AQUATIC CONSERVATION: MARINE AND FRESHWATER ECOSYSTEMS

Aquatic Conserv: Mar. Freshw. Ecosyst. **26**: 549–561 (2016)

Published online 21 December 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/aqc.2586

Monitoring deep Mediterranean rhodolith beds

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ABSTRACT

1. The protocols available for sampling and monitoring shallow subtidal rhodolith beds (RBs) are inadequate for the deep Mediterranean analogues, and need calibration in order to attain comparable results.

2. After reviewing the present knowledge of the specificities of Mediterranean RBs, and in the framework of the ongoing international effort for their conservation, a two-step approach is suggested for their definition, identification, delimitation, description, and monitoring.

3. Regional mapping should be improved, and RBs should be identified and delimited as those areas of the sea floor with >10% cover of live rhodoliths over a minimum surface of 500 m^2, on 1:10000 scale. More detailed scales (at least 1:1000) should be used for monitoring selected RBs, in order to detect significant changes through time.

4. Beside location and areal extent, the description of RBs should include the occurrence of macroscopic sedimentary structures of the sea floor, thickness of live cover, mean percentage cover of live thalli and surface live/dead ratio, cover of dominant morphologies of rhodoliths (simplified on a ternary diagram), and volumetrically important calcareous algal species.

5. For the purpose of assessment of the ecological status and the evaluation of human-induced impacts, quantitative data about community composition are required. The comparative assessment of ecological status and the identification of RBs of high conservation value for special protection should consider the natural geographic and seasonal/annual variability of RBs.

Received 25 May 2015; Revised 20 July 2015; Accepted 9 August 2015

KEY WORDS: sublittoral; benthos; algae; habitat mapping; EU marine strategy; biodiversity

INTRODUCTION

It was only in recent times that maerl and rhodolith beds (RBs) were recognized as a non-renewable resource that is threatened by human activities (Barberá *et al.*, 2003; Nelson, 2009; Aguado-Giménez and Ruiz-Fernández, 2012; Basso, 2012). The increasing awareness of the importance and fragility of the benthic habitats characterized by red algal concretions led to several international initiatives, legally binding or not, aimed at their conservation (Council of the European Union, 2006; UNEP-MAP-RAC/SPA, 2008).

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For historical reasons and because of a more focused research effort, our knowledge, definitions, and evaluation criteria on RBs are largely derived from shallow-water, extra-Mediterranean examples (among others Pruvot, 1897; Cabioch, 1970; Bosellini and Ginsburg, 1971; Bosence, 1983a, b; Birkett *et al.*, 1998), despite some remarkable early Mediterranean contributions, primarily devoted to their species richness and bionomy (Huvé, 1956; Parenzan, 1960; Jacquotte, 1962; Pérès and Picard, 1964).

Mediterranean RBs are coastal to offshore macro-, meso- or mega-habitats (*sensu* Greene *et al.*, 1999) frequently occurring in the mesophotic zone, mostly at about 40–60 m of water depth (Foster *et al.*, 2013). Knowledge of their species diversity is presently undergoing a considerable improvement due to advances in molecular genetics which is also revealing clear latitudinal and biogeographic patterns in coralline species distribution (Pardo *et al.*, 2014). In spite of this fluid taxonomy, Mediterranean RBs appear to possess more diverse species assemblages of coralline and peyssonneliacean algae than their Atlantic counterparts, and to be structured by a suite of combinations of rhodolith shapes and coralline compositions: from monospecific branched growth-forms, to multispecific rhodoliths (Basso, 1998). Therefore, the protocols available for sampling and monitoring RBs in shallow subtidal waters (Steller and Foster, 1995; Peña and Barbara, 2008; Hall-Spencer *et al.*, 2010; Nelson *et al.*, 2012) cannot be applied as such, and require calibrating to the Mediterranean specificities.

Despite their ecological importance and conservation value, understanding of the composition, structure, distribution and natural variability of Mediterranean RBs is still inadequate, and a standardized protocol to monitor deep Mediterranean RBs in a proper manner has not yet been defined (UNEP-MAP-RAC/SPA, 2008; UNEP, 2011). In light of this gap in knowledge between international environmental policy (European Parliament and Council of the European Union, 2008; UNEP-MAP-RAC/SPA, 2008, 2010) and Mediterranean research progress, a two-step protocol, aimed at optimizing the resources through a clearly focused research strategy is proposed here. This contribution is based on a critical review of the published methods for studying and monitoring RBs, with the aim of clarifying the baseline concepts for the definition, identification, delimitation and monitoring of those Mediterranean RBs that lie below the safe limits for investigation by scuba diving.

