MONITORING PROGRAMME FOR POSIDONIA OCEANICA BEDS

Common chapters

ENGLISH NAME (NATURA CODE)

*1120 *Posidonia oceanica* beds (priority habitat type)

Equivalents in different classifications

- EUNIS, National habitats classification

CORINE Biotopes
11.34 [Posidonia] beds

EUNIS
A5.535 : [Posidonia] beds

National habitat classification
G.3.5. Naselja posidonije
G.3.5.1. Biocenoza naselja vrste *Posidonia oceanica* (= Asocijacija s vrstom *Posidonia oceanica*)
(source: Bakran-Petricioli, 2007)

Phytocoenological conversion

- Alliances and association*

EUNIS
A5.5351 : Ecomorphosis of striped [*Posidonia oceanica*] meadows
A5.5352 : Ecomorphosis of "barrier-reef" [*Posidonia oceanica*] meadow
A5.5353 : Facies of dead "mattes" of [*Posidonia oceanica*] without much epiflora
A5.5354 : Association with [*Caulerpa prolifera*] on [Posidonia] beds

Barcelona Convention UNEP(OCA) / MED WG.143/5 (Hyères experts report, 1998)
III. 5. *Posidonia oceanica* meadows
III. 5. 1. *Posidonia oceanica* meadows (= Association with *Posidonia oceanica*)
III. 5. 1. 1. Ecomorphosis of striped meadows
III. 5. 1. 2. Ecomorphosis of "barrier-reef" meadows
III. 5. 1. 3. Facies of dead "mattes" of *Posidonia oceanica* without much epiflora
III. 5. 1. 4. Association with *Caulerpa prolifera*

Manual for Interpretation of habitats according to Habitats Directive (HD)
Associations and facies:
G.3.5.1.1. Ekomorfoza naselja u "prugama"
G.3.5.1.2. Ekomorfoza naselja koja tvore "barijeru"
G.3.5.1.3. Facijes mrtvih naslaga rizoma posidonije bez epiflore
G.3.5.1.4. Asocijacija s vrstom *Caulerpa prolifera*

National Classification of Habitats (NCH)
G.3.5.1.1. Ekomorfoza naselja u «prugama»
G.3.5.1.2. Ekomorfoza naselja koja tvore «barijeru»
G.3.5.1.3. Facijes mrtvih naslaga rizoma posidonije bez epiflore
G.3.5.1.4. Asocijacija s vrstom *Caulerpa prolifera*
(source: Bakran-Petricioli, 2007)

Range

- Total world and European range of the habitat type with description of its **distribution pattern** (including maps)
- Remarks on phytocenological variability

Posidonia oceanica meadows - *Posidonietum oceanicae* (Funk 1927) Molinier 1958 - are exclusive to the Mediterranean Sea; depth range varies from the surface to -40 m according to light availability and nature of the substratum (coarse and medium sands; rock) (Relini & Giaccone, 2009). It is estimated that they cover an area varying from 25,000 to 45,000 km^2 (Borum *et al*., 2004) that means about 1-2% of the Mediterranean seabed (VV.AA., 2008) and about 25% of the sea bottom between 0 and 45 m in the Mediterranean basin (Pasqualini *et al*., 1998) (Figure 1).

Posidonia oceanica meadows are in considerable regression both in the northern and Middle Eastern parts of the basin, due to extensive human impacts and pressures.

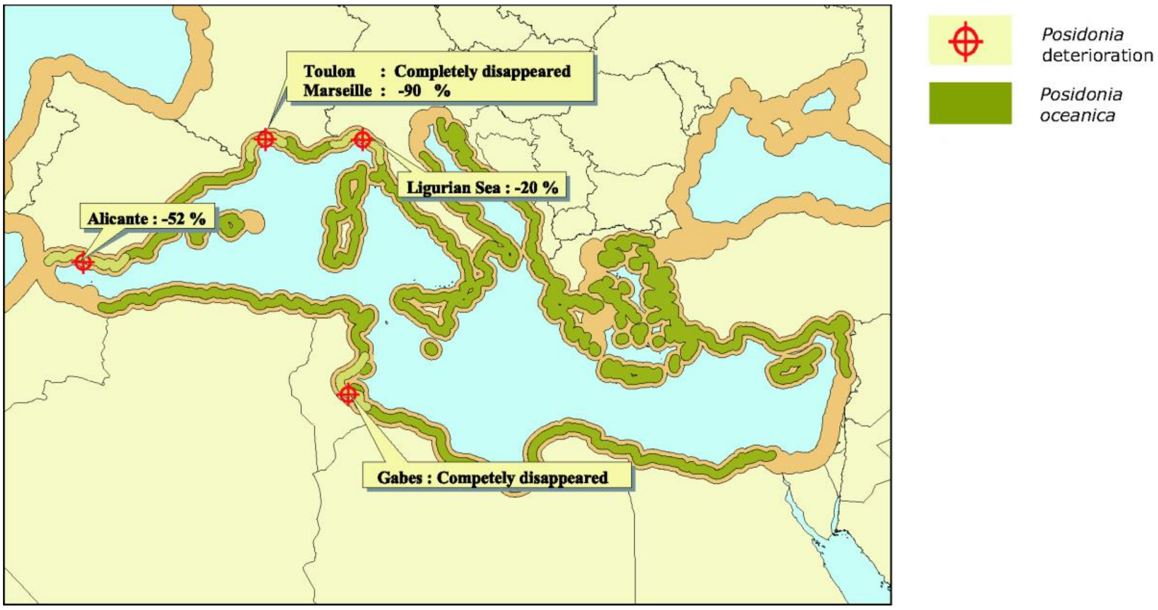

Figure 1. Distribution of the marine angiosperm *P. oceanica* in the Mediterranean (modified from http://www.eea.europa.eu/data-and-maps/figures/distribution-of-the-marine-angiosperm-posidonia-oceanica-and-zostera-sp-in-the-mediterranean).

P. oceanica includes two other distinct associations: the *Myrionemo-Giraudietum sphacelarioidis* Van der Ben 1971 that develops in epibiosis in the upper part of leaves, and the *Flabellio-Peyssonnelietum squamariae* Molinier 1958 located on the rhizomes in the substratum (Relini & Giaccone, 2009).

Distinct facies and ecomorphosis form part of the biocoenosis: a) ecomorphosis of striped meadows, b) ecomorphosis of "barrier-reef" meadows (both are priority habitats of SPA/BD Protocol), c) facies of dead "mattes" of *Posidonia oceanica* and d) association with *Caulerpa prolifera* (Relini & Giaccone, 2009).

Distribution in Croatia

- Summary of **historical development of distribution** (add maps if available in cooperation with SINP)

Posidonia oceanica meadows are common along the Croatian coast (Bakran-Petricioli, 2007; 2011). In general, *Posidonia* beds are better developed in mid and south Adriatic (Gamulin-Brida, 1967); however they have been recorded in several areas in the north (Benacchio, 1938; Zavodnik, 1983; Zavodnik *et al*., 2005). *P. oceanica* meadows have been scarcely investigated in the Adriatic Sea and accurate data on their distribution and status are poor (Kružić, 2008). The distribution of *Posidonia* beds in Croatia is still unknown and data related to range for *P. oceanica* are partial. Existing information is mainly based on the application of the spatial modelling (Antonić *et al*., 2005; Bakran-Petricioli *et al*., 2006); however findings of modelling the spatial distribution of the habitat type are not completely supported by subsequent field surveys (Zavodnik *et al*., 2005, for Senj archipelago) and they should be considered as preliminary (Bakran-Petricioli, 2007). Distribution shown in Figure 2 is based on available data from literature and most of the data from databases of State Institute for Nature Protection (SINP) which mainly consist of presence-absence data gathered through research/mapping by different experts or institutes, faculties and NGOs in the framework of national or international projects and SINP coordinated research. There are still few recent data to be evaluated and entered into databases. Also, future detailed marine habitat mapping project will result in more precise distribution map of Posidonia meadows in Croatian Adriatic.

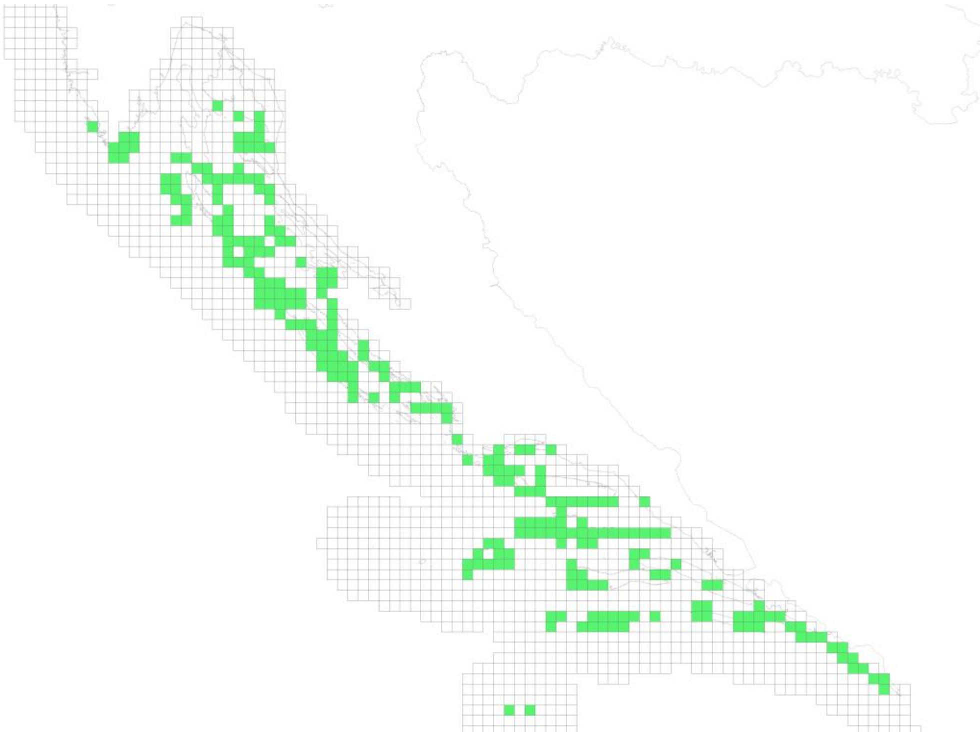

Figure 2. Known distribution of habitat type 1120 *P. oceanica* beds in Croatia (5x5 km grid).

Typical species

- List of typical species of the habitat type (or subtypes – e.g. associations/subassociations)

According to Giaccone *et al*. (1994) *Posidonia oceanica* is the only characteristic species of the association *Posidonietum oceanicae* (Funk 1927) Molinier 1958; *Posidonia oceanica* is the only typical species indicated for the habitat type 1120 in Evans & Arvela (2011; available at http://bd.eionet.europa.eu/activities/Reporting/Article_17/reference_portal); this document indicates structures, functions and typical species which should be considered during the assessment of each habitat type under the Habitats Directive.

