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AMARE: Guidelines and best practices for monitoring the risk of collapse of benthic ecosystems within MPAs

This document is directed to stakeholders involved in conservation of marine coastal biodiversity and in particular to managers of Marine Protected Areas

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Goals and Purpose

Regime shifts, abrupt changes in the structure and functioning of ecological systems are increasingly documented in coastal marine systems following the collapse of habitat-forming species such as macroalgal canopies and seagrasses. Improving the ability to prevent these transitions has profound implications for management and conservation of coastal marine ecosystems, at local, national and transnational level.

This document is intended to provide best practice guidance for estimating spatial indicators of ecosystems collapse at the scale of MPAs for two ecologically relevant habitats: meadows of *Posidonia oceanica* (L.) Delile and underwater forests dominated by brown algae of genus *Cystoseira* spp..

Introduction

Coastal ecosystems are increasingly exposed to anthropogenic and climate stressors that increase the probability of a catastrophic collapse (“regime shift”) (Levin and Mollmann 2014). Regime shifts are policy relevant phenomena because they directly affect wellbeing, human-livelihoods and ecosystem services, on which society relies (Rocha et al. 2014). Assessing the risk of regime shift is a key although challenging endeavor, since natural systems may show only little changes before the collapse.

Within WP 3.5 in the AMARe (Actions for Marine Protected Areas) project, we have developed a new approach to integrate experimental and observational data to model the risk of collapse of key ecosystems, including seagrass beds and macroalgal canopies. This framework is promising, but it relies on high-quality data over a long time-span, that, as it has emerged from AMARe, are scarcely available for Marine Protected Areas. To complement and integrate the modeling approach, we provide here a simple, low-cost field methodology that can be easily applied with MPAs. This approach involves the monitoring of changes in the spatial distribution of selected benthic species to predict the approach to an ecological threshold (Kefi et al. 2014). Spatial snapshots of the status of an ecosystem offer unique advantages; they do not require intense time series and may be often obtained by satellite images and by high-resolution spatial surveys (e.g. scuba diving).

How to use this document

This document provides a stepwise framework which may be used to design a monitoring programme to estimate the risk of collapse of relevant marine benthic ecosystems, from selection of sampling units to data management. Each section provides background information, best practice guidance and recommendations for each stage of the process. An overview of the document's structure and the stepwise framework is presented in Figure 1.