RHODOLITH BED VERSUS MAERL BED

Unequivocal terms and definitions foster the efficacy of legal instruments based on them. The term ‘maerl’ comes from a Breton word, referring to an area of calcareous land or deposits of calcified algae (Grall and Hall-Spencer, 2003). Since Atlantic maerl, mostly composed of *Phymatolithon calcareum* and *Lithothamnion corallioides*, was the original reference material (Lemoine, 1910; Cabioch, 1969), the Breton term was used to identify the same calcareous gravel in the Mediterranean, composed of living and dead coralline branched thalli, twig-like, sometimes interlocking (Huvé, 1956; Jacquotte, 1962). Beside the maerl-type coralline branched thalli, the Mediterranean occurrence of coralline nodules had already been observed (Walther, 1885).

The first use of the term rhodolith in literature is found in Barnes *et al.* (1970), but its formal definition is due to Bosellini and Ginsburg (1971) as ‘rhodolite’, later corrected to ‘rhodolith’ (Ginsburg and Bosellini, 1973). Since then, rhodoliths (sometimes erroneously reported as ‘rhodolithes’, i.e. www.jncc.gov.uk; EUNIS habitat classification; Davies *et al.*, 2004), are intended as unattached nodules formed by calcareous red algae and their growths, as part of a continuous spectrum of forms, with size spanning from 2 to 250 mm of mean diameter (Bosellini and Ginsburg, 1971; Ginsburg and Bosellini, 1973). On the basis of this definition, the term rhodolith beds also includes maerl and calcareous *Peyssonnelia* beds (Lanfranco *et al.*, 1999; Steller *et al.*, 2003; Foster *et al.*, 2013; Figure 1). Although rhodoliths include maerl, the opposite is not true, and the use of the two words as synonyms led to the paradox of listing RBs as a subcategory of maerl beds, which is inconsistent with the origin of the terms (Davies *et al.*, 2004; Council of the European Union, 2006). The misleading use of maerl as a collective term to

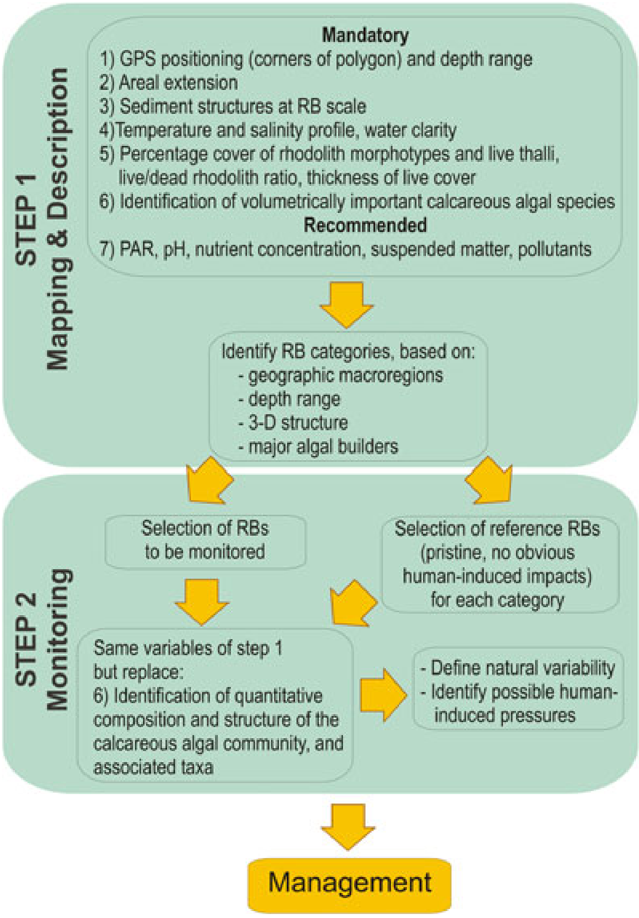

Figure 1. Synopsis of the proposed activity-flow. Basic information should be obtained about RBs located below the safety limits of scuba-enabled investigation, by mapping and describing their main features and structure (Step 1). Main categories of RBs can then be defined based on geographic macroregions, depth-range, tridimensionality, and major calcareous algal builders. Within each category, unimpacted RBs are selected as reference for comparative assessment of ecological status and monitoring (Step 2).

include all kind of assemblages of unattached calcareous red algae dwelling on sedimentary bottoms, implies that they are dealt with as an artificially homogeneous category – despite their different structural complexity, species composition, etc. – and consequently, to a misleading interpretation of their ecology (i.e. Martin *et al*., 2014).