Habitat types generally associated in the field

- Enumeration of habitat types that come in mosaics or are exposed to succession (phytodynamic successions, zonations or mosaics)

G.3.2. Fine sands with more or less mud

G.3.2.1. Biocenosis of fine sands in very shallow waters

G.3.2.2. Biocenosis of well sorted fine sands

G.3.2.2.1. Associations with *Cymodocea nodosa*

G.3.2.3. Biocenosis of superficial muddy sands in sheltered waters

G.3.2.3.4. Associations with *Cymodocea nodosa*

G.3.3. Coarse sands with more or less mud

G.3.3.1. Biocenosis of coarse sands and fine gravels mixed by the waves

G.3.5.1.4. Association with *Caulerpa prolifera*

G.3.6. Hard beds and rocks

G.3.6.1. Biocenosis of infralittoral algae

Structures and functions

- Description of physical components of the habitat type
- Description of structural and functional characteristics of the habitat type important for

typical/threatened/indicator species

- Description of ecological processes occurring at a number of temporal and spatial scales including notes on fragmentation

Posidonia oceanica meadows are considered among the most representative and important Mediterranean coastal ecosystems (Buia *et al*., 2004); they grow on sandy and rocky beds and are able to modify the bottom building their own substratum, the *matte*. The meadows are developed with different morphologies (ecomorphosis); according to depth, topography, substrate type, hydrodynamics and sedimentation rate, meadows can be flat and smooth or more or less heterogeneous (steep, striped, barrier-like, hill-shaped), continued or interrupted by unvegetated areas (*intermattes*). *Intermattes* can be structural or erosive and their occurrence, abundance and size depend on natural (e.g. storms, hydrodynamism) or human-mediated (e.g. mechanical impacts, pollution) factors (Boudouresque *et al*., 2006).

Meadows play several ecological roles of relevance for Mediterranean coastal ecosystems (Boudouresque *et al*., 2006 and literature therein). Because of the high rates of primary production, meadows are the basis of many food chains that support species living both within and outside the meadow (Boudouresque *et al*., 2006; Evans & Arvela, 2011); *P. oceanica* meadows are spawning and nursery areas for several species and support high levels of biodiversity (Boudouresque *et al*., 2006; Evans & Arvela, 2011); they are of relevance for water oxygenation (Boudouresque *et al*., 2006; Evans & Arvela, 2011) and are considered among the most efficient vegetated coastal systems for fixing CO_2 as organic matter (Duarte *et al*., 2010; McLeod *et al*., 2011; Pergent *et al*., 2012), eliminating it from the atmosphere; they are able to reduce the hydrodynamics as well as the resuspension of sediments (Boudouresque *et al*., 2006), thus protecting from coastal erosion and maintaining water transparency (Evans & Arvela, 2011).

Human activities can reduce shoot density and cover of sea bed, leading to the regression of the meadow limits and the occurrence of wide areas of dead *matte*, as well as to the change of its key functions (Boudouresque *et al*., 2006). The close relationship between structure and function in these ecosystems is evident in the study of trophic relationships of the different compartments and the associated communities. The food web in the meadows consists of multiple relations among the plant, the epiphytes, the herbivores and other consumers (detritivores, carnivores) (Boudouresque *et al*., 2006): a quarter of all Mediterranean species, including many species of conservation importance (e.g. *Pinna nobilis*) and commercial value (several molluscs, crustaceans and fish), are trophically connected to *Posidonia* (Boudouresque *et al*., 2006).

Pressures and threats

- Detailed and precise description of known important **influences** from the present and past (pressures) and prospective ones (threats)

Posidonia oceanica is sensitive to changes in the marine environment caused by anthropogenic disturbances. In general, pollution, oversedimentation, 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 has been documented to be caused by human activities in many areas of the Mediterranean (Zavodnik & Jaklin, 1990; Boudouresque *et al*., 2006; Di Carlo *et al*., 2011): coastal development (new marinas, ports and seaside complexes), beach nourishment, dredging, dumping at sea of construction materials, dispersion of pollutants from urban and industrial wastewater, water and sediment enrichment with organic matter and nutrients from fish farms and outfalls, changes in fluvial and sedimentary flows, have direct or indirect effects on the meadows (VV.AA., 2008; Boudouresque *et al*., 2006).

The mechanical impacts resulting from boat anchoring and mooring systems (dead weight and crawling chains), placement of submarine cables and pipelines and the use of invasive fishing tools (e.g. trawling) are the main factors that threaten the structure of the meadow at a small spatial scale (Boudouresque *et al*., 2006). Mechanical impacts remove *P. oceanica* leaves and rhizomes largely reducing plant density and cover (Francour *et al*., 1999; Milazzo *et al*., 2004); moreover, they cause re-suspension of the sediment, increased turbidity and nutrient concentrations in the water column (Boudouresque *et al*., 2006).

Increasing land-based sources of pollution and nutrient loading into the sea, as well as the unpredictable effects of global changes (e.g. strong temperature changes of sea water, spread of alien species) are the main potential threats (Borum *et al*., 2004; Boudouresque *et al*., 2006; Marbà & Duarte, 2010). Invasive species that most affect *P. oceanica* meadows, are the green algae *Caulerpa taxifolia* and *C. racemosa*. Although these species apparently do not penetrate into dense healthy meadows, they have often been found in low density meadows and on dead *matte* (Boudouresque *et al*., 2006; Klein & Verlaque, 2008) and they may reduce meadow recovery when associated with other perturbations (Montefalcone, 2009). Climate change effects, such as high temperatures and prolonged heat waves, affect *P. oceanica* shoot growth and increase shoot mortality (Díaz-Almela & Duarte, 2008).

Recovery from damage takes decades and the destruction of the habitat is often irreversible (Boudouresque *et al*., 2006).

An assessment of the main pressures and threats was done for 22 water bodies along Croatian coasts (VV.AA., 2011). Pressures and threats are gathered in different types including punctual sources of pollution (e.g. organic matter, nutrients, metals from municipal and industrial wastewater), diffuse sources (e.g. drainage of nutrients from inland agricultural crops, atmospheric depositions from industrial and urban areas) and other types of pressures (e.g. water pumping stations, power plants, dams, jetties, coastal constructions, fish/mussel farms, vessel transports) and threats (e.g. invasive/alien species, probability of sudden pollution). An overall assessment of their influence on the biological and chemical quality of each water body is also provided: pressures and risks are considered as "not significant" in 8 water bodies, "probably not significant" in 10 water bodies and "probably significant" in 4 water bodies, these latter in very confined urban and industrial areas close to Pula, Bakar Bay and Split.

Whatever their influence on the quality of the whole water body, these pressures and threats could affect the conservation status of *Posidonia oceanica* meadows at a local scale. For instance, anchoring of leisure boats can be locally notable also when pollution and eutrophication are not; this is the case of several sites that are well known as boaters destinations within marine parks: Skrivena Luka, Zaklopatica, Makarac and Pasadur in the Nature Park Lastovo Islands, Kravljačica and Vrulje in Kornati, Lokva and Polače in Mljet National Park, Čuška Dumboka, Kobiljak, Lučica, Sestrica in Telašćica (Font-Gelabert, 2013; Guala *et al*., 2012a; 2012b).

Conservation measures

- Detailed and precise description of measures already **realized as well as needed** to avoid pressures and threats

Posidonia oceanica meadows are directly protected by international conventions ratified by most countries of the Mediterranean: the Berne Convention (on the Conservation of European Wildlife and Natural Habitats in Europe), the Barcelona Convention (on the protection of areas and species in the Mediterranean) and the Habitats Directive (HD) of the European Community (1992/43/EEC); most of the marine Sites of Community Importance (SCIs) in the Mediterranean belonging to the ecological network Natura 2000 are established for the protection of the habitat 1120 *Posidonia oceanica* beds.

Regulation on Ecological network in Croatia has been proclaimed in October 2013 (Official Gazette 124/2013). There are 104 sites of Ecological network in which *Posidonia oceanica* beds are target habitat type (Figure 3).

Posidonia oceanica (L.) Delile is a strictly protected species in Croatia (Ordinance on strictly protected species (Official Gazette 144/2013))

Many indirect measures also protect *P. oceanica*: the establishment of marine reserves and sites of community importance in areas with well-developed *Posidonia* meadows, the obligation to carry out impact studies and to request authorization for projects that could harm the environment, measures to restrict emissions of pollutants, to restrict certain fishing techniques such as bottom trawling, to avoid aquaculture facilities over *Posidonia* meadows, to regulate boat anchoring, to increase surveillance of illegal construction and rock filling near and over *Posidonia* meadows, to increase surveillance service of damaging fishing gears. Marine Fisheries Act (Official Gazette 81/2013, article 76, item 18) prescribes a fine of 40.000 to 75.000 kunas for violation of prohibition of fishing by trawl nets, dredges, surrounding purse seine nets, seine nets, shore seines or similar nets over seagrass bottom, especially *Posidonia oceanica* or other marine fanerogama, defined by Article 4.1 of the Council Regulation (EC) No 1967/2006.

An additional effort should be made in order to prevent *P. oceanica* decline at least in sites where signs of degradation are evident. As Croatian national regulations are in force (*Posidonia* meadows have the status of endangered habitats, Official Gazette 119/2009), what is needed is to implement appropriate measures to ensure their enforcement and compliance, also by means of increasing public awareness. Locally (e.g. within protected areas and managed sites) appropriate measures could be monitoring of pressures and their impacts, surveillance and feasibility study on specific measures (depending on the ongoing pressures) to be implemented (see Font Gelabert *et al*., 2013 as an example of conservation measures for reduction of nautical tourism impact on seabed communities).

Figure 3. Natura 2000 sites for the habitat type 1120 Posidonia beds (*Posidonion oceanicae*) (from SINP, 2014).

Specific chapters for biogeographical regions where the given <u>habitat type</u> occurs

MONITORING PROGRAMME FOR THE MEDITERRANEAN BIOGEOGRAPHICAL REGION

EACH MONITORING PROGRAMME INCLUDES FOLLOWING COMMON PRELIMINARY INFORMATION:

- Short description of **basic scheme of surveillance** (how mapping and monitoring are combined)
- **Sharing the data** or methodologies with other monitoring programmes (for other habitat types or species, other projects or monitoring systems)
- Rules of occupational safety and compliance with all relevant statutory instruments including list of permits needed for research when that is the case (instructions for the field workers).