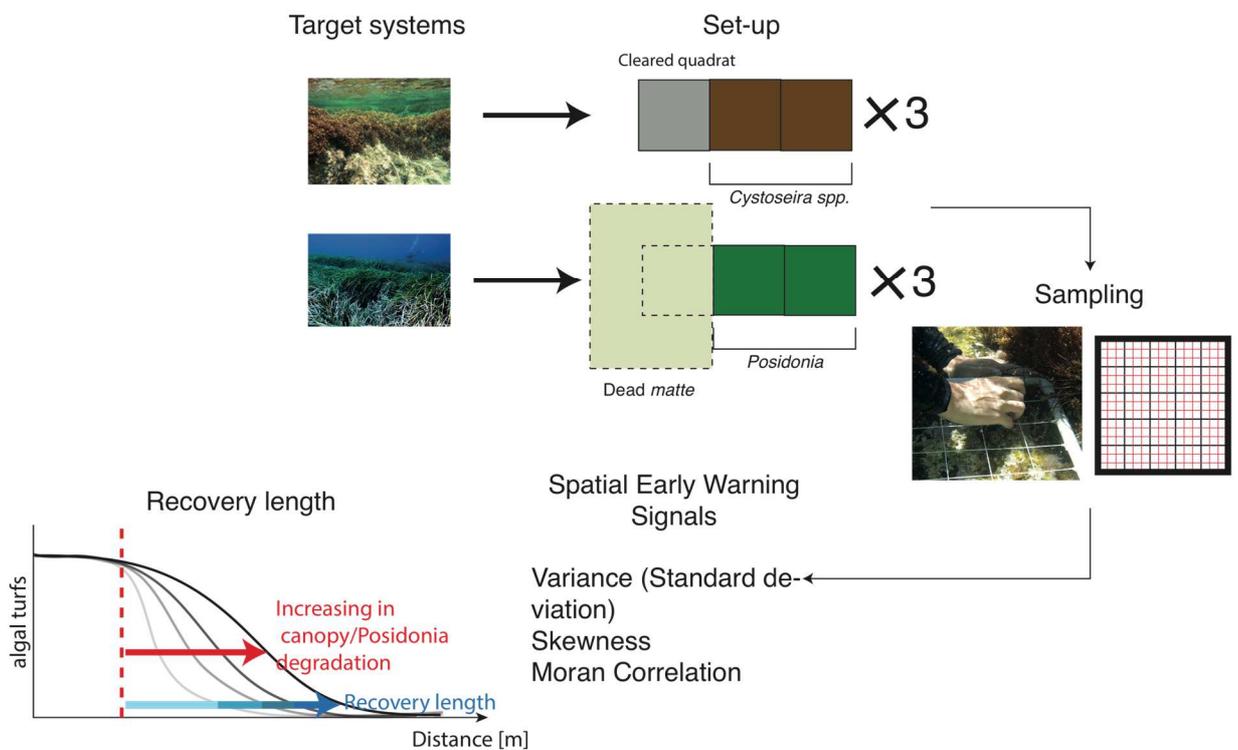


Fig. 1. Workflow illustrating the main steps for monitoring the risk of collapse of two target ecosystems: meadows of *Posidonia oceanica* (L.) Delile and underwater forest dominated by brown algae in the genus *Cystoseira*.

Background

Macroalgal forest in the genus *Cystoseira*.

Canopy forming macroalgae in the genus *Cystoseira* are among the most important foundation species in the Mediterranean Sea (Fig. 2A). These canopies create a complex habitat that maintains a rich understory assemblage of algae and invertebrates and are key for the maintenance of biodiversity and ecosystem functioning in coastal environments. In the last decade structurally complex canopy-dominated communities have been replaced by less complex assemblages dominated by turf-forming species or by barren habitats, due to the combined effect of climate change and anthropogenic pressure (Steneck et al. 2002). Repeated experiments, mimicking natural processes producing gaps in stands of *C. amentacea* such as storms and heat stress, have shown how canopy removal results in the invasion of algal turfs at the expenses of understory assemblages (Benedetti-Cecchi et al. 2015, Rindi et al. 2017). Once established, algal turfs may facilitate their own growth by preventing canopy recruitment and ameliorating physical stress through water retention at low tide as they develop into an intricate mat (Tamburello et al. 2013)(Fig. 2B).





Fig. 2. Alternative states in *Cystoseira amentacea*. Canopy-dominated state (*Cystoseira amentacea* var. *stricta* Bory). (A) Turf-dominated state (B).

***Posidonia oceanica* (L.) Delile**

Seagrasses are important components of coastal marine ecosystems and are involved in a wide range of ecosystem services, such as protection against wave action and coastal erosion, abatement of seawater pollution from human-originated bacteria and provision of a nursery habitat for commercially important fish and invertebrates (HilleRisLambers et al. 2001, Orth et al. 2006, Waycott et al. 2009). In the Mediterranean Sea, coastal areas are extensively populated by a key primary producer: the endemic seagrasses *Posidonia oceanica* (L.) Delile that can develop meadows extending from the surface to 40-50 m depth (Fig. 3A). Increasing water turbidity due to coastal urbanization has been recognized as one of the most important factor driving the collapse of *P. oceanica* and its transition into a degraded state constituted by sediment and dead roots (hereafter dead matte) (Ruiz and Romero 2003, Erftemeijer and Lewis 2006) (Fig. 3B).

