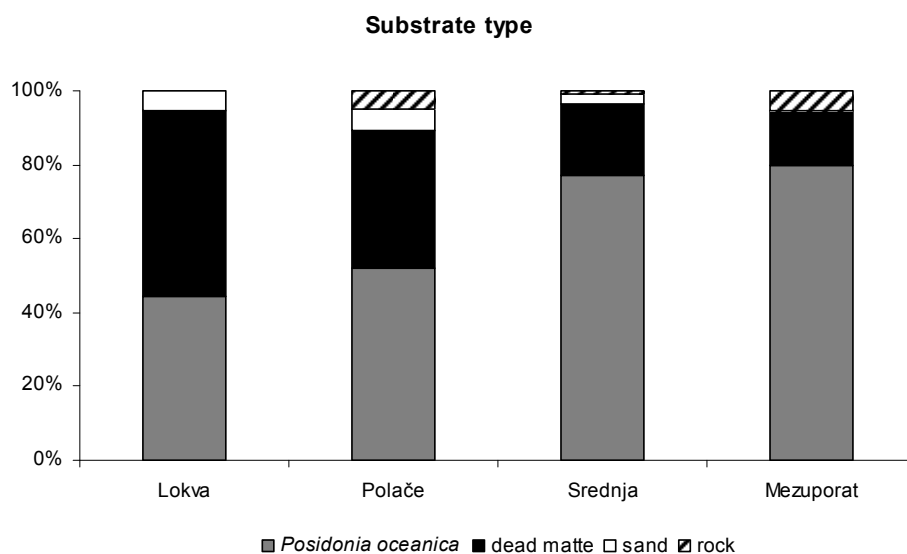
Figura 22. Percentage cover of different substrata and live *Posidonia oceanica*.

The Conservation Index ranges from 0.5 (Lokva) to 0.8 (Mezuporat; figure 23). The analysis of variance reveals significant differences ($p < 0.001$) in the conservation status between impacted (CI = 0.54 ± 0.03) and reference meadows (CI = 0.81 ± 0.04) (Table 10). Significant differences were recorded also among locations; *post-hoc* comparison (SNK) highlights that both impacted locations have significantly lower values of CI than reference conditions.

Conservation Index

Figura 23. Mean values (+se) of Conservation Index at each location. Black & white bars are impacted and reference locations, respectively.

Table 9. Results of 1-way ANOVAs on *Posidonia oceanica* shoot density: (a) test for pressure (Im = Impacted; Re = Reference) and (b) locations (Lo = Lokva; Po = Polače; Sr = Srednja; Me = Mezuporat).

(a)	shoot density				
Source of variation	SS	df	MS	F	p
Pressure	17481	1	17481	6.5705	0.011409
Residual	377788	142	2660		
Chocran's C-test	C = 0.526, p ns				
trasformation	no transformation				
SNK test	Im < Re				

(b)	shoot density				
Source of variation	SS	df	MS	F	p
Location	48539	3	16180	6.5330	0.000362
Residual	346730	140	2477		
Chocran's C-test	C = 0.307, p ns				
trasformation	no transformation				
SNK test	Lo < Po=Sr=Me				

Table 10. Results of 1-way ANOVAs on Conservation Index: (a) test for pressure (Im = Impacted; Re = Reference) and (b) locations (Lo = Lokva; Po = Polače; Sr = Srednja; Me = Mezuporat).

(a)	Conservation Index				
Source of variation	SS	df	MS	F	p
Pressure	1.24428	1	1.24428	36.6545	0.000000
Residual	2.37623	70	0.03395		
Chocran's C-test	C = 0.511, p ns				
trasformation	none				
SNK test	Im < Re				

(b)	Conservation Index				
Source of variation	SS	df	MS	F	p
Location	1.40552	3	0.46851	14.3832	0.000000
Residual	2.21498	68	0.03257		
Chocran's C-test	C = 0.325, p ns				
trasformation	none				
SNK test	Lo=Po < Sr=Me				