The direct, SCUBA-based protocol here proposed, is a traditional protocol using the most fundamental descriptors that are present in programmes to measure conservation status of *Posidonia* in nearly all Mediterranean countries. The basic scheme of surveillance includes periodic monitoring of reference descriptors as indicators of habitat type conservation. The descriptors, to be detected with SCUBA diving without involving the removal of biological material, are listed below:

- <u>physical and physiographical descriptors</u>: depth range; position of typology of upper and lower limits; substrate type (mud, sand, *matte*, rock/coralligenous formations); distribution with respect to the nature of the bottom (flat and continuous beds, terraced beds, belts, stripes, patches, hills, reefs, atolls; presence/abundance/distribution of other plants;
- <u>structural descriptors of the habitat</u>: shoot density (no. of shoots per m^2); coverage (percentage of seabed covered with live plants of *P. oceanica* compared to that non-covered and consisting of sand, rock or dead *matte*);
- <u>biological variables of the associated communities</u>: abundance of *Pinna nobilis* as indicator of meadow health (Díaz-Almela & Duarte, 2008); presence/abundance of invasive or alien species (i.e. Caulerpales).

Monitoring will be carried out in different sites, zones and sampling stations. A site is defined as a continuous area where the meadow is homogenously distributed for at least 1 km in length; a zone is an area, within each site, in which monitoring is carried out at different bathymetric intervals; a sampling station is an area, within each zone, characterized by a single depth interval (details will be provided in the following section "Monitoring on plots").

As the monitoring programme has to cover the entire national territory, the whole geographical range of the habitat has been divided into 10x10 km squares and meadows to be monitored are selected within squares where most probably there is *Posidonia oceanica*. The meadows to be monitored could then be chosen accordingly to different environmental conditions providing a broader view on the status of the habitat.

It is highly recommended that the following information is available before the monitoring is conducted:

- distribution of the meadows and geomorphological features of sea bottom;
- position and depth of lower and upper limits of the meadows;
- existence of previous information on the meadows;
- especially well conserved meadows should be selected as reference;
- meadows under clearly recognizable direct or indirect anthropogenic disturbances are worth to be selected in order to assess the impact of these disturbances.

In the Mediterranean, under the Water Framework Directive (WFD, 2000/60/EEC), a number of indexes have been developed by using *Posidonia oceanica* as a biological element for the definition of the ecological status of coastal water bodies (Romero *et al*., 2007; Fernandez-Torquemada *et al*., 2008; Gobert *et al*., 2009; Lopez y Royo *et al*., 2009; 2010a; Montefalcone, 2009). The index POMI (*Posidonia oceanica* Multivariate Index, Romero *et al*., 2007; Mascarò *et al*., 2012) is used for the monitoring programme for coastal waters in Catalonia since 2005; more recently, it has been applied to Croatian coast (Nikolić *et al*., 2009; UNEP-RAC/SPA, 2011; Mascarò *et al*., 2012).

Some descriptors here suggested for the assessment of conservation status of the habitat type 1120 under the Habitats Directive are also used as metrics for monitoring the ecological status of coastal water by means of POMI Index (Romero *et al*., 2007). However, sharing the data or methodologies between these two monitoring programmes can only be partial and it is not recommended because of different timing and requirements of the monitoring.

The Marine Strategy Framework Directive (MSFD, 2008/56/EEC) aims to achieve good environmental status (GES) of the EU's marine waters by 2020. It came into force in 2008 and, to present, an initial assessment of the state of the environment and the definition of environmental objectives were completed. The elaboration of a monitoring programme and its implementation are ongoing and are scheduled for 2016 at least in France (Bellan-Santini, 2013) and Italy (Tunesi, 2013). Although potential overlaps in the monitoring requirements of the different Directives (MSFD and HD) do exist (http://ec.europa.eu/environment/nature/natura2000/marine/docs/FAQ%20final%202012-07-27.pdf), specific approaches for monitoring *P. oceanica* meadows are still not defined. In France, *P. oceanica* meadow is one of the ecosystems for which the Ministry of Ecology and Sustainable Development and experts are currently defining concepts, identifying parameters to use and elaborating monitoring programmes (Bellan-Santini, 2013). So far work on monitoring in the scope of the MSFD in Croatia is in preparation and *Posidonia* meadows will be included in the proposed monitoring programme.

For safety purposes, in particular for field activities to be realized in SCUBA diving, field workers (or operators) involved must have specific competencies for scientific diving and have a comprehensive understanding of equipment and emergency procedures; hence, they must be skilled with/in survey methods, both surface and sub-surface, capable of accurately locating and marking sites, basic rope work, including the deployment of transects, sampling techniques appropriate to the scientific discipline being pursued; moreover, they must be fully competent with/in SCUBA rescue techniques and management of casualties, the use and user maintenance of appropriate SCUBA diving equipment (ESDP, 2009).

All the activities must be implemented in accordance to the national laws, regulations and permissions. Permission for diving to carry out the monitoring activities should be obtained from the Ministry of Environmental and Nature Protection.
For monitoring in sites inside protected areas permission from management board of MPA or Park is needed.

Field mapping

- Detailed description how the habitat type is surveyed during system of mapping for all habitat types prepared directly by SINP (if no mapping is planned the whole chapter "field mapping" has to be deleted)

According to the guidelines for preparation of monitoring programme (VV.AA., 2012) "*mapping is not a method of monitoring but a way to get a baseline data and data serving for other (e.g. scientific) purposes*". For this reason, and since it requires expensive tools and time consuming methods, mapping is not a objective of this protocol.

However, it is worth to note that mapping should be a priority whereas there is no comprehensive and systematic knowledge on *Posidonia oceanica* meadows distribution in Croatia. Mapping is a helpful tool to understand how the meadows are distributed (Thomas *et al*., 1999) and to supply information on their depth range, position of the limits, characteristic of the bottom (e.g. slope, type of substrate, deterioration signs). Mapping is also of relevance for selecting sampling sites and for management targets (e.g. identify areas to be subjected at some degree of protection, identify mooring areas, safe anchoring areas or areas suitable for landing of recreational and service vessels).

Mapping and monitoring should be combined in order to complete the areas with the occurrence of *Posidonia oceanica* meadows with information on structural features of the meadows (e.g. covering, shoot density, fragmentation).

A synthesis of main survey tools for seagrass mapping is reported in UNEP-RAC/SPA (2011); aerial photography, remote sensing, acoustic techniques are suitable for larger-scale studies (Thomas *et al*., 1999; Kenny *et al*., 2003); however, these methods are expensive and time consuming, and need to be ground truthed by field surveys (e.g. boat-based videography, SCUBA diving). Mapping of *Posidonia oceanica* meadows should be planned as a long-term goal due to extension of Croatian coasts.

Comprehensive field mapping is planned to be conducted with EU Structural Funds. The earliest date would be 2015. The Ministry of Environmental and Nature Protection will apply for technical assistance in order to prepare the project documentation before it starts.

Monitoring on plots

Objectives

- Clarification why monitoring is chosen for habitat type surveillance (if not the whole chapter "monitoring on plots" has to be deleted) and **what outputs are awaited**

- Description of connection with monitoring of other habitat types

The overall objective of the monitoring is to obtain information on the conservation status of *Posidonia oceanica* meadows in Croatia and to identify any degradation or habitat changes over time. Monitoring is chosen in order to estimate the ecosystem status and changes at each monitored site by means of ecological synthetic indices that are informative, sensitive to stress, easily measured and not destructive.
Expected outputs are:
a) collection of physical and physiographical data (depth range; position of typology of upper and lower limits; substrate type; meadow type) on each sampling site;
b) collection of structural data of the habitat (meadow density and coverage);
c) identification of conservation status of the habitat (according to the classifications commonly used in the Mediterranean);
d) identification on presence or absence of habitat disturbances (e.g. pollution, mechanical impacts, invasive/alien species).

No connection with monitoring of other habitat types is feasible.

Field work instructions

- Determination of field workers specialization
- **Detailed instructions** for the field work including:
- period for monitoring (+ other limits like temperature if appropriate);
- character of plots to be chosen in the field and how to mark it;
- description of data recording.

Field work on monitoring must be coordinated by marine biologists with very good knowledge of plant species and methodology for assessing the state of health of the meadows and other features of the habitat type. Coordinators have to provide clear instructions on field methods to other field staff; they also are responsible of data quality and they are in charge for preserving raw data once they are collected and after input into the database (e.g. taking pictures of each dive slate in the field, doing photocopy to preserve raw data after input into the database).

Field staff can be other persons (biologists, ecologists, agronomists, students of these disciplines, rangers, also volunteer divers) trained on the standard methods to be applied; it is essential that workers are motivated and interested in achieving quality outcomes.

All field staff must be skilled in scientific diving and have a comprehensive understanding of diving equipment and emergency procedures. SINP is the responsible agency for coordination and appointing coordinators and other field workers involved in the monitoring.

Monitoring sites should be examined annually at approximately the same time of year. However, sampling frequency depends on the number of sites and the budget available; if the budget is not sufficient for the monitoring of all sites each year, two options are suggested: (a) carry out monitoring of all sites every two years; (b) sites are randomly distributed within two sets and each year the sites of one set are monitored (Mayot *et al*., 2006). The latter allows splitting the monitoring in two following years; a similar approach is currently implemented in Croatia for monitoring of *Posidonia oceanica* meadows under the WFD (Nikolić V., personal communication). Both options allow implementing the monitoring three times for each meadow over the six-year official period of reporting.
Alternatively, as there is no baseline data, monitoring can be implemented annually at the beginning of the programme (for 3-5 years) in order to acquire basic information on the status and trend of the meadows; then it can be adapted according to the results of the first 3-5 years and the availability of financial resources.
For practical and logistical reasons, it is preferable to schedule the monitoring in late spring or early summer (May to early September), when the weather conditions are more favourable.

Different types of data will be collected at different spatial scales (sites, zones within sites and stations within zones). General information will be recorded at the scale of meadow; at each site following data should be recorded:

- date of monitoring;
- name/code of the site;
- name of the coordinator;
- name of the operators;
- exposure;

- presence of evident potential pressures in the area.