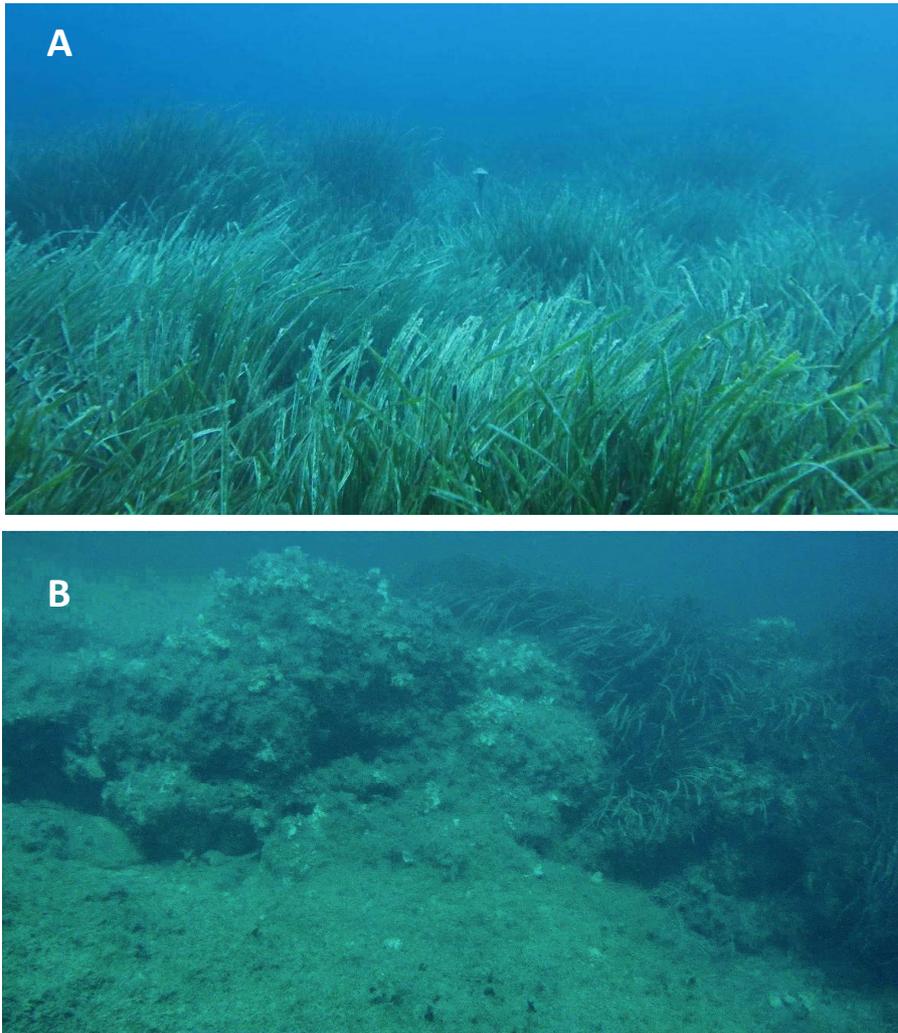


Fig. 3. Alternative states in *Posidonia oceanica*. *Posidonia oceanica* meadow (pristine state) (A). Dead *matte* (degraded state) area at margin with *Posidonia oceanica*. Dead *matte* is completely colonized by filamentous, coarsely branched, articulated coralline algae (B).

Early warning signals

The thresholds for regime shifts are often unknown until surprising shifts occur. Establishing measures of the distance to a threshold may allow anticipating an impending transition. Recent studies have proposed that changes in the spatial

distribution of species may signal the approach to a threshold and thus may be used as early warning indicators of regime shift (Kefi et al. 2014).

In this document, we introduce the application of spatial early warning signals of regime shift and we provide a step-by-step methodology for detecting and estimating EWS for two ecologically relevant ecosystems within MPAs. We first provide a brief overview of the main spatial EWSs. A detailed description of the indicators and theory is provided elsewhere (Kefi et al. 2014).

Indirect indicators

- **Spatial variance:** as an ecosystem approaches a threshold the amplitude of spatial fluctuation is expected to increase. This can cause spatial variance of the variable of the system (e.g. % cover of algal turf) to increase. Spatial variance can be estimated as standard deviation and coefficient of variation.