3.5. COMPARISON AMONG MPAs

In figure 24, centroids of each location calculated averaging the values of each variables, are plotted in the nMDS ordination. The distances among points correspond to the similarity among locations, thus no clear trend of similarity is evident among MPAs.
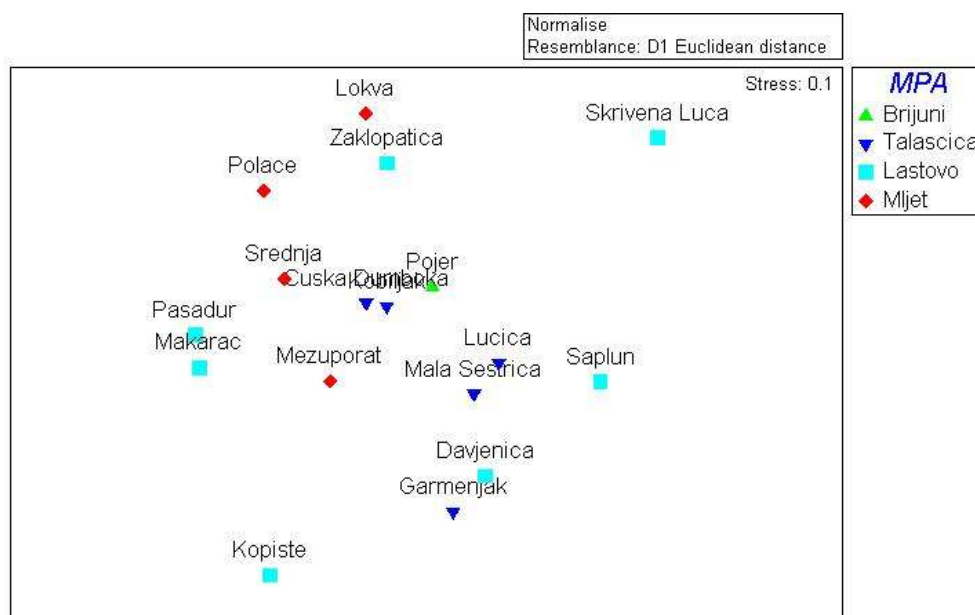
Figura 24. nMDS ordination of the investigated locations plotted according to their belonging to MPAs.

On the contrary, plotting the two levels of pressure (i.e. impacted and reference), the nMDS ordination confirms that, among reference locations, Garmenjak (Telašćica), Kopište and Davjenica (Lastovo) are somewhat separated to all the others, while Pojer (Brijuni), Srednja and Mezuporat (Mljet) are closer (i.e. more similar) to the impacted locations (Figure 25).
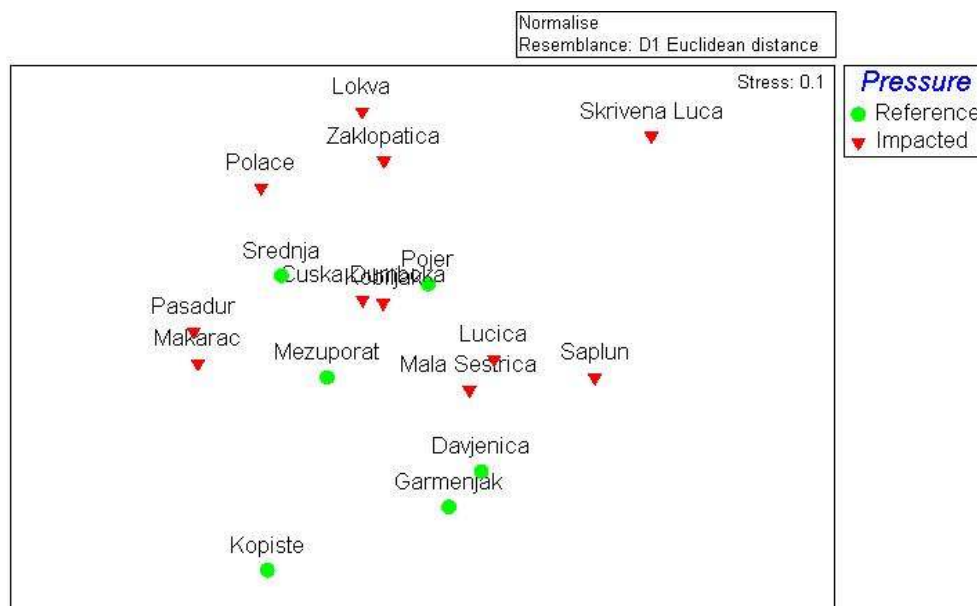
Figura 25. nMDS ordination of the investigated locations plotted according to their level of pressure.