ECOSYSTEM VALUES AND HUMAN PRESSURES

The economic value of an ecosystem can be estimated by identifying and valuing the goods and services that it provides (Costanza *et al*., 1997). Mediterranean RBs are providers of production, regulation, and supporting services, with the addition of currently unknown potential future uses (Beaumont *et al*., 2008; Salomidi *et al*., 2012). Production services correspond mainly to the economic importance of Mediterranean RBs as essential fish habitats (EFH) (Barberá *et al*., 2003, 2012; Bordehore *et al*., 2003; Valavanis and Smith, 2007; Ordines and Massutí, 2009). Unlike the Atlantic maerl beds, corallines are not extracted from the Mediterranean for soil improvement, since they are mostly neither abundant nor shallow enough to support an economically viable activity. However, they may undergo extraction as relict sand for beach nourishment by dredging (Nicoletti *et al*., 2006). Rhodolith beds are also important in climate regulation, through their role as hot-spots of carbonate production and deep benthic primary production (Martin and Gattuso, 2009; Nelson, 2009; Basso, 2012). Finally, the high species richness associated with long-lived rhodoliths and the build-up of their dead remains is attributed to their three-dimensional structure (Foster *et al*., 2013). Rhodolith beds provide a supporting service as ecosystem engineers, thus fostering complex ecological interactions (Barberá *et al*., 2003; Nelson, 2009; Cavalcanti *et al*., 2014).

Mediterranean RBs, as a biogenic calcareous habitat, are a matter of conservation concern because they represent a non-renewable resource owing to their slow rate of growth and carbonate deposition (about 1 mm year^{-1}) (Martin and Gattuso, 2009; Nelson, 2009; Basso, 2012). The physical damage caused to RBs by humans (i.e. from dredging, fishing gear, bottom trawling) is a severe pressure, since habitat modifications can change the species diversity and functional relationships (Bordehore *et al*., 2000, 2003). Other pressures on Mediterranean RBs include: degradation of water quality (i.e. pollution from sewage or from aquaculture effluents; Sanz-Lázaro *et al*., 2011; Aguado-Giménez and Ruiz-Fernández, 2012); smothering effects resulting from changes in sedimentation rates; ocean warming and acidification (Grall and Hall-Spencer, 2003; Wilson *et al*., 2004; Martin and Gattuso, 2009; Basso, 2012; McCoy and Ragazzola, 2014). Moreover, the spread of invasive alien species is a particularly serious threat for the numerous Mediterranean endemic species (Sciberras and Schembri, 2007; Salomidi *et al.*, 2012).

THE LEGAL FRAMEWORK FOR THE PROTECTION OF MEDITERRANEAN RBs

The vulnerability of this habitat has been recognized in various European and international frameworks, through the adoption of a range of protection instruments such as Directives, Regulations and Conventions. Within European legislation, two main tools have been put in place: the Habitats Directive 92/43/EEC and the Council Regulation 1967/2006. In particular, the Habitats Directive includes *Phymatolithon calcareum* and *Lithothamnion corallioides* in Annex V, among those species subject to exploitation and for which Member States have to ensure appropriate management measures. The Council Regulation 1967/2006 (Council of the European Union, 2006), concerning management measures for the sustainable exploitation of fishery resources in the Mediterranean Sea, establishes the banning of specific fishing gear on coralligenous or maerl beds. The latter are defined as '…a collective term for a biogenic structure due to several species of coralline red algae (Corallinaceae), which have hard calcium skeletons and grow as unattached free-living branched, twig-like or nodule coralline algae on the sea bed, forming accumulations within the ripples of mudflats or sandflats sea beds. Maerl beds are usually composed of one or a variable combination of red algae, in particular, *Lithothamnion corallioides* and *Phymatolithon calcareum*' (sic!). Although bottom trawling is prohibited over maerl beds, the insufficient information available on their spatial distribution hampers effective application of the EC regulation (Council of the European Union, 2006; Barberá *et al.*, 2012). Moreover, owing to the diverse and distinctive taxonomic composition of coralline algae forming Mediterranean RBs (Ballesteros, 1988; Basso *et al.*, 2014; Falace *et al.*, 2014), where *L. corallioides* and *P. calcareum* may be absent or just minor components of the rhodolith-forming association, the need to grant legal protection to other characteristic species of the Mediterranean assemblages should be considered (Barberá *et al.*, 2003, 2012).

