Conservation of Mediterranean seascapes: analyses of existing protection schemes

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Abstract

Marine protected areas (MPAs) are aimed at managing and protecting marine environments. Their design, however, often disregards both a thorough knowledge of the distribution of habitats and assemblages and the use of proper experimental evaluations of the efficacy of MPAs by comparing protected vs. unprotected zones. About 200 MPAs have been recently instituted in the Mediterranean area, but the evidence of their efficacy is scant. The MPA of Torre Guaceto (Southern Adriatic Sea, Italy) is one of the rare cases of effective protection enforcement. The reserve was instituted more than 10 years ago, a period currently considered as sufficient to show responses by organisms to protection. The MPA is divided into a C zone, the general reserve, where many activities are permitted, a B zone, the partial reserve where restrictions increase and two A zones, the integral reserve where access is prohibited. The goals of the paper were to map the distribution of benthic assemblages to assess if they were properly represented in the differently protected zones, and to test the efficacy of protection by quantifying possible differences between the assemblages in two control areas and in the two A zones, where human impact is completely excluded. The analysis of habitat and assemblage distribution within the MPA showed that the zones with total protection do not include most valuable environmental types. Most of the considered variables (i.e. cover of substratum, number of taxa, and average abundance of the most common taxa) were not significantly different in and out of the A zones, at each time of sampling. Results, however, suggested a possible effect of protection in modifying patterns of abundance of sponges under Cystoseira canopy (more abundant in the fully protected zone). In the subtidal habitat, differences were found in the structure of the whole assemblage and in the abundance of encrusting coralline
red algae (more abundant outside the fully protected area). Notwithstanding the correct
general methodology employed in the study, a lack of statistical power could have a role in
preventing the detection of ecologically relevant effects of protection. In some instances, data
pooling allowed a discrimination between cases where there was clearly no effect of protection
and cases where there might be. On this basis, the optimization of this experimental design
should be considered in further studies. In any case, if the goals of MPAs have not been clearly
stated, efficacy of protection might prove very difficult to test even with the use of sound
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efficacy; Benthos; Rocky shores

1. Introduction

Research on marine protected areas (MPAs) has been very intense over the last
decades (Allison, Lubchenco, & Carr, 1998; Boero, Briand, & Micheli, 1999;
Halpern & Warner, 2002; Palumbi, 2001). Meta-analysis has suggested that, in re-
spect to adjacent fished areas, reserves of any size harbor more diversity, higher
abundance, and organisms of larger size, if no-take zones are effectively managed
(Halpern, 2003). Protection can also have potential regional effects by enhancing
production of eggs and larvae inside reserves due to increase biomass of spawners
(Castilla & Bustamante, 1989; Jennings, 2000) and by improving fisheries yields
outside reserves due to the spillover of adults emigrating from MPAs (Branch &
Odendaal, 2003; Sluka, Chiappone, Sullivan, & Wright, 1997). Protection, further-
more, provides an opportunity to test the top-down impact of predators and, as
indirect effects, the impact of fisheries at ecosystem-level (e.g. McClanahan & Shafir,
1990; Sala, Boudouresque, & Harmelin-Vivien, 1998; Shears & Babcock, 2002,
2003). Considering the strong constraint of observation scales in manipulative field
ecology, MPAs can be regarded as large-scale human exclusion experiments allowing
the understanding and predicting of changes associated with human impacts and
management interventions (Edgar & Barrett, 1999; Micheli et al., in press).

To date, however, most research on the role, effectiveness and potential of MPAs
has mostly focused on exploited taxa (fish and few invertebrates), considering
management of fisheries as a synonym to conservation of diversity of species and of
habitats. Thus, MPA effectiveness in respect to biodiversity remains mostly unclear
due to lack of reliable information on habitat distribution at the time the reserve was
established and of the quantification of the effect of protection across a range of taxa,
other than fish.