Physical and physiographical data will be recorded at the scale of zone; at each zone following data should be recorded:

- position and depth of upper limit: to be recorded by means of GPS and echo sounder from the boat or depth gauge in SCUBA diving;
- position of lower limit: by means of GPS from the boat marking the position of safety buoy of divers;
- depth of lower limit: by means of depth gauge in SCUBA diving;
- typology of lower limits: based on the description of Pergent *et al*., (1995) integrated by Montefalcone (2009) and UNEP-RAC/SPA (2009):
  - ✓ progressive limit: with plagiotropic (horizontal) rhizomes beyond the limit oriented toward the bottom, absence of *matte*, coverage decreasing regularly (it indicates colonization of the meadow in the depth);
  - ✓ sharp limit: the meadow stops abruptly with the presence of vertical rhizomes but in the absence of *matte*; it can has high (<25%) or low (<25%) cover (these limits usually indicate a status of equilibrium but the low percent cover may indicate deterioration of the environment and an early imbalance);
  - ✓ erosive limit: the meadow stops abruptly with the presence of a pronounced step of *matte* and cover > 50%;
  - ✓ sparse limit: density is lower than 100 shoot per m^2 and cover lower than 15% (in general it reflects degradated conditions);
  - ✓ regressive limit: presence of dead *matte* beyond the limit, within the dead *matte* a few isolated shoots or residual patches of *P. oceanica* alive may persist, with or without step of *matte*, isolated or connected to the meadow (it testify a decline of the meadow).

Depth range and slope of the meadow can be calculated in function of the depths of upper and lower limits and their mutual distance.

Since seagrass abundance typically varies with depth (Pergent *et al*., 1995; Duarte & Kirkman, 2001) the sampling design has to incorporate different depths according to the bathymetric distribution of each meadow. Hence, the whole depth range of the meadows has to subdivided into depth intervals where sampling stations are fixed (Duarte & Kirkman, 2001) and their position marked with GPS; stations should be randomly chosen the first year, but remain fixed each monitoring event (dGPS equipment should be used to minimize the positional error). Within each station, sampling units (transects and quadrats) are placed randomly, so no marking procedure is requested.

In Figure 4, the sampling scheme suggested for the monitoring of each station is shown. Sampling units are different for each of the variables to measures. For percentage cover, four transects (Line Intercept Transects or LITs, Bianchi *et al*., 2004), each of 10 m length (Marcos-Diego *et al*., 2000), will be positioned; transects extend radially in opposite directions from a fixed point in the middle of the sampling station (e.g. the anchor of the boat or the ballast of a safety buoy). Plant and substrate cover are assessed along each transect (see section "Description of data recording"). The four transects delimit a surface of approximately 400 m^2 m in which plots for the detection of shoot density (quadrats 0.16 m^2, Buia *et al*., 2004) are randomly positioned. Ten quadrats are placed at each sampling station (see Pergent *et al*., 1995; Marcos-Diego *et al*., 2000; Cicero & Di Girolamo, 2001; Duarte & Kirkman, 2001; Cancemi *et al*., 2003; Pergent-Martini *et al*., 2005, Borg *et al*., 2006; Cossu *et al*., 2006; Costantino *et al*., 2006; Flagella *et al*., 2006; Lasagna *et al*., 2006, Dante, 2010, Sghaier, 2013).

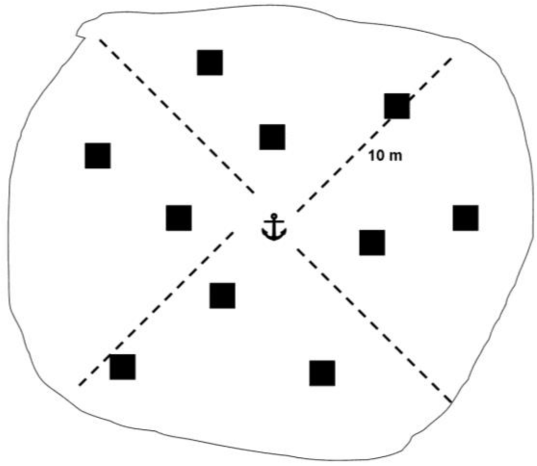

Figure 4. Sampling scheme proposed for each sampling station.

Habitat and community data at the scale of stations; at each sampling station following data should be recorded by means of SCUBA diving:

a) habitat data:

- distribution with respect to the nature of the bottom (e.g. flat/step and continuous/discontinuous beds, terraced beds, belts, stripes, patches, hills, reefs, atolls) by means of visual observation;
- main substrate type (mud/sand, *matte*, rock/coralligenous formations, mixed): by means of visual observation;
- presence of other seagrass species (e.g. *Cymodocea nodosa*, *Zostera noltii*): by means of visual observation;
- presence of alien species (e.g. *Caulerpa racemosa*, *Womersleyella setacea*): by means of visual observation;
- evidence of mechanical pressures (e.g. mooring systems, concrete blocks, pier, chains, ropes, trash): by means of visual observation;
- signs of impacts (e.g. detached shoots, detached plates of *matte*, damages due to trawling or anchoring): by means of visual observation;
- depth: it should be recorded with a depth gauge at the beginning and end of each transect and at each quadrat;
- shoot density (no. of shoot per m^2): 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 0.16 m^2 is considered the optimal sampling unit for estimating the density of *P. oceanica* (Panayotidis *et al.*, 1981). So, at each station, 10 replicated quadrats (40x40 cm) are launched randomly at a distance of at least one meter from the other. Subsequently, the values of the single count are reported to the m^2 and averaged.
- % coverage of live *P. oceanica*: within each of four transects (LITs) 10 m long;
- % coverage of dead *matte*: within each of four transects (LITs) 10 m long;
- % coverage of unvegetated muddy/sandy patches: within each of four transects (LITs) 10 m long;
- % coverage of unvegetated rocky patches: within each of four transects (LITs) 10 m long.
- All percentage cover values will 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 are recorded the occurrence of live *P. oceanica* and the nature of the substrate (sand, rock, dead *matte*). Four LITs, each of 10 m length and randomly positioned, were carried out in each station; for each LIT, the intercept to the nearest centimetr 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

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

Thus, percentage cover data provides information of the amount of different substrata and live *P. oceanica* covering the sea bed (Figure 5). Seagrass cover is a more sensitive indicator of eutrophication at intermediate water depths and in deep water, where light plays a major regulating role, than in shallow water, where physical exposure has a marked influence (Borum *et al*., 2004).

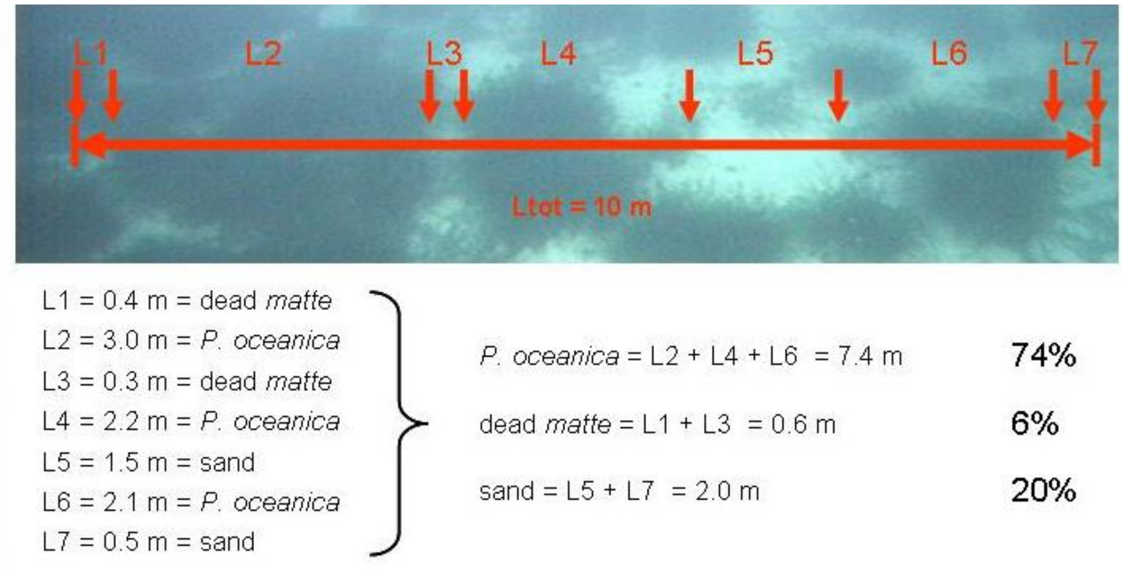

Figure 5. Explanation of the LIT method for the assessment of percentage cover.

b) community data:

- % coverage of substrate colonized by other seagrasses (*Cymodocea nodosa*, *Zostera noltii*): as within each of four transects (LITs) 10 m long;
- % coverage of substrate colonized by alien species (e.g. the green algae *Caulerpa taxifolia* and *C. racemosa*): within each of four transects (LITs) 10 m long;
- presence of target invertebrate fauna: the bivalve *P. nobilis* (listed in the Annex IV of HD) is exclusively dependent on seagrasses, and is therefore affected by physical impacts on the meadows (e.g. boat anchoring); presence of *P. nobilis* is a characteristic of healthy *P. oceanica* meadows (Borum *et al*., 2004). Count of all individual encountered within a 2 m corridor for both sides of each of four transects 10 m long (see "Belt Transect" technique in Bianchi *et al*., 2004; Coppa *et al*., 2010) and evaluation of their status (dead or alive).

Sampling design

- Detailed description of the **selection of plots** (classification, random choice etc.) for monitoring including distribution in time (respecting the six-year official periods of reporting) and a shape file in GIS (should be added later)
- Specification of the number of field workers (or man days per year) needed
- Particularities of potential pilot project (if relevant)

Monitoring should be carried out in different sites distributed over the whole Croatian coastline. A **site** is defined as a continuous area (segment of coastline) where the meadow is distributed for about 1 km in length. At each site, three **zones** about 100 m apart are to be selected, and in each zone three **sampling stations** are chosen at different bathymetric intervals (Figure 6).

When the meadow extend between 7 and 30 m depth (in Croatia the upper limit is typically at 7-10 m depth and the lower one at 25-30; Nikolić V., personal communication) the following depth intervals should be considered: shallow (<10 m), intermediate (12-18 m), deep (as close as possible to the lower limit). However, the sampling design needs to take into account the natural variability and the spatial distribution of seagrass meadows.

Stations should be randomly chosen the first year, but remain fixed over the six-year monitoring programme; therefore, the GPS coordinates of each station should be marked (for shape files in GIS). Within each station, sampling units (transects and quadrats) are placed randomly as described above (see section "Character of plots to be chosen in the field and how to mark it").

As described above (see section "Period of monitoring"), the monitoring should be carried out at least three times during the six-year official periods of reporting, preferably once every year.

Each sampling station is monitored by two independent workers, one to assess the density within the quadrats, the other the percentage cover of *P. oceanica* and all the other variables along the transects. A team composed of three pairs of divers and one operator on board (the boatman) can cover every day the whole monitoring for one site (a total of nine monitoring stations, three dives for each pair of diver, one at deep, one at intermediate and the last at the shallow station).

So, seven field staff per year can cover the whole monitoring effort assuming up to 50 sites to be monitored.