- **Spatial correlation:** close to a threshold, the neighboring spatial units are likely to become more similar to each other (they become increasingly correlated). Increasing spatial coherence can be quantified by the spatial correlation function, or Moran's I computed, for example, along transects.

- **Spatial skewness:** Spatial fluctuations can become increasingly asymmetric as the system approaches a critical threshold. This is because the fluctuations in the direction of the degraded state (e.g. algal turf or dead *matte*) take longer to return back to the non-degraded state than those in the opposite direction. This asymmetry can also arise due to local flickering events (i.e. occasional jumps of local units between their current and alternative state). The spatial asymmetry can be measured

by spatial skewness, which is the third central moment scaled by the standard deviation.

Recovery length

Recovery length, a novel spatial indicator, is defined as the spatial distance from a perturbation in space at which a population recovers (Dai et al. 2013). When approaching a critical threshold, an ecosystem is expected to become increasingly sensitive to perturbations. For instance, a forest of *Cystoseira* spp. approaching the threshold in canopy cover (~75% of canopy removed) should progressively become more susceptible to invasion by algal turf (Benedetti-Cecchi et al. 2015, Rindi et al. 2017). The larger is the spatial scale of propagation of algal turf, the closer the system is to the critical threshold. To estimate this indicator in a macroalgal forest, turf abundance would need to be measured in a range of adjacent areas at increasing distances from a *Cystoseira*/turf boundary. This information could then be mapped to reveal the recovery length (Fig. 4).