4. DISCUSSION AND CONCLUSION

This monitoring is the first ever conducted on *Posidonia oceanica* meadows in Croatia and it will be critical to provide a first baseline to ensure that habitat changes are monitored and managed appropriately. It is worth to emphasize that interpretation of data limited to one occasional survey can provide highly uncertain findings. Further observations on long term temporal scale are needed, as well as more information on boat frequentation and other possible sources of impact should be gathered in following years to get reliable conclusions. However, below, some specific remarks for each MPAs and more general recommendations are suggested.

4.1. NATIONAL PARK BRIJUNI

The data of density and CI provide results somewhat contrasting. Shoot density is critically low and corresponds to a semi-bed according to Giraud (1977); generally, these meadows are located close to the lower limit at depths greater than 20 m, or on sand or mud in extreme environmental conditions for the species to survive (Buia *et al*., 2004). The latter seems to be the case of the investigated meadow, although the CI is not so low.

On the other hand, since local anthropogenic impacts (namely, mechanical damages by anchoring) are lacking (Dujmović, personal communication), the main causes of the poor state of the meadow can be attributable to not optimal environmental conditions or disturbances that work on a broader scale (i.e. Northern Adriatic scale). The presence of fine sediments of continental origin and their frequent re-suspension may seriously affect the development of the meadow because of the high turbidity of the water column that restricts the photosynthetic activity of the plant, as well as for mechanical problems of the hypogean part (rhizomes and roots) (Cancemi *et al*., 2000; Cancemi *et al*., 2003).

Certainly, *Posidonia oceanica* is not representative of this area, but just because of the small size of the meadow and its abnormal density, the conservation of this habitat should be a priority.

4.2. NATURE PARK TELAŠĆICA

The values of *Posidonia oceanica* shoot density match with the *a priori* assumption that impacted locations are subjected to mechanical disturbance: in fact, only the reference location (Garmenjak) has normal values of density while all impacted locations have a lower number of shoots. On the contrary, CI values vary also among impacted meadows,

suggesting potential different responses of the meadows to diverse levels of disturbance. This hypothesis should be corroborated by the assessment of boat frequentation aimed to quantify the real pressure on the meadows in the Telašćica Nature Park.

4.3. NATURE PARK LASTOVO ISLANDS

In Lastovo, the combined use of shoot density and Conservation Index is an effective tool to get information on conservation status of the meadows. Neither density nor CI provide comprehensive information when they are used separately. In fact the lower densities were recorded in Pasadur and Makarac, where CIs were comparable with those recorded in the reference locations and only CI reveals the higher detrimental effects of anchoring in Zaklopatica and Skrivena Luka.

In Davjenica the highest value of shoot density was recorded, but it corresponds to disturbed meadow for that depth; it should be verified whether the small number of observations (only two sites and ten replicates quadrats) can be the cause of this unexpected result.

4.4. NATIONAL PARK MLJET

Despite significant differences between impacted and reference locations, values of density are very low in both conditions, on average half of those considered normal for these depths. These values correspond to a semi-bed according to the classification of Giraud (1977) that are generally found in deep beds, close to the lower limit, or in poor environmental conditions for the species to survive (Buia *et al*., 2004).

On the contrary, CI shows more clearly the differences between impacted and reference locations. Indeed, the percentage cover of dead *matte* in impacted meadows of Mljet is the highest recorded in the whole study.

These results suggest that anchoring is not the only source of perturbation for *Posidonia oceanica* meadows in Mljet.