The former problem has been tackled by focusing on the spatially explicit de-
scription of patterns of biodiversity in the assemblages, with the goal of establishing
networks of MPAs (Airamé et al., 2003; Banks & Skilleter, 2002; Day & Roff, 2000;
Leslie, Ruckelshaus, Ball, Andelman, & Possingham, 2003; Sala et al., 2002). Cabeza
and Moilanen (2001) reviewed improvements to algorithms leading to proper design of reserves, but lamented a general lack of proper data to analyze (data uncertainty), since biodiversity cannot be evaluated by considering just a few taxa, as is usually done. Leslie et al. (2003) considered habitats as a surrogate of taxa (Vanderklift, Ward, & Phillips, 1998; Ward, Vanderklift, Nicholls, & Kenchington, 1999) and applied site selection algorithms to map 26 benthic habitats in the Florida Keys National Marine Sanctuary. Similarly, Sala et al. (2002) surveyed the rocky shores in the Gulf of California producing a map of benthic habitats to identify priority sites for conservation and to ensure connectivity among them. The geographic extent of these and similar studies (www.ecology.uq.edu.au/marxan.htm) is so large that their resolution is necessarily coarse.

Another issue, besides proper evaluation of biodiversity is that, once MPAs have been established, effectiveness is seldom adequately investigated by using proper experimental designs (Benedetti-Cecchi et al., 2003; Edgar & Barrett, 1999; Fraschetti, Terlizzi, Micheli, Benedetti-Cecchi, & Boero, 2002b; Gell & Roberts, 2003; Guidetti, 2002; Lasiak & Field, 1995; Russ, 2002). Assessment of environmental change involves the estimate of the mean abundance of some relevant taxa and/or of the structure of assemblages at impacted areas in comparison to multiple reference areas (Glasby, 1997; Underwood, 1991, 1993). This approach is widely applied to detect impacts of human activities (e.g. Chapman, Underwood, & Skilleter, 1995; Guidetti, Terlizzi, Fraschetti, & Boero, 2003; Hewitt, Thrush, & Cummings, 2001; Lardicci, Rossi, & Maltagliati, 1999; Roberts, 1996; Terlizzi, Fraschetti, Guidetti, & Boero, 2002), but is less widespread in the evaluation of the impact of protection (Benedetti-Cecchi et al., 2003; Garcia-Charton & Pérez-Ruzafa, 1999; Gell & Roberts, 2003).

In the Mediterranean Sea, about 200 MPAs have been established (Badalamenti et al., 2000). In most cases, however, as elsewhere in the world (Francour, Harmelin, Pollard, & Sartoretto, 2001; Roff & Evans, 2002), their planning is based on little common sense, with ad hoc evaluations and little scientific justification for site selection. Scant quantitative information is available on biodiversity distribution within MPAs (but see Villa, Tunesi, & Agardy, 2002), the variety of protected habitats and assemblages being not adequately acknowledged. Also, time series of pre-protection data are usually not available.

Here we use fine scale data from the Italian MPA of Torre Guaceto (about 10 km of coastline), in the Southern Adriatic Sea. Unlike many Italian MPAs, protection is not just nominal and entry and fishing restrictions are effectively enforced. The MPA, furthermore, was established more than 10 years ago, a long-enough period to show biological responses to protection (Halpern & Warner, 2002). As with all Italian MPAs, the area is divided in three zones (A, the no-take and no-access zone with total protection; B, the partial reserve where restrictions decrease; C, the buffer zone), varying with respect to the degree of restriction of human activities; in this MPA, there are two A zones.

The goals of the paper are: (1) Map the distribution of benthic habitats and assemblages to assess if they are properly represented in the differently protected zones. (2) Test the efficacy of protection by quantifying possible differences between the
assemblages characterizing the dominant habitats in two reference locations and in the two fully protected locations, where direct effects of anthropogenic disturbance are completely excluded.

The experiment to test the efficacy of protection in the A zones was focused on two dominant assemblage types: shallow subtidal, algal-dominated benthic assemblages (at 5 m depth) and the understory assemblages of the *Cystoseira amentacea* Bory var. *stricta* Montagne fringe (~0.1 to 0.1 m with respect to the mean low water level of rocky coast). The *Cystoseira* canopy increases local biodiversity providing space for both vagile and sessile species (Bulleri, Benedetti-Cecchi, Acunto, Cinelli, & Hawkins, 2002; Chemello & Milazzo, 2002; Fraschetti et al., 2002a). Experimental studies suggested that the loss of *Cystoseira* in the northwestern Mediterranean, determined by a suite of different disturbances, could lead to increased cover of turf-forming algae and declining abundances of invertebrates (Benedetti-Cecchi, Menconi, & Cinelli, 1999; Benedetti-Cecchi et al., 2001; Bulleri et al., 2002). In temperate areas, algal-dominated benthic assemblages in the shallow subtidal habitats have been the subjects of several studies with the conclusion that variation in their structure is often caused by a complex suite of indirect trophic interactions driven by fishing activities (Sala et al., 1998).