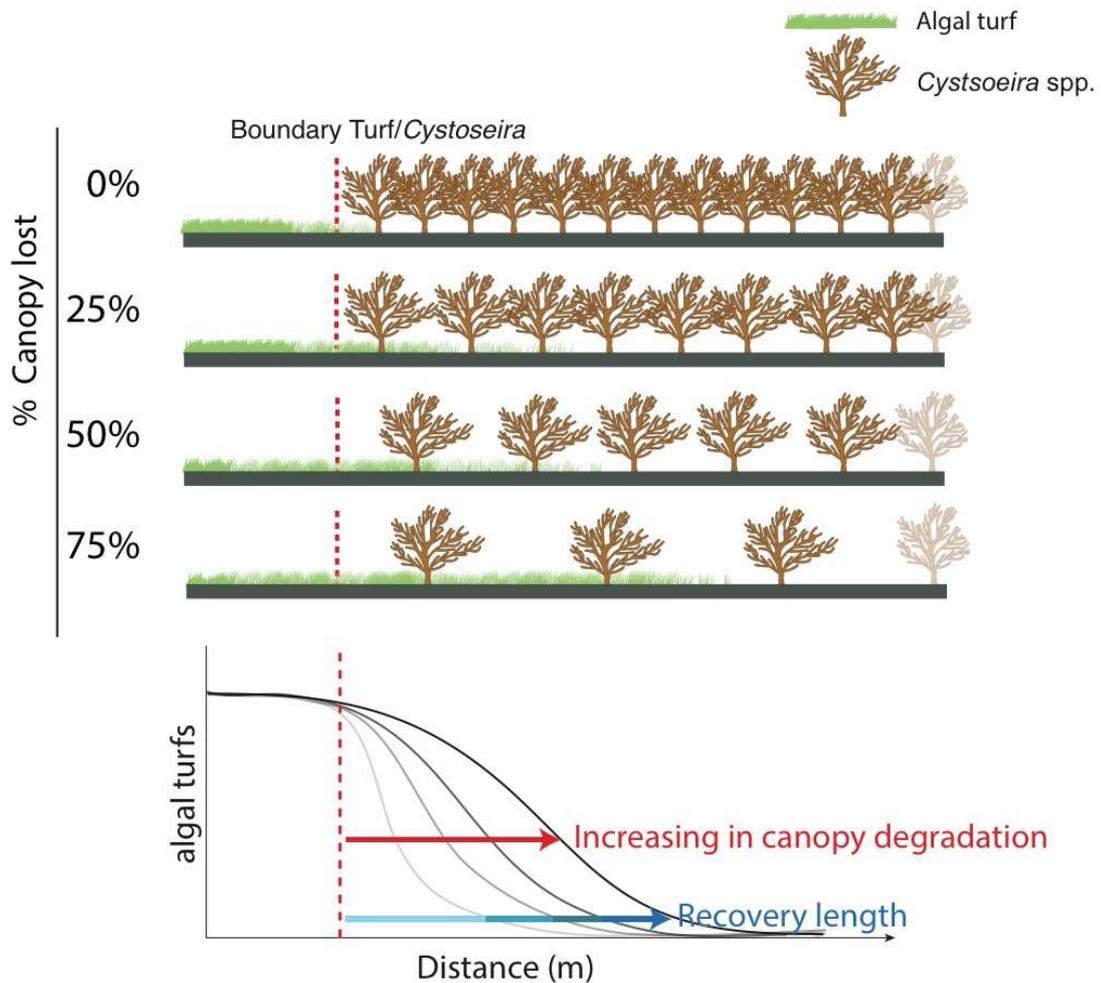


Fig. 4. Hypothetical propagation of algal turf for different levels of canopy degradation (expressed as canopy removed %). *Cystoseira* transect at different levels of degradation (from 0% to 75% of canopy removed, the latter corresponding approximately to the threshold in canopy cover) at the margin of an area colonized by algal turf. Algal turf is expected to propagate vegetatively from the surrounding area within the transect proportionally to the level of canopy degradation.

Setup

The guidance of each step is given separately for the two target-ecosystems: *Posidonia oceanica* (L.) Delile and macroalgal forests.

Posidonia oceanica (L.) Delile

The setup is carried out in an area colonized by *P. oceanica* at the margin with a dead *matte* area colonized by filamentous and turf-forming algae (Fig. 5). Three transects of 50x150 cm (3 contiguous quadrats of 50x50cm) are established at the boundary between *P. oceanica* and dead *matte*. The first quadrat of each transect must be placed within the dead *matte* and the remaining two quadrats are within the *P. oceanica* meadow (Fig. 5). Transects should be marked at each corner by means of metal sticks for future relocation.

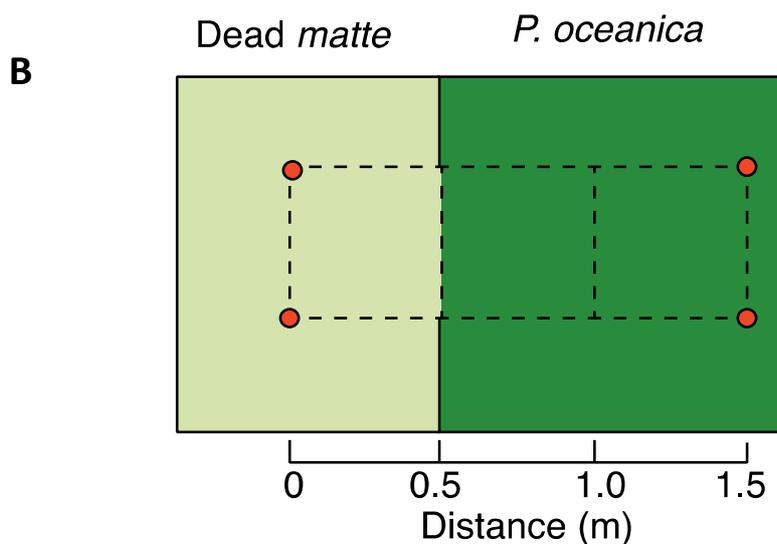
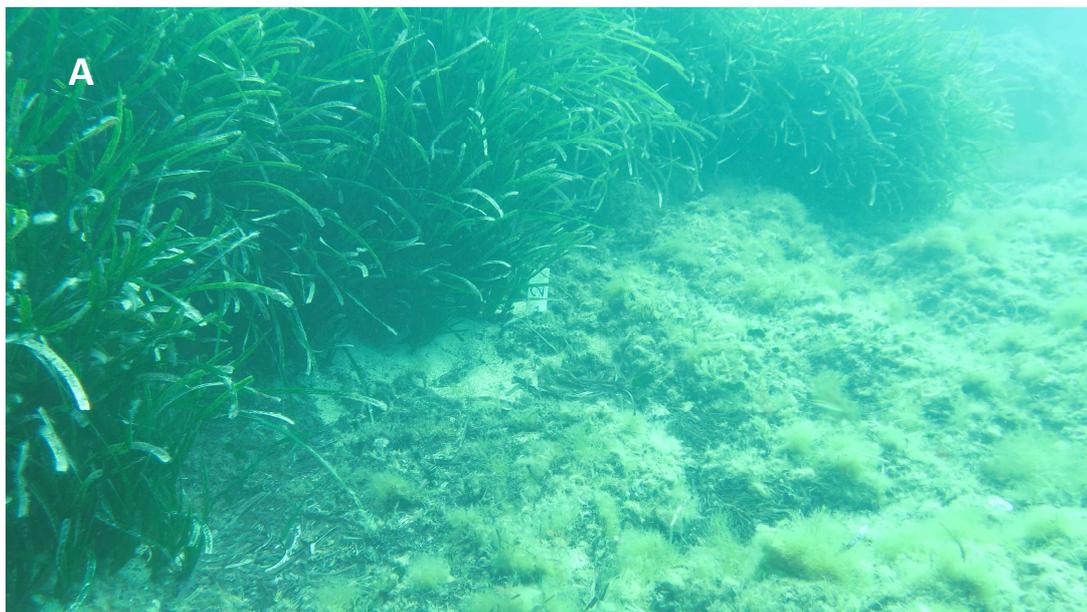