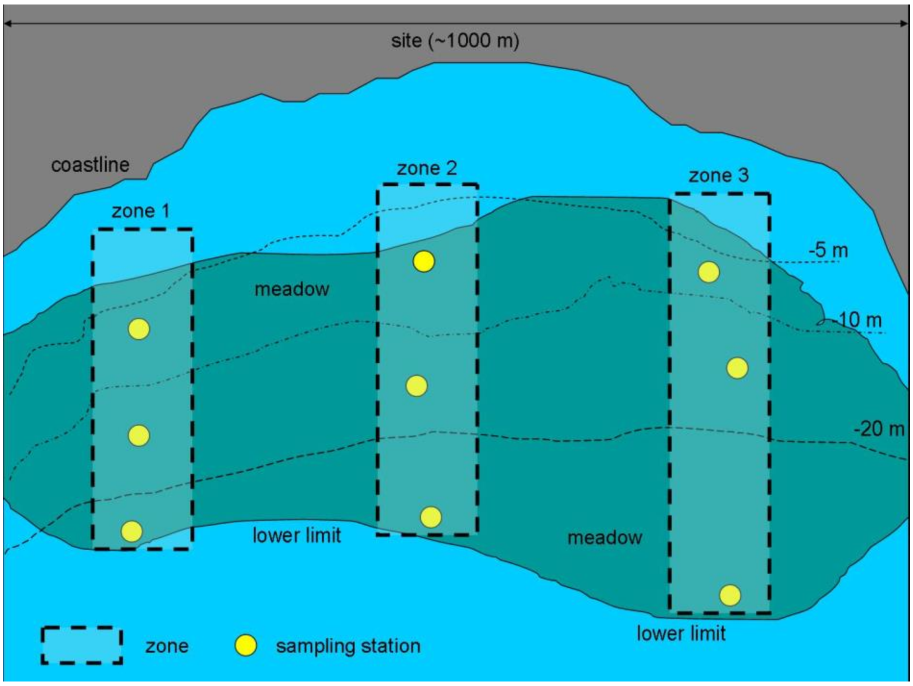

Figure 6. Example of distribution of sampling stations in the three zones and three different bathymetric ranges.

In order to cover as much as possible the whole geographical range of the habitat, 50 sites are proposed for the monitoring.

According to the Annex III of Habitats Directive (HD) that defines the criteria for selecting areas eligible for identification as Sites of Community Importance (SCIs), the following factors should be considered:

(a) Degree of representativity of the natural habitat type on the areas.

(b) Surface of the area covered by the natural habitat type in relation to the total surface covered by that natural habitat type within national territory.

(c) Degree of conservation of the structure and functions of the natural habitat type concerned and restoration possibilities.

(d) Global assessment of the value of the area for conservation of the natural habitat type concerned.

This means that baseline data are necessary for selection of areas according the HD. In the absence of this information (but ensuring that *Posidonia oceanica* meadow is the dominant habitat at the site) the following factors could be considered for selecting potential sites to be monitored:

- priority 1. the site is relevant for conservation purposes (e.g. it is located inside Natural Parks, Marine Protected Areas; important Natura 2000 areas) or data on conservation status of the meadows already exist;
- priority 2. the site is subjected to various anthropogenic pressures;
- priority 3. the site is relevant for other monitoring purposes (e.g. other monitoring programmes within the WFD and/or MSFD); currently, under the WFD, 15 monitoring stations exist and 26 are planned in the next 2 years (Nikolić V., personal communication).

In figure 7 the Croatian coastal area is divided into squares of 10x10 km and a colour code is assigned to each square of the grid:

- red, MPAs, important Natura 2000 areas, sites with data available;
- orange, a mix with pressure/no pressure sites;
- green, existing WFD sites;

- blue, possible future WFD sites (2014-2016);
- yellow, further filling gaps (potential additional sites).

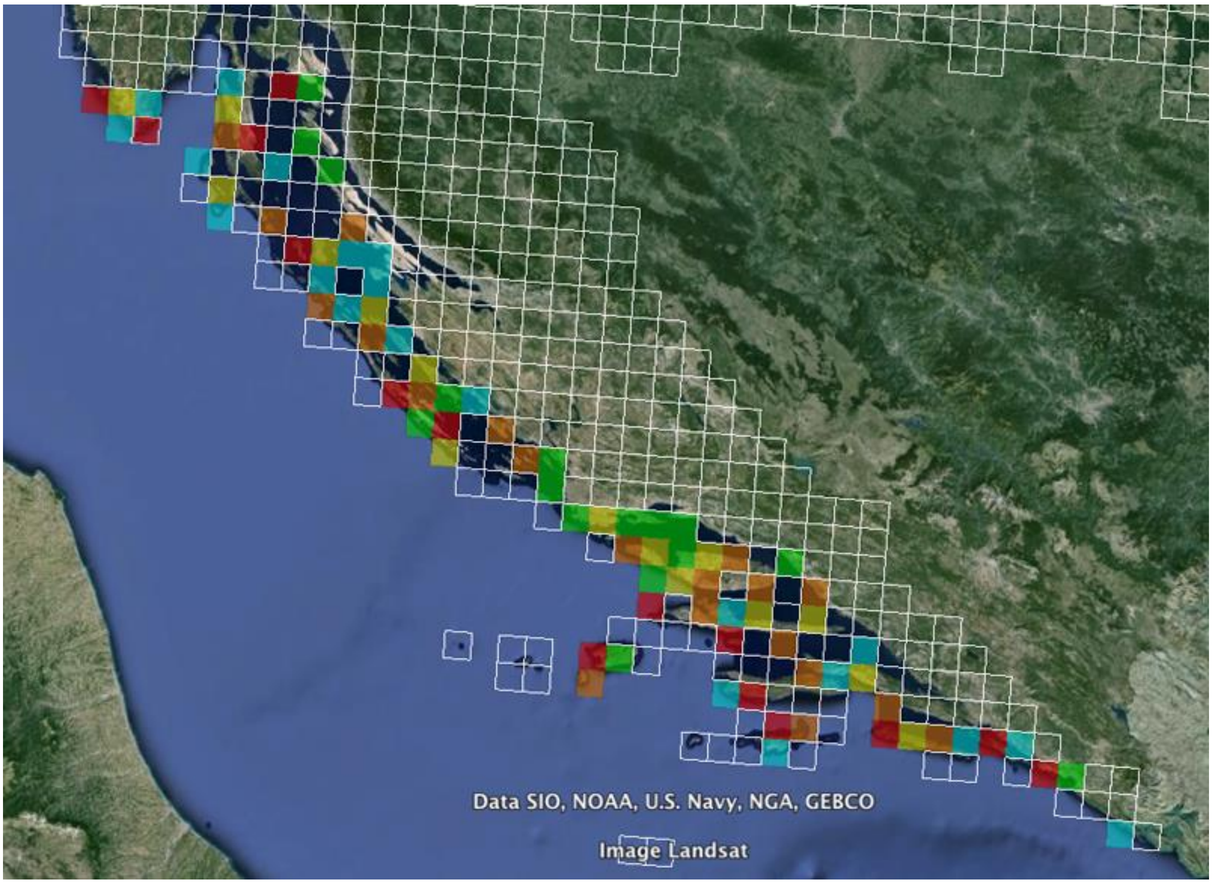

Figure 7. Mediterranean biogeographic region of Croatia divided in area with different priority (10x10 km grid). Colours identify different priorities (see text).

Based on priorities, 50 monitoring sites could be selected as follows (but note that the total number of sites is tentative because it depends on the budget availability):

- 15 sites from priority 1 (red squares in figure 7),
- 20 sites from priority 2 (orange),
- 15 sites from priority 3 (blue and green), selected through the whole habitat range.

It is worth to take into account that sites should be logistically feasible (e.g. weather, access, safety) and that, in any case, *Posidonia oceanica* has to be the dominant habitat at the site. Selection procedure should involve "Google map" investigation, interviews to local people, preliminary field investigation.

In general, it is appropriate to begin the surveys of a limited number of meadows in order to ensure they are regularly monitored over time. At a later stage, according to budget availability, additional monitoring sites may be added for filling gaps throughout the habitat range.

Below the list of sites proposed within the Project Eu IPA 2007: Identification and setting-up of the marine part of Natura 2000 network in Croatia – marine NATURA 2000 (SINP, 2012):

<u>Protected areas</u>

- Nature Park Telašćica (Islet Mali Garmenjak)
- Nature Park Lastovo (Skrivena Luka bay, Lastovnjaci and Vrhovnjaci Islets)
- National Park Brijuni
- National Park Kornati (Vrulje bay, Islet Rašip Mali, Islet Piškera)
- National Park Mljet (Lastovska bay, Islet Glavat)

<u>Other sites</u>

- Southern part of Istria peninsula (rt Kamenjak)
- Island of Krk (Baška bay)
- Island of Cres (Islets Veliki and Mali Ćutin)

- Island of Silba Island (Sveti Ante bay, Južni rat)
- Island of Vir (Duboka Bay)
- Island of Dugi otok (Lagnići Islets, Veli rat, Brbinjšćica bay, Mežanj islets)
- Island of Žut (Pinizel Bay)
- Islet Tetovišnjak (Kablinac)
- Island of Žirje (Stupica Bay, Tratinska Bay)
- Island of Drvenik Veli (Solinska Bay)
- Island of Brač (Smrka Bay)
- Island of Hvar (Pakleni Islands)
- Island of Mljet (Velike i Male Blace bays)
- Cavtat (Mrkan and Bobara Islets)

In table 1 the first proposal of the list of sites for monitoring in accordance with Habitats Directive is reported.

Table 1. List of proposed sites. Colour code: red – 1st priority; orange – 2nd priority; green – WFD stations with data; blue – future WFD sites (2014-2016).

no.	site	main pressure	Natura 2000 (1120*)
1	Brijuni (MPA)		x
2	Kamenjak	anchoring	x
3	Krk-Baška	anchoring	x
4	Cres-Ćutin		x
5	Silba		x
6	Telašćica (MPA)		x
7	Kornati (MPA)		x
8	Pakleni otoci	anchoring	x
9	Vis		x
10	Hvar-Šćedro		x
11	Korčula-jug		x
12	Lastovo (MPA)		x
13	Mljet (MPA)		x
14	Šipan		x
15	Lokrum		x
16	Lošinj-jug		x
17	Pag-zapad		x
18	Dugi otok-Veli rat		x
19	Žut		x
20	Dugi otok-sredina		x
21	Prvić		
22	Šolta-zapad		x
23	Brač-jugozapad		x
24	Brač-sjever		
25	Hvar-sjever		x
26	Hvar-jug		x
27	Korčula-sjever		
28	Lastovo-lastovnjaci		x
29	Pelješac-Žuljana		
30	Mljet-sjever		x
31	Makarska		
32	Brač-jugoistok		
33	Biševo		x
34	Murter-jug		
35	Cres-Zeča		x
36	Unije		x
37	Pag		
38	Vir		x
39	Molat		

40	Ugljan	urbanization	x
41	Zlarin	urbanization	
42	Rogoznica	urbanization	
43	Split (Žnjan)	urbanization	
44	Baška voda	urbanization	
45	Korčula		
46	Pelješac (Marčuleti)		x
47	Lastovo (Skrivena luka)		x
48	Elafiti (Koločep)		x
49	Cavtat	urbanization	x
50	Molunat		x

Figure 8. Tentative positions of proposed sites (see text).