The two selected assemblages are appropriate to show evidence of protection because their accessibility makes them susceptible to a variety of impacts. Before hypotheses concerning specific patterns or processes can be drawn, good mensurative observational experiments are extremely useful for individual response variables or and for multivariate assemblages as a whole. Here we expected that, regardless of specific models about relevant ecological processes, human exclusion in the A zones could be able to cause differences in pattern of distribution and abundance of populations and assemblages between fully protected versus control locations.

2. Materials and methods

2.1. Study site

The MPA of Torre Guaceto (40°42′N; 17°48′E) has a surface of about 2.207 ha and is embedded within a human-dominated landscape. The terrestrial portion of the Protected Area is a naturalistic oasis of European interest, included within the list of Special Areas of Conservation of the Habitat Directive, with a wetland area of national importance according to the Ramsar Convention of 1971. The MPA extends off shore to the bathymetry of 50 m. The two A zones cover a combined area of 183 ha, the B zone covers 149 ha, and the C zone covers 1808 ha (Fig. 1).

2.2. Evaluation of habitat distribution

The PC GIS software (Surfer® version 7) was utilized to map the benthic habitats of the whole MPA. The grid-based map was produced by digitizing the shoreline from a 1:25,000 scale IGM map (Military Geographic Institute) and by the
interpolation of more than 8000 bathymetric data points. Data were collected during three extensive field surveys (September–November 2001) along a series of transects (maximum 200 m from each other) parallel and orthogonal to the shoreline. The number of transects was occasionally increased in case of sharp discontinuities (in terms of substrate morphology) for a more precise definition of the map. For each data point geographic coordinates were identified by a Global Position System (GPS), and depths were detected by a digital echo sounder. The georefering was provided according to the GAUSS-BOAGA coordinate system.

The navigation corrected XYZ data file was gridded using a Minimum Curvature algorithm (Surfer tutorial, Smith & Wessel, 1990) with a grid density of 1024 rows and 1102 columns (spacing = 10). For large data sets (>1000 observations), the gridding method of Minimum Curvature is quite fast, producing valuable map representations.

Contextually, 3500 data on the distribution of dominant habitats were collected up to a depth of 50 m. Visual censuses were carried out by snorkelling (at shallow depths) and SCUBA diving (between 15 and 50 m depth). Longitude, latitude, physical (depth and type of substratum) and biological (the presence of particularly abundant taxa) attributes were assigned to each data point, in the definition of GIS.

Data, after GIS transferring, were visualized by overlapping of a classed post map on a contour map obtained from the grid. Base maps were produced using the program SURFER, digitizing the boundaries of each habitat, assemblage and

![Study area](image.png)

Fig. 1. Study area. A zone: no take area; B zone: buffer zone; C zone: general reserve area. A1, A2: sampling locations in the A zone; C1, C2: control locations, outside the A zone.
substratum. Information was encoded into separate layers, and final maps (scale 1:5000) could be extracted from the GIS as combination of any of the layers. The obtained raster map (Urbanski & Szymelfenig, 2003) was utilized for a quick determination of the area extent of each habitat. For each habitat, the total percentage cover (area extent) in relation to the whole MPA, and its proportion in the three MPA zones were calculated.

2.3. Effectiveness: sampling design

Sampling was undertaken at four locations in May and October 2002: two locations corresponded to the two A zones and two locations were selected as reference areas (controls) outside the A zones (Fig. 1). Controls were chosen at random from a set of possible locations, to provide comparable habitats to those occurring at the fully protected locations (in terms of type and slope of the substratum and exposure to waves). Three sites (approximately 100–300 m apart from each other) were randomly selected at each of the four locations.