Fig. 5. Margin between *P. oceanica* and dead *matte* (A). Schematic representation of the transect. Red dots indicate markers (metal sticks or plastic tags).

Macroalgal forest

The setup is carried out in area dominated by canopy forming macroalgae in the genus *Cystoseira*. Three transects of 30x60 cm (2 contiguous quadrats of 30x30cm) are established in an area colonized by *Cystoseira* spp. A cleared quadrat of 30x30 cm, where all *Cystoseira* is completely removed (including the understory assemblage), is produced at one end of each transect (Fig. 6A). Fronds and holdfasts of *Cystoseira* spp. are removed by hammer and chisel (Fig. 6B). A 60 cm wide border from the boundary of *Cystoseira* forest must be left, to avoid possible edge effect. It has been shown that, 30x30cm (the size of the cleared patch) represent the minimum gap size that can be become successfully colonized by algal turf within one year (Tamburello et al. 2013). If algal turf fails to colonize the cleared patch, we recommend to increase the size of the cleared patch (and quadrats) to 50x50 cm. Transects should be interspersed and 10s of m apart and should be marked at each corner with epoxy putty (e.g., Veneziani, SUBCOAT A+B) for future relocation. We recommend, for all the duration of the study, to remove, from cleared patches, possible recruits and/or juveniles of *Cystoseira* that could prevent the propagation of algal turf.

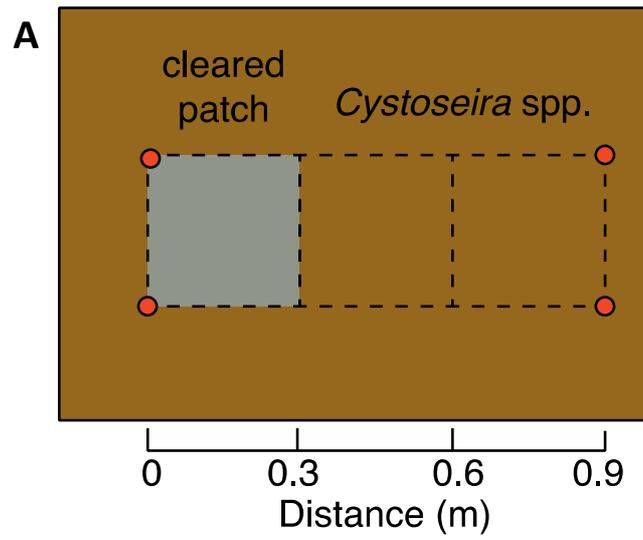


Fig. 6. Schematic representation of the transect. Red dots are markers (patches of epoxy putty) (A). An example of cleared patch produced in an intertidal belt of *Cystoseira amentacea* (B).