The positions marked in Figure 8 are only tentative. The exact position will be determined during the first round of monitoring activities and based on additional data which will become available in the meantime. Kmz files are considered an integral part of this document.

Data forms

- Forms as sheet tables, check lists etc.
- Have to be user-friendly and contain **clear data structure** – no latitude making misinterpretation possible and minimum of free text fields
- Communication with SINP needed because of the structure of actual and prepared official databases (CroHabitat etc.) and the forms prescribed

Suggested forms for data collection and remarks for their filling out are reported in Annexes I and II.

Scientific research

Objectives

- Clarification why also scientific research is chosen as a part of species surveillance (if not the whole chapter "Scientific research" has to be deleted) and what outputs are awaited

Most of the knowledge on biological and ecological features of *Posidonia oceanica* is based on data obtained from NW Mediterranean meadows and most of national and regional monitoring programmes refers to this sector of the basin (Lopez y Royo *et al*., 2007). Moreover, some indicators here suggested (density, Conservation Index) for defining the status of *Posidonia oceanica* meadows have been defined at NW Mediterranean level (Pergent *et al*., 1995; Moreno *et al*., 2001; Buia *et al*., 2004; Pergent-Martini *et al*., 2005; Montefalcone *et al*., 2006; 2008; UNEP-RAC/SPA, 2011). Because of the paucity of historical data on distribution, ecological traits and status of *Posidonia oceanica* meadows in the Croatian Adriatic Sea, scientific research is needed to obtain baseline data (although baseline data exist for some sporadic areas).

The outputs expected by the research should clarify if descriptors and classification methods commonly used for NW Mediterranean meadows (Pergent *et al*., 1995; Moreno *et al*., 2001; Buia *et al*., 2004; Pergent-Martini *et al*., 2005; Montefalcone *et al*., 2006; 2008; UNEP-RAC/SPA, 2011) are suitable for Croatian meadows, or whether others should be considered. This part of research should take into account the spatial variability of main descriptors (e.g. shoot density) as well as the presence of different environmental conditions along the Adriatic coast (e.g. climatic differences at different latitude, exposure, hydrodynamics, topography, substrate type) and various anthropogenic impacts.

Additional research should be devoted to assess the potential influence of alien species (e.g. *C. racemosa* and *Womerseleyella setacea*) on the meadows.

Further research should also be addressed to test the effectiveness of videography as additional method for assessing the conservation status of *Posidonia oceanica* meadows. Videography is commonly used worldwide as ground truth tool for acoustic, aerial and satellite mapping; it is advocated to be suitable for mapping and monitoring of *Posidonia oceanica* meadows in the Croatian Adriatic because of its precision, high statistical power and cost-effectiveness in detecting meadow cover (Schultz 2008; Puhr *et al*., 2014). However, although videography is able to identify position, depth and type of meadow limits, it is not able to evaluate density (number of shoot per m^2) that scientific community throughout the Mediterranean considers to be the most important descriptor of vitality and dynamics of the *Posidonia oceanica* meadows (Pergent-Martini *et al*., 2005) and the variable most commonly used in the Mediterranean monitoring systems (Lopez y Royo *et al*., 2007; UNEP-RAC/SPA, 2011). Moreover, the capability of videography to distinguish substrate types in different conditions should be explored in order to avoid uncertain assessment of meadow cover (and Conservation Index) due to misidentification of dead *matte* whenever it is covered with sediment; in fact, dead *matte* covered with sediment is very common in Croatian meadows (Nikolić V., personal communication) and increased sedimentation is one of the main reasons for reduction of *Posidonia oceanica* cover. Aslo, videography could provide misleading information in case of unvegetated areas masked by leaf litter or dense leaf canopy.

Below, suggestions for implementing videographic method are reported, if pilot projects will be chosen for testing the effectiveness of videography for the reliable monitoring of the conservation status of *Posidonia oceanica* meadows and its consistency with the direct, traditional SCUBA-based protocol here proposed.

The main advantages of videographic method are (1) the ability to sample large areas in a short time (Puhr et al., 2014); (2) the production of a permanent high resolution photographic record of the site; (3) the low cost of field labour; (4) the ability to observe and identify bottom habitat with none of the fatigue or discomfort (Ardizzone, 1991; Ardizzone et al., 2006); (5) the ability to conduct quantitative image analysis to derive habitat variables with high precision (Puhr et al. 2014); and (6) sufficient statistical power to detect small changes in seagrass cover at individual sampling sites (Schultz, 2008; Puhr et al. 2014). The ability of videography to detect small changes within sites allows the design of BACI studies to quantify the magnitude of anthropogenic disturbance.

The field protocol of DGPS-assisted videography for *Posidonia oceanica* monitoring in Croatia is a hybrid of the methods used by Ardizzone et al., (2006), Norris et al. (1997), and Gaeckle et al. (2008). Two video cameras are attached to a towfish. One camera (Multi SeaCam, DeepSea Power and Light) sends an analog color video signal via cable directly to an onboard video overlay box that overlays DGPS position, depth of the sea bottom, and GPS time onto the analog video display. The analog signal from the video overlay box is sent to a SIIG analog-digital converter to a laptop, where a video capture programme displays the video image real time on the monitor and records it into an AVI file. The second camera is a high density (HD) color video camera (GoPro Hero 3) that records another image of the benthos simultaneously, but does not send the video signal to the boat. The GoPro video is synchronized with the GPS by recording the GPS time from a GPS datalogger onto the video. The DGPS must be high (submeter) accuracy real-time, as for example any Trimble DGPS device including the Trimble Pro-XRS or later. The DGPS (using EGNOS satellite) records the differential position of the boat every one second into a downloadable file in the datalogger.

The towfish can be any design that is streamlined and a minimum of 20 kg in weight. Attached are the two cameras, and two laser pointers directing two parallel beams of light to the sea bottom. The towfish is raised and lowered by a suitable winch or pulley system.

On the boat are three persons: driver, navigator, and towfish operator. In case of combined expertise, the driver can also be the navigator, and a minimum of two persons are necessary. The driver follows a pre-planned trajectory provided by the navigator and displayed either on the GPS datalogger screen, or on the display of a second laptop. The towfish operator maintains the towfish at the target distance above the sea bottom by constantly adjusting the line with a winch or pulley system while monitoring the video display.

Within-site sampling. Each sampling session comprises a minimum of 3 hours in the field. Standard sampling sites are those requiring only 3 hours of field time. Priority sampling sites are those requiring up to 12 hours sampling time. For a standard site, six video transects are taken of the site. These transects are of two kinds: 1) parallel and 2) perpendicular to the isobaths. Each parallel transect is one kilometer long and at one of three depths: 20 m, 15 m, and 10 m. The perpendicular transects run from 1 m to 30 m depth or until *Posidonia* is no longer seen. The number of perpendicular transects is variable, but number at least three per sampling site.

At a speed of 0.5 meter per second, this method produces over 4 km of video transects during a 3-hr field session. Because of the high sample size per site, video transects can be chosen randomly each monitoring event, rather than revisited, while still providing sufficient statistical power to detect a decline in cover of 10% (Schultz 2008). However, it is advised that previous transects are revisited as closely as possible in order to increase power by providing some control over spatial variation in cover (Schultz, 2008). Transects are revisited by the driver while reading a GPS compass and observing the real-time positional track superimposed on the previous monitoring track.

Image analysis. The video data is converted into one still frame per second, with each frame scored with DGPS coordinates, depth, speed, and GPS time. All images can be permanent stored, and will be made available in perpetuity to interested parties for study and analysis.

All images will be subjected to digital texture analysis which identifies *Posidonia oceanica* presence/absence with high accuracy. Images with *Posidonia* present will be further digitally analyzed using *k*-means clustering on pixel intensities in the green band to obtain cover estimates.

A subset of images will be chosen for human analysis using random sampling, stratified by depth. The size of this subset can vary, depending on the number of person-hours available for image analysis. These images will be scored for cover of *Posidonia*, dead *matte*, type of margin, and any other visually identifiable information of conservation importance, such as presence of *Pinna nobilis* or other macrofauna of conservation interest, evidence of specific kinds of disturbances affecting *Posidonia* cover, epiphytic cover, grazing damage. This information will be entered into a spreadsheet whose rows are number of seconds into the video, and whose columns are DGPS coordinates, GPS time, depth, and digital cover of *Posidonia*. The intraclass correlation coefficient (ICC) is calculated between the human and machine scores to demonstrate the accuracy of the latter.

Conservation status. The following variables will be used as indicators of *Posidonia oceanica* conservation status. Note that items 7-8 and the standard error for items 1-3 can be measured only by videography.

1. Depth of the lower limit of *Posidonia* meadow. Videography allows a robust measure of the lower limit as the intercept of a regression of depth on seagrass cover. This is achieved in a regression of the depth of each image on the *Posidonia* cover as expressed on that image.
2. Depth of upper limit of *Posidonia* meadow. This descriptor is also highly variable in space, and will be measured as mean (±SE) of the upper limit depth as indicated by the perpendicular transects.
3. Relationship between depth and *Posidonia* cover. A fundamental parameter of every seagrass meadow is the rate at which cover declines as depth increases. Videography allows calculation of this rate as the slope of the regression of cover with depth, where each independent observation is derived from a different image as above.
4. Type of lower limit. This is directly observed on the images. The type may vary spatially, and if so then the type of lower limit is presented as a table of frequencies of each type.
5. Type of upper limit. Same comments as for 4.
6. Mean cover of *Posidonia* at the sampled site, across all transects.
7. Spatial autocovariance and autocorrelation of cover at the sampled site, across all transects. How cover varies across space is as important as the mean cover. Spatial autocorrelation and autocovariance are measures of the spatial scale at which cover varies (along and across depth isobaths), and allow an estimate of the degree of fragmentation of the meadow, and the scale of fragmentation, and may provide critical information concerning identity and frequency of anthropogenic processes causing meadow disturbance.
8. The mean cover of dead *matte* and its spatial autocorrelation and autocovariance.
9. The relationship between dead *matte* cover and depth, calculated as the regression slope.