2.4. Sampling methods

2.4.1. The understorey assemblages of the C. amentacea var. stricta

At each site, ten 20 x 20 cm random quadrats were used to evaluate in situ the percentage cover of sessile organisms. This was made by dividing the quadrat into twenty-five 4 x 4 cm subquadrats to facilitate counting. The percentage cover was evaluated after the visual estimation of the cover of the Cystoseira canopy and the cut of its stipes. The presence of each taxon within each of these subquadrats was evaluated by giving a score from 0 to 4 to each subquadrat. Final values were expressed as percentages (Dethier, Graham, Cohen, & Tear, 1993; Meese & Tomich, 1992). Most taxa were identified at species level. However, the adopted non-destructive sampling procedure allowed a taxonomic resolution ranging from species to genera, family, order, and morphological group. The abundance of some mobile invertebrates (i.e., the gastropods Patella sp. and Columbella rustica) and of sea anemones was assessed as number of individuals in each quadrat. Small and fast-moving animals such as amphipods and polychaetes were not considered.

In order to obtain sufficiently large values for univariate analyses, taxa were grouped, according to their morphology, into: filamentous dark algae (mostly red algae belonging to the order Ceramiales), encrusting coralline red algae (including Lithophyllum sp. and Peyssonnelia spp.), articulate corallines (Amphiroa rigida, Corallina elongata), erect algae (Halimeda tuna, Wrangelia verticillata, Hypnea sp., Laurencia complex and the order of Dactyliota), filamentous green algae (Bryopsis sp., and the order of Cladophorales). The sponges (Ircinia foetida, Cliona spp., Haliclona sp., Phorbas spp.) were also grouped into a single taxon.

2.4.2. Shallow subtidal, algal-dominated benthic assemblages

Photographic samples were taken at each site. The photographic equipment consisted of a Nikonos V underwater camera, 28 mm focal length, close-up macro-
system and two SB 105-Nikon electronic strobes. To prevent problems due to loss of samples, 13 randomly located quadrats of 16 x 23 cm (total area 0.4 m²) were photographed at each site and 10 of them were used in analyses. The slides were analyzed in the laboratory, under a stereomicroscope by superimposing a transparent grid of 24 equally sized squares. Percent cover values of each taxon were then estimated according the procedure described above for the understorey assemblages of *Cystoseira*. Also in this case some taxa were grouped according to their morphology, to obtain sufficiently large values for univariate analyses: erect algae (*Flabellia petiolata*, *H. tuna*, *Padina pavonica*, *Laurencia* complex and the order of Dyctiotales), dark filamentous algae (*Ectocarpus* spp. and *Sphacelaria* spp.), filamentous green algae (Cladophorales), encrusting coralline red algae (such as *Lithophyllum frondosum*, *L. incrustans*, *Mesophyllum alternans*). All sponges (*Chondrilla nucula*, *Chondrosia reniformis*, *I. variabilis*, *Cliona* sp., *Phorbas* spp.) were also grouped.

2.5. Statistical analyses

2.5.1. Univariate analyses

A three-way analysis of variance (ANOVA) was used on data from each assemblage and from each time separately to analyse effectiveness of protection on total number of taxa and on cover of the most abundant taxa and morphological groups above described. The analyses treated the factor protection (P, 2 levels) as fixed, location (L, 2 levels) as random and nested within P and site (S, 3 levels) as random and nested within L. Prior to analyses, the homogeneity of variances was assessed by Cochran’s test and data were appropriately transformed, if required. When there was no suitable transformation, analyses were done on untransformed data and results interpreted with a more conservative level of $\alpha = 0.01$ (Underwood, 1997). Pooling procedures were also used when appropriate, according to Winer (1971). ANOVAs were done using GMAV 5 software (University of Sydney, Australia).

2.5.2. Multivariate analyses

Four distinct non-parametric multivariate analyses of variance (one for each of the two assemblages at each of the two times) (NPMANOVA, Anderson, 2001a) were used to test for differences between fully protected assemblages and reference assemblages. NPMANOVA is a hypothesis-testing method for multivariate data able to include the full three-factor model in the analyses. The analyses were based on Bray–Curtis dissimilarities (Bray & Curtis, 1957) on untransformed data (42 and 40 taxa respectively in Time 1 and Time 2) for the understorey assemblages of the *Cystoseira* canopy, and 52 and 49 taxa respectively in Time 1 and Time 2 for shallow subtidal habitat (algae-dominated benthic assemblages at 5 m depth). Each term in the analyses was tested using 999 random permutations of the appropriate units (Anderson, 2001b; Anderson & ter Braak, 2003). For some terms in the analysis, there were not enough permutable units to get a reasonable
test by permutation, so a P-value was obtained using a Monte Carlo random sample from the asymptotic permutation distribution (Anderson & Robinson, 2003). The analyses were made by DISTLM2.exe program (courtesy of M.J. Anderson).