Sampling

The estimation of EWS and recovery length requires measurements of algal turf abundance at high spatial resolution for each transect.

***Posidonia oceanica* (L.) Delile**

Visual estimates of percentage cover are used to assess the abundance of algal turf in each of the 50 × 50 cm plots along the transects. The cleared patch and the two adjacent quadrats in a transect are sampled by dividing each of the 50 × 50 cm plots into a grid of 5 × 5 sub-quadrats of 10 × 10 cm, yielding 75 observations for each of the transects (Fig. 7 and 8). A score from 0 to 4% is given to each sub-quadrat depending on the abundance of algal turf: if absent; 1 if turf occupies 1/4 of the sub-quadrat; 2 if it occupies 2/4 of the sub-quadrat; 3 if it occupies 3/4 of the sub-quadrat; 4 if it occupies the entire sub-quadrat (Fig. 7).

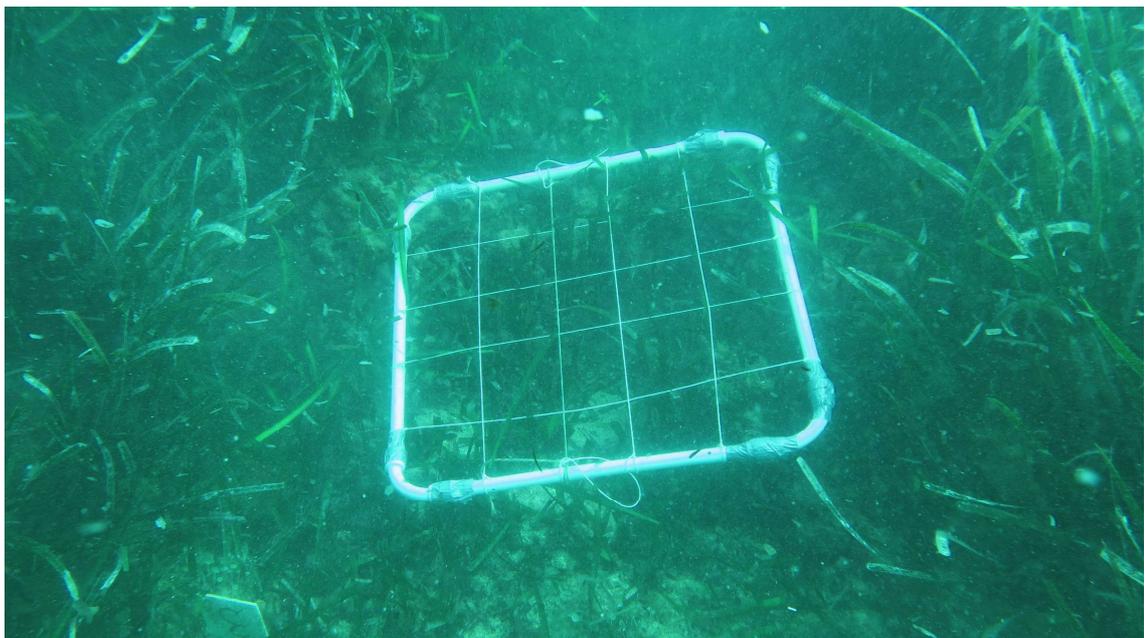


Fig. 7. Sampling device. 50x 50cm plastic frame divided into 25 10x10 cm sub-quadrats.