A special plan for the protection of the Mediterranean RBs is present within the framework of the United Nations Programme's Mediterranean Action Plan (UNEP-MAP-RAC/SPA, 2008; Agnesi *et al.*, 2009), which, however, is not mandatory for the national governments.

More recently, the Marine Strategy Framework Directive (MSFD; European Parliament and Council of the European Union, 2008; European Commission, 2010) aims at achieving the 'Good Environmental Status' (GES) of all marine waters by 2020, by protecting the resource base and the biodiversity upon which marine-related economic and social activities depend. In order to achieve GES, each Member State is required to develop a strategy of knowledge-based sustainable management for its marine waters. On the basis of the Barcelona Convention (1995) and other international initiatives for the environmental protection of the Mediterranean (UNEP-MAP-RAC/SPA, 2008, 2010; UNEP-MAP, 2011), a group of special habitat types has been identified and selected by each member state as being of special scientific or biodiversity interest (MSFD, Annex III, Table 1). Among them, RBs have been included in the national initial assessment process by several European countries, including France, Spain, Italy, Malta and Greece (EIONET, 2015). Consequently, management and GES assessment must be kept up-to-date and reviewed every 6 years.

It is noteworthy that the overall idea of protection of RBs as defined in these legal instruments is derived from human use of this resource in the Atlantic, where RBs are actively exploited (Nelson, 2009; Salomidi *et al.*, 2012 and references therein).

A key conservation measure should therefore include the upgrade of RB-forming coralline algae protection status to a higher category under the Habitats Directive, becoming Annex II species (species of community interest whose conservation requires the designation of special areas of conservation). Alternatively, the habitat type as a whole should be protected, in order to prevent disturbance and/or destruction (ecosystem-based approach: Barberá *et al*., 2012; Salomidi *et al*., 2012), by RBs being listed as an Annex I Habitat type (natural habitat types of community interest whose conservation requires the designation of special areas of conservation). Whichever conservation and management strategies are decided on to be put in place, a programme for monitoring the state of Mediterranean RBs is required, based on the knowledge of their distribution, biodiversity, and 3-D structure.

CONSERVATION AND MANAGEMENT OF MEDITERRANEAN RBs

The sustainable utilization and monitoring of resources is a top priority in marine ecosystem conservation and management (Birkett *et al*., 1998). The protection of a specific habitat type cannot be achieved effectively without access to sound geospatial data and monitoring plans (Salomidi *et al*., 2012). To this purpose, modern advances in remote sensing and acoustic habitat-mapping provide effective tools for assessing the distribution, extent and state of the RBs (Georgiadis *et al*., 2009; Barberá *et al*., 2012; Savini *et al*., 2012).

Beside mapping, evaluation of the GES of the RBs should include: (1) characterization of the 3-D structure of the bed and identification of the main habitat-forming red calcareous algae; (2) measurement of the physical–chemical variables correlated with the RBs occurrence and status (PAR, temperature, salinity, pH, nutrient concentration, suspended matter, hydrodynamics, sediment grain-size and composition, bed sedimentary structures, pollutants in the water column and in the sediment) (Sciberras *et al*., 2009; Barberá *et al*., 2012); (3) comparative assessment of the natural intra-bed and inter-bed variability in pristine conditions, in order to set limits outside of which management action is needed; and (4) identification of possible pressures and impacts, with emphasis on the pressures to which RBs have been proven to be vulnerable. Since no comprehensive report is available about occurrence, composition and variability of Mediterranean RBs, and in consideration of the heterogeneous policies and research effort dedicated by the 22 countries bordering the Mediterranean, the GES evaluation of Mediterranean RBs is a challenging task.

The most feasible way to address the problem is to follow a cost-effective, two-step strategy (Figure 1). All RBs should be mapped (first step), but the available resources (researchers and funding) of most Mediterranean countries make the monitoring (second step) of all of them unrealistic. A priority group of RBs must be identified, after considering the different geographic areas, with at least one of each different type of RB, as identified from the preliminary descriptions. The second step should include study of the ecosystem structure and functionality, and the identification of threats that may affect it.

Most Mediterranean RBs occur beyond the safe limits of standard scuba-based sampling designs and methods (Basso, 1998; Sciberras *et al*., 2009; Barberá *et al*., 2012), thus, investigations have been affected by logistic and technological constraints. Therefore, the available techniques for the investigation of deeper settings that are likely to produce valuable and comparable data are discussed here. The proposed sampling protocol is conceived to minimize the impact on the sea floor, and to provide quantitative and comparable data; thus dredging is not considered.