Trend estimation. Trend is the net change in any descriptor from one monitoring event to another monitoring event. There are two sampling designs and therefore two fundamental methods for defining trend. These are the random-plots and the fixed-plots designs. In the random-plots method, each transect is chosen independently of transects followed the previous monitoring event. In the fixed-plots method, the monitoring team revisits all transects from the previous year using real-time submeter-accuracy DGPS navigation. Note that, because of the very large sampling intensity of videography, this method does not require a fixed-plots method (Schultz, 2008); however, a fixed-plots method will increase statistical power and simplify the statistical analysis.

<u>Random plots method</u>. In this sampling design, transects are chosen independently of those the previous monitoring event. Each monitoring event new random coordinates are drawn representing the starting position, and transects are situated relative to that starting position. In this method, the overall mean for the descriptor is calculated each monitoring event, and trend is measured simply by the slope of the linear regression with monitoring time. If there are only two monitoring times to be compared, then the estimated trend is the overall difference in the sample mean of the descriptor between the two monitoring times.

The trend calculated as above, however, may not be real, but may be an artefact of sampling or measurement error. To test for significance of a linear trend across several monitoring events, the significance probability of the linear regression is calculated. To test the significance of a sample mean difference between two monitoring times, the two-sample *t*-test or its non-parametric equivalent is used.

<u>Fixed-plots method</u>. In this sampling design, the same fixed transects are re-navigated each monitoring event. Note that these transects do not need to be re-navigated exactly, and any error in renavigation will have the effect of introducing sampling error that is similar in magnitude to that in the random-plots method (Schultz, 2008). Because of the potential for reducing spatial sources of error, the fixed-plots sampling method is strongly advised.

In this design, the mean seagrass cover for each transect is calculated at each monitoring event. Then for each transect, the slope of the linear regression against time is calculated. This produces a sample of slopes. Across all transects, the mean slope and its standard error and confidence interval are calculated. If the confidence interval does not span zero, then we conclude that there has been a significant trend, and its estimate is provided by the mean slope and its confidence interval.

<u>Precision and statistical power of the descriptors</u>. During a typical 6-hour field day, the videography method can detect a 5-10% decline in seagrass cover within any sampled site defined as a segment of shoreline 1 km in length, as shown by both a theoretical model (Schultz, 2008) and a field study in a *Posidonia oceanica* meadow in Croatia (Puhr et al. 2014). Variance components in *Posidonia* cover measured in the field (Puhr, et al. 2014) indicate a very low standard deviation in mean cover per site, approximately 0.1 or lower. Thus to detect an overall decline of 0.1 across all sampling sites in Croatia at a statistical power of 0.8 in a paired design, a sample size of just 10 randomly chosen sampling sites is necessary. We recommend however, at least 20 sites per monitoring event across Croatia for the any method, because a sample size of less than 20 would generally not be a publishable study. An annual sample size of 20 sites would yield a statistical power of 0.99 of detecting a decline in cover of 0.1, at an alpha probability of 0.05. Thus, the videography method is a very powerful monitoring method capable of detecting small changes in *Posidonia* cover as required under the HD.

<u>Framework assignment</u>

- Basic instructions with a hypothesis to be tested
- Proposal of academic institutions or other subjects able to do the research

In order to evaluate the effects of anthropogenic activities on meadows supposed to be under pressure (i.e. impacted), reference areas that are representative of the same type of environment to be monitored, should be selected (Benedetti-Cecchi *et al*., 2004). These meadows have environmental characteristics similar to impacted ones (e.g. substrate, depth, exposure), but they are not subject to the impact that has to be evaluated (e.g. in the case of recreational boating, meadows located in areas where navigation and anchoring are interdicted or, however, negligible). If it is possible identify an impact occurring within a defined time period, the BACI approach (Before/After-Control/Impact, Underwood, 1992; 1993; Benedetti-Cecchi *et al*., 2004; Montefalcone *et al*., 2008) is recommended.

List of academic institutions with competence to conduct the research above:

- Institute for Oceanography and Fisheries, Split
- Rudjer Boskovic Institute, Center for Marine Research, Rovinj
- University of Zagreb, Faculty of Science, Department of Biology
- University of Zadar, Maritime Department

THE WAY OF EVALUATION NEEDED FOR ALL FOUR COMPONENTS OF THE CONSERVATION STATUS IS DESCRIBED:

Evaluation of the conservation status components

Range

- description of the data use and interpretation for preparation of the **distribution map** (map will be prepared for all biogeographical regions together)
- description of the data use and interpretation to filling in 10 x 10 km **grid cells** (map will be prepared for all biogeographical regions together)
- proposal of rules for **gap closure** to join grids together where appropriate according to the environmental parameters

Range should be calculated based on the map of the actual distribution. Because of the lack of precise maps coming from complete surveys, the surface area of range could be estimate based on partial data with some extrapolation and/or modelling or on expert opinion with no or minimal sampling (Evans & Arvela, 2011).

Distribution data will be provided as presence on a 10 x 10 km grid; polygons created by adjacent cells will define the outer limits of the overall area in which a habitat type occurs, namely the range (Evans & Arvela, 2011). Major discontinuities due to natural factors (e.g. for *P. oceanica*, terrestrial areas and areas over 30 m depth) should be excluded to the range (Evans & Arvela, 2011).

Discontinuity in the range will be calculated considering the recommended gap distance of 40 km between distribution cells or polygons (Evans & Arvela, 2011).

The monitoring sites are scattered throughout the Mediterranean biogeographic region of Croatia and cover almost the whole range of the habitat. At each monitoring event, presence/absence of the habitat should be recorded and projected on the map grid in order to estimate changes of the whole range over time.

Area covered by habitat type

- description of the data use and interpretation to determination of:
- the **surface area** incl. methodology of analysis
- the **distribution pattern,** if possible

Because of the lack of precise maps coming from complete surveys, the surface (in km^2) area covered by the habitat type 1120 could be estimated on the base of partial data with some extrapolation and/or modelling or on expert opinion with no or minimal sampling (Evans & Arvela, 2011). Reliable data for determination of the surface area and the distribution pattern of *P. oceanica* meadows should come from mapping (see previous section "Field mapping"). A real assessment of the area covered by habitat type would only be possible after mapping in higher resolution, e.g. with minimum mapped surface area ranging from 1 to 25 m^2 as suggested by UNEP-RAC/SPA (2011).

Specific structures and functions

- description of the data use and interpretation to determine the **conditions** and status of **typical species**

As previous reported *Posidonia oceanica* is the only characteristic species of the association *Posidonietum oceanicae* (Funk 1927) Molinier 1958 (Giaccone *et al*., 1994; Evans & Arvela, 2011).

As there is no baseline for Croatia, standard thresholds of following descriptors are suggested to be used to get immediate information on conservation status of the meadows.

Lower limit depth. Due to its well-described relationship with water clarity (transparency and nutrient concentrations) and the relative ease with which it can be estimated precisely, colonisation depth is one of the best-known seagrass indicators of water quality (Pergent *et al*., 1995; Borum *et al*., 2004). In Table 2 a recent classification, that meet the requirements of the WFD (UNEP-RAC/SPA, 2009), is reported.

Table 2. Status of the meadow in function of lower limit depth (UNEP-RAC/SPA, 2011 modified).

	High	Good	Moderate	Poor	Bad
depth (m)	> 34.2	34.2 to 30.4	30.4 to 26.6	26.6 to 22.8	< 22.8

Type of the lower limit. The features of the lower limit may be indicative on the health status of the meadow. An interpretation (UNEP-RAC/SPA, 2009) is proposed in Table 3.

Table 3. Status of the meadow in function of lower limit depth (UNEP-RAC/SPA, 2011 modified).

	High	**Good**	**Moderate**	**Poor**	**Bad**
Lower limit	progressive	sharp (cover > 25%)	sharp (cover < 25%)	sparse	regressive

Cover at the lower limit. The percent cover of living *Posidonia oceanica* provide information on the health status of the meadow (Table 4, UNEP-RAC/SPA, 2009).

Table 4. Meaning of meadow cover at the lower limit (UNEP-RAC/SPA, 2011 modified).

	High	**Good**	**Moderate**	**Poor**	**Bad**
% cover at lower limit	> 35%	35% to 25%	25% to 15%	15% to 5%	< 5%

Shoot density. The number of shoots per m^2 is one of the most used descriptors to assess the ecosystem health (Pergent-Martini *et al*., 2005; UNEP-RAC/SPA, 2011) because it provides information on vitality and dynamic of the meadows, also revealing changes due to human influence (Pergent *et al*., 1995) when measured on a pluriannual time scale (Buia *et al*., 2004). Changes of shoot density over time may be early indicators of change or negative trends that are happening in the habitat type.

Since the meadow density is strongly affected by the depth, Pergent *et al*. (1995) identify four classes, which are a function of the theoretical average densities calculated for each depth, and that reflect the ecological conditions of the meadow (Buia *et al*., 2004).

Recently this classification has been updated and adapted to *P. oceanica* meadows of Southern and Eastern countries of the Mediterranean (Algeria, Tunisia, Libya, Turkey) with the creation of five classes (bad, poor, moderate, good and high, Table 5) that meet the requirements of the WFD (UNEP-RAC/SPA, 2009). This classification is suggested for the interpretation of monitoring data (UNEP-RAC/SPA, 2011).