Taxa comprising algal turfs colonizing the dead-*matte* include: *Corallina elongata* J. Ellis et Solander, 1786 and *Dyctiota* spp., *Padina pavonica*, *Acetabularia acetabulum* (Linneaus) P.C. Silva, the coarsely branched algae *Laurencia* spp. J.V. Lamouroux,

1813. Agardh, 1817 and *Gastroclonium clavatum* (Roth) Ardissonne, 1883, and several species of filamentous algae (e.g. *Sphacelaria* spp. and red algae in the family *Ceramiales*). As an alternative to visual sampling, algal turf abundance can be estimated using photographic sampling. This consists of taking photographs in an area delimited by a frame. Percentage cover of algal turf can then be obtained later by projecting images onto a grid on a screen and computing cover as described earlier for the visual sampling.

Sampling should be repeated at least once in a year, preferably during the summer season.

Date:.....

Trasect n°:..... Long: Lat: Depth:

Quadrat 1 (Dead matte)					Quadrat 2 (Posidonia)					Quadrat 3 (Posidonia)				

Fig. 8. Filed data-sheet used to record algal turf cover in *Cystoseira* spp. transects. Percentage cover of algal turf must be reported for each sub-quadrat in the the transect.

Macroalgal forests

The propagation of algal turf within transects should be evaluated at least after 12 months from the production of the cleared patch. This period of time is needed to let algal turf to fully colonize cleared patches. Visual estimates of percentage cover are used to assess the percentage cover of the algal turf and the canopy in each of the 30 × 30 cm plots along the transects. The cleared patch and the two adjacent quadrats of the nearby transect are sampled by dividing each of the 30 × 30 cm plots into a grid of 5 × 5 sub-quadrats of 6 × 6 cm, yielding 75 observations for each of the transects with cleared patches (Fig. 9 and 10). A score from 0 to 4% is given to each sub-quadrat depending on turf cover: 0 if the turf is absent; 1 if it occupies 1/4 of the sub-quadrat; 2 if it occupies 2/4 of the sub-quadrat; 3 if it occupies 3/4 of the sub-quadrat; 4 if it occupies the entire sub-quadrat. The abundance of algal turf in the un-manipulated quadrats is assessed by moving the canopy of *Cystoseira* spp. aside.



Fig. 9. Sampling device. 30x30 cm plastic frame divided into 25 6x6 cm sub-quadrats.

Date:.....

Trasect n°:..... Long: Lat: Depth:

Cuadrado 1 (Cleared cuadrado)						Cuadrado 2 (Canopy)						Cuadrado 3 (Canopy)										

Fig. 10. Filed data-sheet used to record algal turf cover in *Cystoseira* spp. transects. Percentage cover of algal turf must be reported for each sub-quadrat of the transect.

Taxa belonging to algal turfs includes: *Corallina elongata* J. Ellis et Solander, 1786 and *Halimtilon virgatum* (Zanardini) Garbary and H.W. Johansen, 1982, the coarsely branched algae *Laurencia* spp. J.V. Lamouroux, 1813, *Chondria* spp. C. Agardh, 1817 and *Gastroclonium clavatum* (Roth) Ardissonne, 1883, and several species of filamentous algae (Benedetti-Cecchi et al. 2001). As an alternative to visual sampling, algal turf abundance can be estimated using photographic sampling as described for *P. oceanica*.

After sampling, we recommend to remove all recruits and juvenile stages of *Cystoseira* spp. from the cleared patch that could eventually prevent the vegetative propagation of algal turf within the transect. Sampling should be repeated at least once in a year, preferably during the summer season.

Excel data-sheet

An Excel spreadsheet for data archiving is provided. After returning from the field, sampling teams should report all sampling data in the Excel spreadsheet.

Tab.1. Explanation of the information that must be reported in the Excel spreadsheet.

Colum	Description
Long	Longitude of the study site with a minimum of 4 decimal points
Lat	Latitude of the site with a minimum of 4 decimal points
Site	Name of the site.
Date	Date of the sampling.
Transect_n°	ID number of the transect (ranging from 1 to 3).

Once the spreadsheet has been filled, should be emailed to (lbenedetti@biologia.unipi.it and lrindi@biologia.unipi.it). Early warning signals will be estimated by UNIPI and all the result about the risk of collapse of target ecosystems will be shared with the MPA.

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