4.5. SYNTHESIS OF RESULTS AND RECOMMENDATIONS

Results highlight the effectiveness of the combined use of shoot density and Conservation Index to get information on conservation status of the meadows.

Also, results highlight the sensitivity of *Posidonia oceanica* to mechanical damage and reveal the high level of disturbance in the most frequented locations. Nonetheless, in some cases

(at least in Brijuni and Mljet) other sources of perturbance probably affect the structural variables of the meadows.

The implementation of management measures seems to be necessary for reducing human pressures on the meadows. Nonetheless, in the absence of information on long/medium time scale, the management measures should also refer to general knowledge coming from other areas of the Mediterranean.

In Brijuni, management measures for the conservation of the meadow are rather tricky to identify. This survey provides a first baseline to ensure that habitat changes are recognize by assessing the variability of structural variables over time. Moreover, the **monitoring of lower limits** by means of fixed concrete blocks or metal poles (*balisage*, figure 26) would be extremely informative on the dynamics (progress / regression) of the meadow and, at the same time, easy to do given the low depth of the area.

Figura 26. Example of *balisage* with metal poles in Sardinia, Italy (from Guala *et al*., 2009).

In the all MPAs, at present, at least three main actions should be undertaken in order to improve the knowledge on *Posidonia oceanica* meadows and begin to reduce mechanical impacts of anchoring:

- ✓ **continue monitoring of *Posidonia oceanica*** meadows in order to get a database on long-term changes of habitats and their conservation status;

- ✓ start up the **monitoring of boat frequentation**, at least during the summer months, in order to assess the effective pressure of anchoring on the meadows. This monitoring could be critical to implement effective management measures aimed to minimize the impacts of anchoring on the meadows;

- ✓ **increase the awareness** of boaters on the most correct methods of anchoring is also essential to minimize the impacts;

- ✓ install **ecological mooring systems** that would lower the pressure of anchoring on meadows.

4.5.1. Monitoring of *Posidonia oceanica*

In order to improve the knowledge on *Posidonia oceanica* meadows of Croatian MPAs and to get valuable information on their conservation status their monitoring, with the same approach used in this study, is crucial. Nonetheless some changes may have taken to get sound results and more useful tools for the management.

For example, a balanced design that uses the same number of location, sites and replicates, can increase the power of statistical test. On the other hand, the differences among MPAs, and within each MPA among locations, sites and meadows features, do not allow a fully balanced design. Anyway, at least the use of the same number of replicates for each site (i.e. quadrats and transects) is recommended.

Monitoring sites should be examined at least once every 12 months, at approximately the same time of year, generally in spring or summer. Conclusions based upon short term data may not be accurate or definitive. On the contrary, a long term monitoring allows to (i) assess the temporal trend of habitat changes, (ii) to plan appropriate measures to minimize impacts, (iii) assess the effect of selected measures, (iv) if necessary, re-fix strategies according to the concept of adaptive management based on the immediate integration of the monitoring results.

In order to make a helpful temporal comparison, the sampling effort in subsequent years should be at least the same of previous year, or increased, in terms of number of areas, sites and replications.

A key tool for understanding the presence and status of *Posidonia oceanica* meadows, as well as for their management, is the cartography. Maps provide a general framework on the meadow distribution and surrounding habitats, supply information on depth range, substrate type and structural features of the meadows (e.g. covering, shoot density, fragmentation), allow to identify deterioration signs, environmental values and vulnerability conditions. Associated with Global Positioning System (GIS), maps are crucial to achieve proper management actions: identify zoning areas to be subjected at different degree of protection, identify proper constrains, solve conflicts between users and management targets, assess disturbances and possible actions for protection, identify mooring areas (Figure 27), safe anchoring areas or areas suitable for landing of recreational and service vessels.

Figura 27. Mooring system used at Penisola del Sinis – Isola di Mal di Ventre MPA, Italy (from Cancemi *et al*., 2008).

Mapping itself is a monitoring method that enables to identify the status of seagrass on a broad scale, but also to detect local changes in meadow distribution and the dynamics of upper and lower limits.