For each assemblage and for each time, non-metric multi-dimensional scaling ordinations (nMDS) (e.g. Kruskal & Wish, 1978) were done on the basis of a Bray–Curtis dissimilarity matrix calculated from untransformed data. Ordinations were obtained by plotting centroids of the 12 S(L(P)) cells to limit the number of observation points within the framework of the plot. To obtain the four plots, principal coordinates were calculated from the Bray–Curtis dissimilarity matrices among all pairs of the 120 observation units. Centroids, as arithmetic averages, were therefore calculated using these principal coordinates. The Euclidean distance between each pair of centroids was then calculated and used as the input distance matrix for the nMDS algorithm (e.g. Anderson, 2001a; Terlizzi et al., in press). Stress values were shown for each MDS plot to indicate the goodness of representation of differences among samples (Clarke, 1993).

3. Results

3.1. Evaluation of habitat distribution

The whole area is characterised by a gently sloping bottom. Six major habitats were identified (Table 1 and Fig. 2). Sandy substrata accounted for the 39% of the total area, and were mostly in the partial reserve zone (B zone). Muddy substrata have been found in a small percentage (about 7%) and only below 45 m depth, in the general reserve zone (C zone). Biogenetic formations characterized the seafloor between 14 and 40 m depth and were present only in the general reserve zone. They were present in two forms, distinguished on the basis of the relative importance of concretioning organisms (i.e. mostly *Peyssonnelia* spp. and encrusting coralline red algae, or mainly bryozoans, serpulids and sponges). In these habitats, small patches supporting dense assemblages of the sea fans *Eunicella singularis* and *E. cavolinii* were also found.

Table 1
List of habitats and relative percentage cover in the three zones with different protection levels

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Total</th>
<th>A Zone</th>
<th>B Zone</th>
<th>C Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cymodocea nodosa</em></td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Muddy substrata</td>
<td>6.6</td>
<td>0.0</td>
<td>0.0</td>
<td>6.6</td>
</tr>
<tr>
<td>Rocky substrata</td>
<td>10.2</td>
<td>4.7</td>
<td>1.1</td>
<td>4.4</td>
</tr>
<tr>
<td><em>Posidonia oceanica</em></td>
<td>20.2</td>
<td>0.5</td>
<td>3.0</td>
<td>16.8</td>
</tr>
<tr>
<td>Biogenetic formations</td>
<td>23.8</td>
<td>0.0</td>
<td>0.0</td>
<td>23.8</td>
</tr>
<tr>
<td>Sandy substrata</td>
<td>39.1</td>
<td>3.2</td>
<td>2.9</td>
<td>32.9</td>
</tr>
</tbody>
</table>

The total refers to the total extent of each habitat within the boundaries of the reserve.
The seagrass *Posidonia oceanica* accounted for 20% of the total reserve area, mostly in the general reserve zone, interspersed among sandy patches and dead *matte* (what is left of dead rhizomes and roots, including interstitial sediment), and

Fig. 2. Map representing distribution of habitats in the three zones of the Torre Guaceto Marine Protected Area. Biogenetic formations: concretioning organisms such as *Peyssonnelia* spp., encrusting coralline red algae, bryozoans, serpulids and sponges. White dots indicate the dense assemblages of the sea fans of the genus *Eunicella*.

The seagrass *Posidonia oceanica* accounted for 20% of the total reserve area, mostly in the general reserve zone, interspersed among sandy patches and dead *matte* (what is left of dead rhizomes and roots, including interstitial sediment), and
covered the seafloor up to 17 m depth. Only 0.5% of the *Posidonia* meadows lies within the integral reserve zones, where small patches of the seagrass *Cymodocea nodosa* were also recorded. Rocky substrata accounted for about the 10% of the MPA seafloor, representing the dominant habitat type within the integral reserve zones. They were generally present from the shoreline up to 7–8 m depth. *C. amentacea* was the dominant species in the shallow infralittoral habitat. Below the *C. amentacea* fringe, a patchy algal-dominated assemblage characterised by a mosaic of different patches of turfing and erect algal taxa such as dark filamentous algae, *H. tuna* and *Halophytis incurvans* was found. Inside the A zones, rocky substrata were only occasionally represented by sea urchin barrens.