CRITERIA FOR THE IDENTIFICATION OF RBs

Rhodolith beds are composed of a variable thickness of live and dead thalli of unattached calcareous red algae and their fragments, creating a biogenic, unstable, 3-D architecture typically exposed to bottom currents (Steller *et al*., 2007).

Live surface and minimum spatial extent of RBs

The surface of a living RB is naturally composed of a variable amount of live thalli and their fragments, lying on a variable thickness of dead material and finer sediment. An RB is defined as a habitat (*sensu* MSFD) that is distinguished from the surrounding sea floor by having >10% of the mobile substratum covered by live calcareous red algae (in the Mediterranean, coralline algae and the fully calcified *Peyssonnelia* species) as unattached branches and/or nodules (Steller *et al.,* 2003). The term nodule includes both nucleated and non-nucleated rhodoliths. The non-nucleated rhodoliths originate from vegetative reproduction by fragmentation (Freiwald, 1995), or from spore germination on a microscopic nucleus. In contrast, the nucleated rhodoliths possess a macroscopic nucleus (= detectable with the naked eye in sectioned rhodoliths), as a lithic grain or a non-algal biogenic grain, more or less completely wrapped up by calcareous algae.

Those calcareous algal nodules with non-algal nuclei making up >50% of the total thickness have been defined as coated grains (Steneck, 1986). Coated grains crowded on the sea floor could be considered as a step in RB development, or a steady-state situation, depending on the dynamic equilibrium between available nuclei and coralline growth rate. Steneck's definition (1986) includes a suite of possible intermediate gradings from sub-millimetre-thick, incomplete algal coating of lithic pebbles, to nodules with a 50/50 coralline/nucleus ratio. Sparsely coated grains are extremely common in the Mediterranean infralittoral and circalittoral sedimentary bottoms. A sea floor covered by incomplete algal coatings of lithic pebbles and shell remains should not be considered as an RB, although there has been no evaluation so far of the ecosystem service value of coated grains versus rhodoliths. On the contrary, for conservation and management purposes, any grain completely wrapped up by live calcareous red algae should be equated to a rhodolith, in order to avoid the need for sectioning to properly assess the thickness of the algal coating.

Dead RBs were defined as the portion of the sea floor where >10% of the sediment (surface) is composed of remains of calcareous red algae (Tompkins, 2011). However, live RBs are naturally accompanied by a variable quantity of dead rhodoliths and their fragments, thus, most sea floors covered by mixed biogenic sediment with few live calcareous red algae are likely to possess >10% of dead calcareous algal remains (Bracchi and Basso, 2012). Consequently, this definition (Tompkins, 2011) is much wider than that of the live RBs, and probably misleading. Moreover, attention must be paid to transport phenomena that could have led to the accumulation of the algal fragments away from their native biotope. Therefore, a much more restrictive definition of dead RBs is recommended here, by indicating a threshold of >50% surface cover by dead rhodoliths and their fragments as a condition to identify a dead RB (or its fossil counterpart, Basso *et al.*, 2012; Sheehan *et al.*, 2015).

The live algal cover of an RB is not homogeneous across the bed, and the transition to a different benthic association might be sharp or gradual, with live rhodoliths becoming sparser. Moreover, RBs are intrinsically mobile and unstable, with borders that may shift depending on hydrodynamics or possible periodical changes in the sedimentation rate (Steller *et al.*, 2003). Thus, a hypothetical reference spot within an RB may appear to be different at different times, independently of its ecological status. On the contrary, the 'healthy' appearance of RBs (with vivid pink and red hues) could hide the shift from one calcareous algal assemblage to another, following a possible impact. It has been shown that dead RBs cannot always be assumed to have lower conservation value than those with a scattering of live thalli (Sheehan *et al.*, 2015). For these reasons, the visual analysis, or the sole monitoring of the areal surface repeated over defined periods, is not sufficient to describe the ecological status of RBs.

There are no literature data about the required minimum spatial extent for a portion of the sea floor to be defined as an RB. The choice of the minimum spatial extent is constrained by the size of Mediterranean RBs and the map scale, the latter depending on the purpose (i.e. the framework of a regional assessment at basin level versus the monitoring of a specific bed in an MPA) (Burrough and McDonnell, 1998). Present-day geo-spatial data are managed by Geographic Information Systems (GIS), allowing repeated updating and displaying on variable scales. However, GIS is often not the appropriate instrument to convey information to stakeholders (i.e. public agencies, MPA administrators, fishermen), because of the lack of readily available software to visualize spatial geo-data. Therefore, printed cartography, on different appropriate scales, still represents an essential tool for any management plan (Bianchi *et al.,* 2012). Practically, the scale 1:10000 is the best choice, since it is suitable for transfer into most international initiatives for marine habitat mapping. On this scale, it is possible to delimit areas down to about 500 m^2, which is a good compromise between precise RB delimitation and study effort on a regional basis. Conversely, a scale equal to 1:1000 (or larger) is suggested for detailed monitoring studies of selected RBs, where the areal definition and the RB boundaries should be more accurately located and monitored through time. Two adjacent RBs are considered separate if, at any point along their limits, a minimum distance of 200 m separates them (Peña and Barbara, 2008).