Table 5. Classification of *P. oceanica* meadows according to shoot density and depth (UNEP-RAC/SPA, 2011 modified).

depth (m)	High	Good	Moderate	Poor	Bad
1	> 1133	1133 to 930	930 to 727	727 to 524	< 524
2	> 1067	1067 to 863	863 to 659	659 to 456	< 456
3	> 1005	1005 to 808	808 to 612	612 to 415	< 415
4	> 947	947 to 757	757 to 567	567 to 377	< 377
5	> 892	892 to 709	709 to 526	526 to 343	< 343
6	> 841	841 to 665	665 to 489	489 to 312	< 312
7	> 792	792 to 623	623 to 454	454 to 284	< 284
8	> 746	746 to 584	584 to 421	421 to 259	< 259
9	> 703	703 to 547	547 to 391	391 to 235	< 235
10	> 662	662 to 513	513 to 364	364 to 214	< 214
11	> 624	624 to 481	481 to 338	338 to 195	< 195
12	> 588	588 to 451	451 to 314	314 to 177	< 177
13	> 554	554 to 423	423 to 292	292 to 161	< 161
14	> 522	522 to 397	397 to 272	272 to 147	< 147
15	> 492	492 to 372	372 to 253	253 to 134	< 134
16	> 463	463 to 349	349 to 236	236 to 122	< 122
17	> 436	436 to 328	328 to 219	219 to 111	< 111
18	> 411	411 to 308	308 to 204	204 to 101	< 101
19	> 387	387 to 289	289 to 190	190 to 92	< 92
20	> 365	365 to 271	271 to 177	177 to 83	< 83
21	> 344	344 to 255	255 to 165	165 to 76	< 76
22	> 324	324 to 239	239 to 154	154 to 69	< 69
23	> 305	305 to 224	224 to 144	144 to 63	< 63
24	> 288	288 to 211	211 to 134	134 to 57	< 57
25	> 271	271 to 198	198 to 125	125 to 52	< 52
26	> 255	255 to 186	186 to 117	117 to 47	< 47
27	> 240	240 to 175	175 to 109	109 to 43	< 43
28	> 227	227 to 164	164 to 102	102 to 39	< 39
29	> 213	213 to 154	154 to 95	95 to 36	< 36
30	> 201	201 to 145	145 to 89	89 to 32	< 32
31	> 189	189 to 136	136 to 83	83 to 30	< 30
32	> 179	179 to 128	128 to 77	77 to 27	< 27
33	> 168	168 to 120	120 to 72	72 to 24	< 24
34	> 158	158 to 113	113 to 68	68 to 22	< 22
35	> 149	149 to 106	106 to 63	< 63	
36	> 141	141 to 100	100 to 59	< 59	
37	> 133	133 to 94	94 to 55	< 55	
38	> 125	125 to 88	88 to 52	< 52	
39	> 118	118 to 83	83 to 48	< 48	
40	> 111	111 to 78	78 to 45	< 45	

<u>Conservation Index</u>. The coverage is the surface of seabed, expressed as a percentage, covered with live plants of *P. 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 allows to calculate, for each LIT, the conservation index (CI) of the meadows (Moreno *et al*., 2001; Montefalcone *et al*., 2006); CI 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 *P. oceanica* and is expressed by the formula

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

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

Based on the values of CI, meadows can be classified according to the criterion proposed by Montefalcone (2009) that follow the WFD requirements:

1 - CI < 0.3: bad conservation status;

2 - CI between 0.3 and 0.5 excluded: poor conservation status;

3 - CI between 0.5 and 0.7 excluded: moderate conservation status;

4 - CI between 0.7 and 0.9 excluded: good conservation status;

5 - CI > 0.9: high conservation status.

However, the method proposed by Moreno *et al*. (2001) and reviewed by Montefalcone *et al*. (2006) is suggested for classifying CI values at a local or regional scale:

1 - advanced degree of regression: CI < (x – ½ s);

2 - impacted meadow: (x – ½ s) ≤ CI < x;

3 - low-to-moderate conservation status: x ≤ CI < (x + ½ s);

4 - high state of conservation: CI > (x + ½ s)

where the mean (x) and the standard deviation (s) are calculated for each depth taking into account CI values of all the transects at a local or regional scale.

Since the presence of dead *matte* may be of natural origin (Boudouresque *et al*., 2006), the conservation status of *P. 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. Trend is one of the most important components for the assessment of conservations status (Evans & Arvela, 2011). For the density, due to its small seasonal variability, an abrupt decrease (> 20%) in the number of shoots clearly indicates a decline of the meadow conditions (Borum et al., 2004), regardless of the variability due to natural patchiness of seagrass growth (Díaz-Almela & Duarte, 2008). For all the descriptors for which an interpretation scale is available (i.e. lower limit depth, type and cover, shoot density and Conservation Index), the shift to a lower level class can be considered as an indicator of decline, and vice versa.

Additional information can be acquired by detecting some species that are relevant for conservation purpose (i.e. *Pinna nobilis*) or for potential detrimental effects on the meadows (alien species). Presence of *P. nobilis* is a characteristic of healthy *P. oceanica* meadows (Borum *et al*., 2004). A gross assessment of its density and the conservation status of populations can be obtained by counting the number of individuals along the four transects (LITs) of each stations and evaluating their status (dead or alive). To assess the state of preservation of the habitat type, reference value of *P. nobilis* abundance could be obtained by comparing data coming from other Mediterranean meadows that are considered in favourable state of conservation according to HD. However, a single observation is just the baseline for the assessment of conservation status; only by comparing changes in habitat data throughout the time it is possible to describe changes in conditions. For an unfavourable assessment of the situation, changes in the abundance of the typical species should be detected in at least two consecutive measurements. However, it should be considered that usually *P. nobilis* has a patchy distribution (Richardson et al., 2004; Katsanevakis, 2005) and that a proper surveyed area size should be selected (Coppa et al., 2010); a surface of only 160 m^2 could not be enough for an accurate knowledge of abundance and distribution of individuals, and data coming from such a survey should be considered just for a preliminary assessment of species range and potential threats.

Degradation process within this habitat type is indicated by the increasing abundance of alien species, such as *C. racemosa*. Again, by comparing changes in habitat data throughout the time, it is easy to describe negative trends in habitat conditions.

Future prospects

- description of the information use and interpretation to determination of:
- main **pressures** and **threats** and their value
- **conservation measures** and other positive provisions realized to avoid pressures and threats

Future prospects should be evaluated by considering the future trends and likely future status of range, area and structure and functions; future trends of habitats are dependent on pressures and threats (negative influence) and conservation policies (positive influence) (Evans & Arvela, 2011).

Based on existing baseline of main pressures along Croatian coasts (VV.AA., 2011) and further sources of information on their magnitude on a local scale, the relative importance of pressures and threats should be ranked as:

- High: important direct or immediate influence and/or acting over large areas;
- Medium: medium direct or immediate influence, mainly indirect influence and/or acting over moderate part of the area/acting only regionally;
- Low: low direct or immediate influence, indirect influence and/or acting over small part of the area/acting only regionally (Evans & Arvela, 2011).

Expert judgments should be used where real data from sites/occurrences or other data sources are not available (Evans & Arvela, 2011). Only threats that have a reasonable chance of happening are to be considered (Evans & Arvela, 2011).

A number of threats of high or medium importance indicates that the future trend will likely be decreasing; threats of low importance or no threats indicate stable or even increasing future trend (Evans & Arvela, 2011).

Changes in community structure and habitat quality over time will provide quantification of the effects of main pressures and information on the trend of the conservation status of the meadow. An evaluation matrix for future prospects is available in Evans & Arvela (2011).

Evaluating the effects of anthropogenic activities on the meadows is crucial to ensure proper management and conservation measures. Assuming that anthropogenic activities affect *P. oceanica* meadows, differences must be found between areas characterized by those activities and similar reference areas not exposed to the particular disturbance under investigation (Benedetti-Cecchi *et al*., 2004).

The BACI approach (Before/After-Control/Impact, Underwood, 1992; 1993; Benedetti-Cecchi *et al*., 2004; Montefalcone *et al*., 2008) is recommended, whenever it is possible identify impacts occurring within a defined time period, to evaluate the effects of anthropogenic disturbances and identify prevention and conservation measures. BACI studies are also suggested to assess and maximize the effects of management measures.

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Annexes

Annex I. Field sheet for detecting the number of leaf shoots of *Posidonia oceanica* and additional information at each sampling station

Annex II. Field sheet for detecting the percent coverage of *Posidonia oceanica*, substrates and other species and features at each sampling station.

Annex I. Field sheet for detecting the number of leaf shoots of *Posidonia oceanica* and additional information at each sampling station (in blue it is reported as tables should be filled)

***Posidonion oceanicae* (1120)**		**Page: ___ of ___**
Site: name or code **Zone:** number or code **Sampling station:** number or code **Depth:** shallow (<10 m), intermediate (~ 15 m), deep (lower limit) **Geographical position (GPS):** position of the buoy (HTRS, or at least mark which coordinate system is used)		**Date:** **Coordinator:** name and surname **Operators:** name and surname
Evident potential pressures in the area: e.g. anchoring, wastewater, camping area, recreational beaches, mooring sites for small boats	**Upper limit depth:** m **position(GPS):** coordinates	**Lower limit depth:** m **type:** category **position(GPS):** coordinates

DENSITY

Size of Unit Sampling (US): quadrat 40 x 40 cm

Replicate	no. of shoots	depth (m)	notes
1	55	7.3	- for each replicate record depth with diving computer - additional information (e.g. presence of flowering, mucilage, overgrazing)
2	67	7.1	
3	50	7.8	
4	15	7.9	
5	60	8.0	
6	…	7.8	
7	…	…	
8	…	…	
9	…	…	
10	…	…	

Meadow type: e.g. flat/step, continuous/discontinuous, terraced beds, belts, stripes, patches, hills

Main substrate: mud/sand, *matte*, rock/coralligenous formations, mixed

Other seagrasses: yes/no, which (e.g. *Cymodocea nodosa, Zostera noltii*)

Presence of *Pinna nobilis*: yes (number)/no

Presence of alien species: yes/no, which (e.g. *Caulerpa racemosa, Womersleyella setacea*)

Evidence of mechanical pressures: yes/no, which (e.g. mooring systems, concrete blocks, pier, chains, ropes)

Signs of impact: yes/no, which (e.g. detached shoots, detached plates of *matte*, damages of trawling/anchoring)

Annex II. Field sheet for detecting the percent coverage of *Posidonia oceanica*, substrates and other species and features at each sampling station (in blue it is reported as tables should be filled)

***Posidonion oceanicae* (1120)** **Page: ___ of ___**

Site: name or code **Zone:** number or code **Sampling station:** number or code **Depth:** shallow (<10 m), intermediate (~ 15 m), deep (lower limit) **Geographical position (GPS):** position of the buoy (HTRS, or at least mark which coordinate system is used)	**Date:** **Coordinator:** name and surname **Operators:** name and surname

% COVERAGE

Size of Unit Sampling (US): transect 10 m

Replicate		**1**			**2**			**3**			**4**	
starting depth (m)		…			…			…			…	
final depth (m)		…			…			…			…	
	C	**i**	**O**	**C**	**i**	**O**	**C**	**i**	**O**	**C**	**i**	**O**
C = categories P = Posidonia D = Dead *matte* M = Mud Sa = Sand St = Stones/Pebbles R = Rock	M	20										
	P	75										
	D	195										
	S	220										
	D	475										
	P	510										
	D	530										
	S	610										
	P	710										
	S	730										
i = intercept value in cm corresponding to each point of discontinuity encountered along the transect (to be recorded in correspondence of each category)	D	830										
	P	870										
	D	940										
	St	970										
	D	1000										
O = other Pna = *Pinna nobilis* alive Pnd = dead *Pinna nobilis* Cr = *Caulerpa racemosa* Ct = *Caulerpa taxifolia* Ct = *Caulerpa prolifera* Cn = *Cymodocea nodosa* O = Other species / features												