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Optimizing *Posidonia oceanica* (L.) Delile shoot density: Lessons learned from a shallow meadow

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ABSTRACT

Although shoot patchiness has long been studied in *Posidonia oceanica* meadows, small scale spatial structure of *P. oceanica* meadows is poorly known, as very few studies focused on this feature. In order to analyze spatial patterns within *P. oceanica* meadows that appear uniformly dense and undisturbed, we collected shoot density data at the Capo Rizzuto Marine Protected Area (NE Mediterranean, Italy). Intensive sampling was carried out within a square lattice at small spatial scale (i.e. in the 10^{-1}–10^{2} m^2 range) and shoot counts were obtained from sample quadrats of different size (60, 40, 20 cm). Spatial data analysis highlighted high irregularity in shoot density from centimetric to larger spatial scales. Therefore the deviation between shoot density estimates obtained using conventional methods and the overall average of quadrat counts (assumed as the best estimate for true density) was never negligible even when larger counting quadrats or higher numbers of replicates were adopted. While shoot density is regarded as the most important property of a *P. oceanica* meadow and as an indicator of ecosystem health, uncertainty in density estimates and unknown expected errors impair the effectivity of this approach. However, we showed that error could be predicted based on sampling intensity and design in an apparently uniform meadow. Although results from a single case study cannot be generalized, our work is the first attempt at analyzing the problems related to density estimates obtained from shoot counts and it shows how sampling can be optimized to achieve any desired level of accuracy.

1. Introduction

The endemic *Posidonia oceanica* (L.) Delile is the most abundant seagrass in the Mediterranean Sea (Buia et al., 2003) and can be certainly regarded as a key species (Short and Wyllie-Echeverria, 1996). *P. oceanica* meadows play a major ecological (Bell and Harmelin-Vivien, 1983; Gambi et al., 1989; Romero et al., 1992; Cebrian and Duarte, 2001), sedimentary (Jeudy de Grissac and Boudouresque, 1985), and economic role (Duarte, 1999, 2002). As a consequence of a widespread phenomenon of regression of the seagrass meadows in the Mediterranean basin due to increased human pressures (Boudouresque et al., 2009), *P. oceanica* meadows have been listed as a priority natural habitat, whose protection requires Special Areas of Conservation (SACs) to be designated (*sensu* Habitats Directive). Moreover, *P. oceanica* seems to be a reliable bioindicator (Pergent et al., 1995; Montefalcone, 2009) because of its wide distribution (Pasqualini et al., 1998; Procaccini et al., 2003), sensitivity to modifications of littoral zone (Short and Wyllie-Echeverria, 1996; Pergent-Martini, 1998; Ruiz et al., 2001; Ruiz and Romero, 2003) and good knowledge about specific response of the plant and of its associated ecosystem to specific impact (Romero et al., 2007). In the Water Framework Directive (WFD 2000/60/EC) *P. oceanica* was selected as a Biological Quality Element (BQE) to be taken into account in the evaluation of the Ecological Status (ES) of coastal waters and in the Marine Strategy Framework Directive (MSFD 2008/56/EC) *P. oceanica* contributes to the determination of the Good Environmental Status (GES).

Among many descriptors used in the study of *P. oceanica*, shoot density was found to be an effective descriptor of environmental health, conveying the vitality and dynamics of the meadows and revealing the effects of anthropogenic impacts (Pergent-Martini et al., 2005), especially when long-term variations of meadow status are taken into account (Mazzella and Buia, 1989). Shoot density plays an important role when estimates of quantitative

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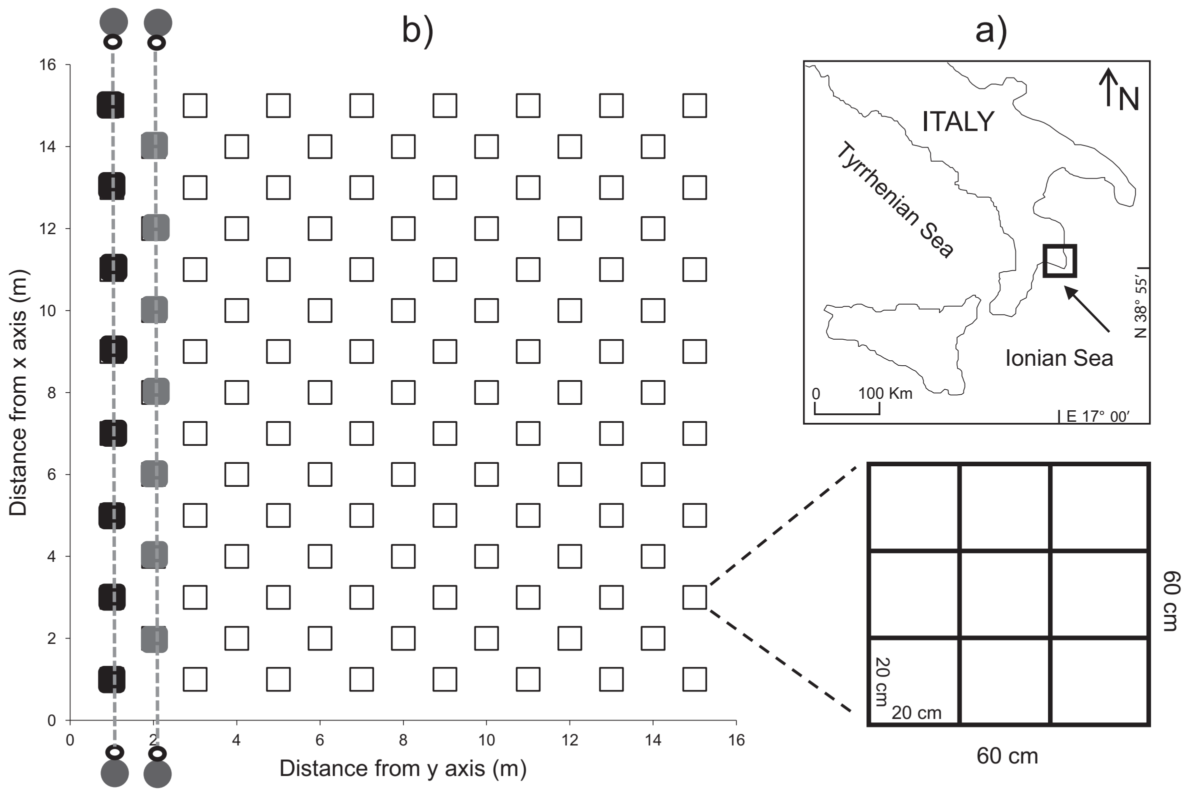