Step 1: RB mapping and preliminary description

This first step is focused to fill the gap of basic information about the occurrence of RBs and their main features (Figure 1).

The comparison of different survey techniques showed that the areal extension of RBs is conveniently defined by the use of acoustic methods (UNEP, 2011). Acoustic mapping technology (side scan sonar and multibeam echosounder) is used for morpho-sedimentary or biological habitat discrimination, or both approaches (Smith and Greenhawk, 1998; McRea *et al*., 1999; Ojeda *et al*., 2004; Panadian *et al.*, 2009), and must be effectively calibrated by ground-truthing (grab sample, box-corer, submarine video, ROV, diving) (Ehrhold *et al*., 2006; Brown *et al*., 2011; Barberá *et al*., 2012; Savini *et al*., 2012).

Sediment texture and sedimentary structures are strictly linked to water movement (such as the type and size of ripple marks, or the occurrence of underwater dunes, channels, etc.) (Bordehore *et al*., 2003; Barberá *et al*., 2012; Nelson *et al*., 2012) and sedimentation rate, that in turn are important factors for understanding the development and fate of RBs (Bosence, 1979; Basso, 1998; Steller *et al*., 2003, 2007; Basso *et al*., 2009). Routine, grain-size wet analysis can be conducted on about 200 g of sediment randomly collected from the upper 10 cm layer of the original sample (Barberá *et al*., 2012). It is worth mentioning that live calcareous algae are supposed to be the main component of the sediment, and must not be removed from the sample before analysis in order to obtain a realistic picture of the kind of substratum available to the live benthos.

The mean percentage cover of live thalli, the live/dead rhodolith ratio (Peña and Barbara, 2010), and the thickness of the live layer are an approximate measure of the algal growth rate and vitality, although this sole observation is insufficient for GES assessment and inter-bed comparison. The percentage cover of live thalli over a wide area can be assessed by ROV dives that are also useful for the detection of meso-scale sedimentary structures. The thickness of the live cover could be measured through the transparent or removable side of a box-corer. Alternatively, a sub-sample could be taken from the recovered box-core using a Plexiglas core of about 10 cm in diameter and at least 20 cm long.

Establishing the degree of 3-D complexity of RBs is a critical feature for understanding heterogeneity and associated biodiversity (Steller *et al*., 2003; Sciberras *et al*., 2009; Villas-Boas *et al*., 2013). Unfortunately, a single RB may include any gradation of shape and structure from coated grains and pralines (Molinier, 1956; Pérès and Picard, 1964) to the largest boxwork rhodoliths (Bosence, 1983a; Basso, 1998; Basso *et al*., 2009; Sciberras *et al*., 2009). Bosence's pioneer classification (1983a) is particularly useful for describing maerl *sensu stricto*, that is to say, the accumulation of mostly non-nucleated branching forms. Sciberras *et al*. (2009) attempted to merge the existing classifications by distinguishing a series of rhodolith morphotypes. However, explorative investigations possibly conducted by non-specialists require a simpler morphological classification of rhodolith forms. All possible variations in growth form, shape, and internal structure of rhodoliths have been already simplified in a scheme with three major categories as focal points along a continuum: compact and nodular pralines, larger and vacuolar boxwork rhodoliths, and unattached branches (Basso, 1998, 2012; Basso *et al*., 2009). Each of the three end-members within rhodolith morphological variability corresponds to a typical (but not exclusive) group of composing coralline species and associated biota and is possibly correlated with environmental variables, among which substratum instability (mainly due to hydrodynamics) and sedimentation rate are the most obvious (Basso, 1998; Hinojosa-Arango and Riosmena-Rodriguez, 2004; Steller *et al.*, 2007; Sciberras *et al.*, 2009). Thus the indication of the percentage live cover by the three rhodolith categories (Figure 2; Basso *et al.*, 2009) at the surface of each RB is a proxy of RB structural and ecological complexity, suggesting the possible identification of a number of different types of RBs (Hinojosa-Arango and Riosmena-Rodriguez, 2004; Barberá *et al.*, 2012). In this sense, it is necessary to underline that these morphological and structural categories have no significance for the GES evaluation; rather, RBs with very different architecture are expected to be sedimentologically, structurally, and biologically non-comparable for GES assessment.