Although mapping techniques need strong expertise and can be quite expensive, the management plan should begin to foresee fund raising and resources aimed to their implementation.

4.5.2. Monitoring of boat frequentation

The quantification of boat frequentation should be addressed to assess the potential impact of anchoring on marine ecosystems and, possibly, to identify strategies and policies for the sustainable management of this activity. In addition, the knowledge of nautical tourism flows can provide useful socio-economic information on the characteristics and the extent of one of the major stakeholder groups (the boaters) dealing with marine protected areas.

Here, based on previous experiences in other Mediterranean protected areas (Milazzo *et al*. 2004; Baroli *et al*. 2008; Cancemi *et al*., 2008; Guala, 2012), guidelines for monitoring the frequentation of recreational boats in Croatian MPAs are proposed.

The procedure for data collection has been greatly simplified in order to be carried out even by unskilled operators and/or volunteers. Nonetheless it is still sufficiently informative for the intended purpose, i.e. the monitoring of boats at anchor. Moreover it can be integrated with the census of boats, and perhaps even their anchors, which are moored in marinas or found in the piers of the main bays. Of course, sampling design and sampling effort might be further adapted according to local conditions and resources available.

For the monitoring of the boats at anchor the following actions are proposed:

- ✓ select areas of investigation with clear and unambiguous boundaries;

- ✓ areas of investigation may be those most frequented by sailors, therefore more susceptible to the pressure of the anchors, and that need the adoption of appropriate management measures;

- ✓ identify possible sources of variation among the selected areas (e.g. bays for daily stands with light winds; bays used for longer periods also with strong winds; bays sheltered from the winds of the third and fourth quadrants of the compass);

- ✓ areas of investigation should preferably be monitored from the boat; moreover monitoring can be done from the coast, also by photo detection;

- ✓ data collection should be carried out in as many days as possible in order to have a representative number of samples (at least 2-3 times a week during the season, distinguishing between working days and holidays); sampling should cover at least the summer months, those most affected by yachting;

- ✓ sampling design and sampling effort should be adapted to local conditions and resources available.

Annex V is the board for data collection. For each survey the following data can be recorded:

- ✓ date*;

- ✓ time of day*;

- ✓ names of the operators;

- ✓ wind direction;

- ✓ estimate of wind intensity **

In the board, for each area, a field with predefined number of boats (up to 30) are defined also differentiating among the following categories:

- ✓ sailing boat;

- ✓ motor boat;

- ✓ less than 6 metres (<18 feet)***;

- ✓ between 6 and 12 meters (<40 feet)***;

- ✓ longer than 12 meters (>18 feet)***.

* use two (or more) board for data collection in the case of two (or more) observations on the same day but with different time of day;

** use multiple board for data collection if wind direction and intensity are different from one sampling area to another;

*** the length of 6 meters is arbitrary; alternatively the boat used for the observations (thereafter called "unit boat") can be helpful as a reference size. So, in case of uncertainty, it is possible to visually assess if the boat to be measured is longer (or not) than the "unit boat" or if it is longer (or not) than twice of the "unit boat".

4.5.3. Increase the awareness

Awareness campaign to increase the awareness on the relevance of *Posidonia oceanica* ecosystems, their vulnerability and the most correct methods of anchoring (e.g. no anchors on the meadow, vertical anchor retrieval, figure 28) should be implemented. Simply the distribution of information leaflets, when boaters are moored in marinas or found in the piers of the main bays, can help to enhance awareness and promote a more sustainable approach of tourism.

Figura 28. People should be informed on the right way for anchor retrieval.

4.5.4. Install ecological mooring systems

In order to reduce impact of anchoring on meadows it is recommended that ecological mooring systems are set up, primarilly in most impacted locations. Appropriate technical soultions could be searched based on experiences of other Mediterranean MPAs (Francour *et al.*, 2006; Boudouresque *et al.*, 2006) and local conditions of each MPA. Alternatively, it may be examined the opportunity to identify areas where *Posidonia oceanica* is lacking because of natural conditions; here, free mooring and/or anchoring areas, adequately signaled, can be set up.

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