Fig. 3 shows the percentage of the different habitats, in proportion to the extent of the three MPA zones. Besides sandy substrata, evenly distributed according to the zonation of the MPA, the other habitat types occupy very different proportions of the three zones. The B zone, furthermore, is on the side of one A zone, the two A zones being mostly surrounded by the C zone.

3.2. The understorey assemblages of the *C. amentacea* var. *stricta*

On the whole, 42 taxa were recognized in the field. Algae were largely more abundant than invertebrates. At both sampling times, NPMANOVA did not
evidence differences in the structure of the understorey assemblages of Cystoseira between the A zones and controls. However, there were significant differences among sites within locations in Time 1, and between locations, and among sites within locations in Time 2, for both fully protected and reference areas (Table 2).

This was also evident in the nMDS plots of the site centroids where no clear separation among protected locations and controls emerged (Fig. 4(a) and (b)).

In both sampling times, the ANOVA on the mean cover of C. amentacea showed no significant differences between the A zones and controls. In the second sampling time, in both protected and reference areas, the percentage cover of Cystoseira was severely reduced due to large annual variations commonly experienced by the canopy formed by this species (Benedetti-Cecchi & Cinelli, 1992). The analysis of variance on mean number of taxa of the understorey assemblages did not reveal significant differences between the A zones and controls. Also, in both levels of the factor protection, there were no significant differences between locations and among sites within locations. Similar patterns were observed for total cover of algae and invertebrates found underneath canopy, where no significant differences were observed between the A zones and controls and between locations. Significant differences, though limited to Time 1, were observed among sites within locations (Table 3).

The effects of protection on most abundant taxa and/or morphological groups were also analyzed by ANOVA. Results are summarized in Table 4. In most cases

Table 2
Results of three-way NPMANOVAs testing for effects of protection on the structure of the understorey assemblages of C. amentacea and of the shallow subtidal, algal-dominated benthic assemblages

<table>
<thead>
<tr>
<th>Source</th>
<th>Time 1</th>
<th>Time 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Understorey assemblages of C. amentacea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protection = P</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Location(P) = L(P)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sites(L(P)) = S(L(P))</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Residual</td>
<td>108</td>
<td>108</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>119</td>
</tr>
<tr>
<td>MS</td>
<td>13,429.48</td>
<td>35,834.50</td>
</tr>
<tr>
<td>F</td>
<td>0.74ns</td>
<td>0.23ns</td>
</tr>
<tr>
<td>Algal-dominated benthic assemblages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protection = P</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Location(P) = L(P)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sites(L(P)) = S(L(P))</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Residual</td>
<td>108</td>
<td>108</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>119</td>
</tr>
<tr>
<td>MS</td>
<td>22,298.73</td>
<td>16,740.15</td>
</tr>
<tr>
<td>F</td>
<td>2.75a</td>
<td>1.29ns</td>
</tr>
</tbody>
</table>

ns: not significant.
Analyses based on Bray–Curtis dissimilarities on untransformed data.
Each term was tested using 999 random permutations of the appropriate units. P-values were obtained using 999 Monte Carlo samples from the asymptotic permutation distribution.

\[ a \ p < 0.05. \]
\[ b \ p < 0.001. \]
there were significant differences between locations and/or among sites within locations. Such differences, however, were independent from the effect of protection (as indicated by the non-significance of the term P). Sponges had greater abundance underneath the *Cystoseira* canopy within protected than unprotected areas in both sampling times, as evidenced by Fig. 5. However, the $F$-test associated to the term $P$ was not statistically significant, even though the obtained $F$ ratio and associated $P$ value approximated to the tabulated one (Table 4). Since these tests could have very low power to detect effects of protection, pooling procedure was used (in case of $P \geq 0.25$). Eliminating terms from the linear model did not change the results obtained with the natural denominator. In cases such as encrusting corallines in Time 1 and dark filamentous algae in Time 2, values of $P$ associated with $F$-tests significantly larger than zero prevented any possibility of pooling.