The high species diversity hosted by RBs requires time-consuming and expensive laboratory analysis for species identification. Unluckily, there are no shortcuts available for obtaining the biological data necessary for RB monitoring. In particular, videos and photos provide no information on RB composition owing to the absence of conspicuous, easy-to-detect species. Moreover, since most coralline species belong to a few genera only, the use of taxonomic ranks higher than species is not useful, as already assessed for other macroalgal assemblages (Ceschia *et al.*, 2007). To overcome this problem, a minimum of three box-cores with opening $\geq$0.16 m^2 should be collected in each RB. One box-corer must be collected within the RB area with the highest percentage of live cover (on the basis of preliminary ROV dives), and the others as far as possible from it, following the depth gradient in opposite directions of the maximum RB extension. In many instances grab samples could be useful, but attention must be paid to sea floor surface disruption and mixing, and the possible loss of material during recovery. In those extreme cases of very coarse material preventing box-core penetration and closure, a Hamon grab could be used instead, although it cannot preserve stratification.

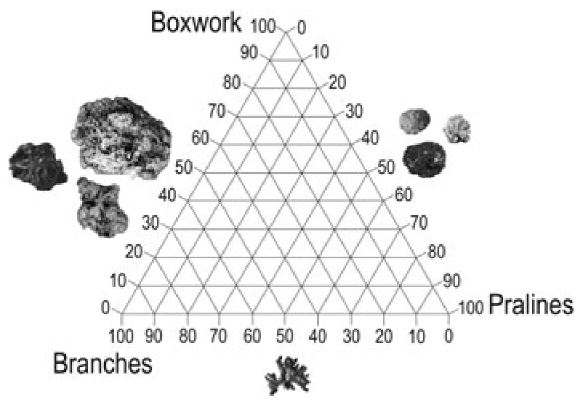

Figure 2. Ternary diagram for the description of the rhodolith bed tridimensionality. The 3-D structure at the sea floor is provided by rhodoliths, disregarding vitality. The percentage cover of each rhodolith morphotype, relative to the total rhodolith cover, can be plotted on the correspondent axis. The three main rhodolith morphotypes (boxwork rhodoliths, pralines and unattached branches) are intended as focal points of a *continuum*, to which any possible rhodolith morphology can be approximately assigned.

The mandatory operations to be performed after box-core recovery are: (1) colour photograph of the whole surface of the box-core, at a high enough resolution to recognize the morphology of single live rhodoliths and other conspicuous organisms. In addition, the possible occurrence of heavy overgrowths of fleshy algae that may affect rhodolith growth rate must be reported; (2) visual definition of the live percentage cover of red calcareous algae; (3) visual definition of the live/dead rhodolith ratio calculated for the surface of the box-core; (4) visual definition of the rhodolith morphologies characterizing the sample, to be plotted into the triangular diagram (Figure 2); (5) measurement of the thickness of the live rhodolith layer; (6) collection and preservation of live specimens for further analysis; and (7) collection, drying and preservation of about 200 g of surface sediment as a whole, including also dead rhodoliths, empty shells, etc. and their fragments.

The live material should be analysed for identification of at least the macroscopic, volumetrically important calcareous algal species, with a semi-quantitative approach (classes of abundance of algal coverage: absent, 1–20%, 21–40%, 41–60%, 61–80%, >81%). The additional identification of conspicuous fleshy algae and invertebrates (as listed in UNEP-MAP-RAC/SPA, 2008) is also useful. For molecular investigations, samples from voucher rhodolith morphotypes should be air-dried, and preserved in silica gel (Pardo *et al.*, 2014). The sediment sample should be analysed for grain-size (mandatory), and carbonate content.

Step 2: full description and monitoring

After identification of the main RB features obtained on the basis of the results of Step 1, it will be possible to define categories of RBs by geographic area, depth range, 3-D structure, and dominant algal engineer-species (Figure 1). Non-impacted sites of each category will serve as reference sites for monitoring the same category of RB (Figure 1). Monitoring should address all the variables indicated in the first step, with the addition of the description of the RB community (Sciberras *et al.*, 2009). At the site of the highest live cover within each RB, three replicate box-cores $\geq$0.16 m^2 each should be collected randomly, at the same water depth (variation should not exceed 1 m). In the event that the depth interval separating upper and lower limits of an RB is >5 m, further replicate sampling, depth-stratified with increments of 3 to 10 m, is needed in order to highlight possible variability or ecotones, in agreement with Steller *et al.* (2007). All live calcareous algae and accompanying phytobenthos and zoobenthos should be identified and quantified, in order to allow for detection of intra- and inter-bed variability in space and time, and any changes after possible impacts. A partitioned sampling by sub-habitats (cryptofauna of rhodoliths, sediment infauna, epibenthos, etc.) could be used for specific research purposes (Steller *et al.*, 2007).