Fig. 1. (a) Location of the study area (MPA-Capo Rizzuto, Italy); (b) position of the sampling stations with details about the square frame used and the sampling methodology adopted.

properties of *P. oceanica* meadows are to be calculated (e.g. for primary production studies: Wittmann, 1984; Romero, 1989; Buia et al., 1992; Cebrian and Duarte, 2001; Dumay et al., 2002). Furthermore, the abundance and diversity of animal communities is strongly related to the density of seagrass shoots (Mazzella et al., 1992; Worthington et al., 1992; Sanchez-Jerez et al., 2000; Garcia-Charton and Perez-Ruzafa, 2001). Therefore, a decrease in shoot density can alter structure and functioning of the communities associated with the meadow (Den Hartog, 1977). The assessment of shoot density (expressed as number of live shoot of *P. oceanica* per m^2) is usually carried out by counting the number of shoots within square frames (i.e. quadrats) randomly located in a study area, and then averaging several counts (Giraud, 1977; Pergent et al., 1995; Duarte and Kirkman, 2001; Buia et al., 2003; Pergent-Martini et al., 2005). Currently, in spite of the widespread usage of shoot density measurements in *P. oceanica* investigations (Pergent-Martini et al., 2005), only large-scale studies (from kilometers to hundreds of meters) have been discussed in detail, focusing on variations on a geographical scale (e.g. among meadows along latitudinal gradients) or, within meadows, across depth gradients (e.g. shallow vs deep stands) (Balestri et al., 2003). Although the existence of density patchiness within *P. oceanica* meadows has long been recognized (Panayotidis et al., 1981), small-scale structural variability in *Posidonia* meadows is poorly known and very few studies have addressed this aspect (Balestri et al., 2003; Gobert et al., 2003; Zupo et al., 2006), so that information about fine grained patterns is still lacking. In particular, the horizontal expansion and exploratory growth of a meadow is controlled firstly by traits of the species, such as rhizome elongation, branching patterns and clonal growth (Marba and Duarte, 1998); secondly, it depends on an extensive range of biological and physical variables such as topography, substrate type, irradiance, temperature, nutrient availability, currents, sedimentation and also anthropogenic human impacts (Pirc, 1986; Duarte, 1991; Buia et al., 1992; Romero et al., 1992; Alcoverro et al., 1995; Lorenti et al., 1995; Pergent-Martini and Pergent, 1996; Zupo et al., 1997; Leoni et al., 2007; Giovannetti et al., 2008). Gradients in shoot density may be driven by some of these features at large spatial scale, but they are not likely to play a role at smaller spatial scale.

The aim of our work was to investigate *P. oceanica* shoot density patterns at small spatial scale, i.e. at a scale that is locally unaffected by some of the above-mentioned drivers, looking for new insights into the accuracy of density estimates and into the way they have to be measured and interpreted, especially in monitoring programs that support environmental decision-making and management. To the best of our knowledge, our work is the first attempt at exploring *P.oceanica* shoot density patterns at spatial scales ranging from 10^{-1} to 10^2 m^2. It is intended as a contribution to a better understanding of the magnitude of the errors that are inherent to any shoot density estimate and to the definition of optimized choices for quadrat size, number of quadrats and sampling area.

2. Materials and methods

2.1. Study area and sampling method

Field activities were carried out in June 2010 at the Capo Rizzuto Marine Protected Area (Central Mediterranean, Italy, Fig. 1a). Sampling was performed in a *P. oceanica* meadow settled on *matte*, within 16 m × 16 m area at 5 m depth (38°55′10″ N, 17°00′05″ E) that looked uniformly dense at repeated visual inspections. While the midpoint of the depth range of *P. oceanica* meadows is about 15 m and therefore this depth is often selected when a single stand must be assumed as representative of a whole meadow (e.g. Romero et al., 2007; Gobert et al., 2009), we selected this rather shallow stand because of the large amount of underwater activity that was required by our sampling design, based on 113 points (minimum distance between points = $\sqrt{2}$ m), regularly distributed over the sampling area (Fig. 1b). In order to allow SCUBA divers