### 3.3. Shallow subtidal, algal-dominated benthic assemblages

On the whole, 61 taxa were recognized in the field. The assemblage was largely dominated by algae such as dark filamentous algae, *Dyctiota* and *Peyssonnelia* spp. Among invertebrates, only sponges (*Cliona* spp. and *Chondrilla nucula*) and...
Table 3
Three-way Analysis of variance testing for effects of protection on mean number of taxa and total cover in the two assemblages for each of the two sampling times

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Number of taxa</th>
<th></th>
<th></th>
<th>Cover</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time 1</td>
<td></td>
<td>Time 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MS</td>
<td>F</td>
<td></td>
<td>MS</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>Understorey assemblages of C. amentacea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protection = P</td>
<td>1</td>
<td>14.01</td>
<td>0.89</td>
<td>ns</td>
<td>0.03</td>
<td>0.00</td>
<td>ns</td>
</tr>
<tr>
<td>Location(P) = L(P)</td>
<td>2</td>
<td>15.71</td>
<td>2.40</td>
<td>ns</td>
<td>31.42</td>
<td>4.71</td>
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<td>1.30</td>
<td>ns</td>
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<td>2.11</td>
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<tr>
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<td></td>
<td></td>
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<td></td>
<td>None</td>
<td></td>
<td></td>
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<td>0.88</td>
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<td>108.74</td>
<td>12.62</td>
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<td>Sites(L(P)) = S(L(P))</td>
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<td></td>
<td>108.09</td>
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<tr>
<td>Total</td>
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<td></td>
<td></td>
<td>88.37</td>
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</table>

ns: not significant.

a Variances were heterogeneous (as indicated by Cochran’s C test) and could not be stabilised by transformations.
b The term is not significant due to the more conservative level adopted in analyses where variances were not stabilised by transformations.
c Tested against the pooled term: L(P) + S(L(P)) (df = 10).
d p < 0.05.
e p < 0.01.
f p < 0.001.
encrusting bryozoans (*Schizobrachiella sanguinea* and *Reptadeonella violacea*) were found to be abundant.

NPMANOVA provided evidence of significant differences in the structure of the assemblage between the A zones and controls in Time 1. Also, there were significant differences between locations and sites within locations, (Table 2). Such patterns were also evident by the visual inspection of nMDS plots where site centroids did separate protected from reference areas only in Time 1 (Fig. 4(c) and (d)).

The ANOVA performed on cover and total number of taxa did not show significant differences between the A zones and controls. Significant differences among sites within locations (Time 1) and between locations (Time 2) were observed in the analysis of mean number of taxa. In Time 1, cover significantly differed among sites within locations; in Time 2, significant differences were observed among sites within locations and among locations (Table 3).

Mean percentage cover of most taxa and morphological groups significantly differed among sites within locations, without significant differences between the A zones and controls (Table 5). The only exceptions to this trend were observed for the encrusting corallines, sponges and dark filamentous algae. Encrusting corallines, though only in Time 1, were found significantly more abundant in controls than in fully protected areas. In Time 2, differences between the A zones and controls were

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**Table 4**

Analysis of variance on mean percentage cover of most abundant taxa and/or morphological groups in the understorey assemblages of *C. amentacea*

<table>
<thead>
<tr>
<th>Source</th>
<th>Protection = P</th>
<th>Location(P) = L(P)</th>
<th>Sites(L(P)) = S(L(P))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time 1</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>C. amentacea</em></td>
<td>681.33</td>
<td>0.25&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>2685.00</td>
</tr>
<tr>
<td>Sponges</td>
<td>1.87</td>
<td>17.31&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.11</td>
</tr>
<tr>
<td>Articulated corallines</td>
<td>5187.67</td>
<td>2.02&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>2566.04</td>
</tr>
<tr>
<td>Erect algae</td>
<td>381.63</td>
<td>0.04&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>9964.83</td>
</tr>
<tr>
<td>Dark filamentous algae&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>22.22</td>
<td>2.66&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>8.48</td>
</tr>
<tr>
<td>Encrusting corallines</td>
<td>149.63</td>
<td>1.88&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>79.62</td>
</tr>
</tbody>
</table>