CONCLUSIONS

Implementation of the Habitats Directive has proved to be problematic, and there is a need for a revision of the list of protected habitats, with the aim to include Mediterranean rhodolith beds regardless of the occurrence of *P. calcareum* and *L. corallioides* (Barberá *et al.*, 2012). For clarity and consistency of definitions, the use of the term ‘rhodolith bed’ is recommended as a generic name to indicate those sedimentary bottoms characterized by any morphology and species of unattached non-geniculate calcareous red algae (incompletely-coated grains excluded) with >10% of live cover (Foster *et al.*, 2013). The name maerl should be restricted to those RBs that are composed of non-nucleated, unattached growths of branching, twig-like coralline algae (Bosence and Wilson, 2003, pl. 1, Fig. a; *unattached branches* in Basso, 1998; Basso *et al.*, 2009; lower left sector of the ternary plot in Figure 2). Accordingly, the EUNIS habitat classification should be improved in order to show rhodolith beds as a parent level that includes also maerl beds, and not the other way round (Galparsoro *et al.*, 2012) (A5.51, EUNIS habitat classification 2007 rev. 2012, http://eunis.eea.europa.eu).

A scale of 1:10000 is suitable for mapping RBs with areal extent $\geq$500m^2 at regional level. Larger scales (e.g. 1:1000) should be used for detailed investigation and specific purposes.

Alternative strategies of investigation are available for shallow vs. deep RBs. Shallow RBs (allowing safe and cost-effective scuba diving for visual description and sampling) can be effectively described and monitored following Steller *et al.* (2007). Deep RBs can be identified, described and monitored following the two-step approach suggested here, in a clearly defined, cost-effective framework. In the first step, the need for specialized taxonomists and time-consuming laboratory analyses is kept to a minimum. The mandatory information here proposed for a first description of RBs includes (Figure 1): GPS positioning (corners of polygon) and depth range, areal extent, sediment analysis and sedimentary structures of the sea floor (such as ripples, mega-ripples and underwater dunes), mean percentage cover of live thalli and thickness of live cover, live/dead rhodolith ratio, dominant morphologies of rhodoliths (Figure 2), and identification of the most common and volumetrically important species of calcareous algae. These data should be accompanied by time-series of *in situ* measurements of temperature, salinity, water motion, water clarity (Secchi disk, nephelometer), PAR, pH, nutrient concentration, suspended matter, pollutants in the sea water and in the sediment (European Environment Agency, 2011).

Acoustic methods are presently the most convenient technique for mapping RBs, associated with ground-truthing by ROV and box-coring. Box-coring with a cross-section ≥0.16 m^2 is recommended because it has the advantage of preserving the original substratum stratification. The use of dredges for sampling RBs should be discouraged, in order to minimize the impact of the investigation. The information obtained after the first step allows for an initial description and identification of categories of RBs (preliminarily by geographic area, depth range, 3-D structure, and major builders among calcareous algal species; Figure 1). Within each of these, monitoring is required at least on particularly valuable RBs (selected for their high live/dead rhodolith ratio and high live percentage cover) by comparative assessment with non-impacted RBs belonging to the same category (Figure 1). Monitoring should address all the variables indicated in the first step, with the addition of the full quantitative description of the RB community, through periodical surveys. The decrease of RB extent, live/dead rhodolith ratio, live rhodoliths percentage cover, associated with change in the composition of the macrobenthic community (calcareous algal engineers and associated taxa) and possibly in sedimentology reveals potential negative impacts on RBs, deserving investigation as to causal factors, and implementation of management actions. A focused and practical planning of the description and monitoring of Mediterranean RBs is an essential tool for optimizing research and conservation efforts and fostering communication, with the final aim to achieve a basin-wide network of biodiverse, ecologically connected and protected marine habitats.

ACKNOWLEDGEMENTS

We acknowledge the financial support from the EU project FP7 *CoCoNet* and from INTERREG Italy-Slovenia *TRECORALA* to AF. We thank two anonymous referees and J. Baxter (editor) for their constructive comments and critical review of the manuscript.

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