**Time 2**

| *C. amentacea*<sup>c</sup> | 5.63           | 4.12<sup>ns</sup>| 1.37                 | 0.76<sup>ns</sup>    | 1.79      | 1.38<sup>ns</sup> |
| Sponges<sup>c</sup>        | 12.67          | 0.59<sup>ns</sup>| 21.51                | 34.41<sup>f</sup>    | 0.62      | 0.63<sup>ns</sup> |
| Articulated corallines<sup>c</sup> | 630.21         | 0.03<sup>ns</sup>| 22,270.40            | 6.43<sup>d</sup>     | 3463.85   | 18.39<sup>f</sup> |
| Erect algae<sup>a,b</sup>  | 35.89          | 3.39<sup>ns</sup>| 8.08                 | 0.72<sup>ns</sup>    | 11.20     | 15.85<sup>f</sup> |
| Dark filamentous algae<sup>c</sup> | 39.67         | 1.02<sup>ns</sup>| 38.71                | 2.00<sup>ns</sup>    | 19.32     | 1.59<sup>ns</sup> |
| Encrusting corallines<sup>a</sup> | 2.71           | 0.12<sup>ns</sup>| 21.73                | 7.14<sup>d</sup>     | 3.04      | 6.18<sup>f</sup>  |

<sup>ns</sup>: not significant.

<sup>a</sup> Data transformed to remove heterogeneity of variance (as indicated by Cochran’s C test).

<sup>b</sup> Tested against the pooled term: L(P) + S(L(P)) (df = 10).

<sup>c</sup> Variances were heterogeneous and could not be stabilised by transformations.

<sup>d</sup> $p < 0.1$.

<sup>e</sup> $p < 0.01$.

<sup>f</sup> $p < 0.001$. 

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evident from the graph (Fig. 6), but the $F$-test associated with the term $P$ was not statistically significant, and pooling was not possible (Table 5). Sponges did not show any significant influence of protection. An opposite pattern was found for dark filamentous algae (pooled data: $F_{1,10} 9.54, P < 0.05$).

In Time 2, the alga *Caulerpa racemosa* (Bryopsidales Chlorophyta), an introduced species, which was absent at Time 1, appeared in the shallow subtidal habitat both in the reserve area and in one control, with a percentage cover of up to 12% ($\pm 5.4$) (Fig. 6). The mean abundance of this species did not show significant differences between protected and reference areas, but was found significantly differing at the scale of sites.
4. Discussion

The mapping of habitat and assemblage distribution showed that the present zonation of the MPA does not represent the full range of habitats present within the reserve boundaries. The comparison between the A zones and controls showed few evident effects of protection on both population and assemblage structure, leading to an impressive record of no-significant results when comparing protected and reference areas.

The first result is not surprising since, especially in the Mediterranean Sea, studies of MPAs highlighted that most zonations are arbitrary (Villa et al., 2002) and that A zones are mostly located near the shore for convenience. The second result has to be carefully discussed even though it is not a novel finding. Species diversity and ecological cascades can have complicated responses to protection, so that the distribution of assemblages has often been found inconsistent with the presence of MPAs (Benedetti-Cecchi et al., 2003, but see also Palumbi, 2001 for general considerations).

Apulia is about 800 km long, the three MPAs established in the last 10 years cover 63 km of coast, about 8% of the total. The choice of the protected sites does not stem from a thorough knowledge of Apulian “regional diversity”. Basic questions such as how many reserves are needed to represent the regional biodiversity?, how large they

<table>
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<tr>
<th>Source</th>
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<th>Sites(L(P)) = S(L(P))</th>
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<tr>
<td></td>
<td>MS F</td>
<td>MS F</td>
<td>MS F</td>
</tr>
<tr>
<td>Time 1</td>
<td></td>
<td></td>
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<tr>
<td>Encrusting corallines</td>
<td>16.49 18.60d</td>
<td>0.88 0.34ns</td>
<td>2.57 6.62f</td>
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<tr>
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<td>1208.82 1.71ns</td>
<td>706.19 4.42f</td>
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<td>Dark filamentous algae</td>
<td>4095.01 9.54e</td>
<td>308.44 0.67ns</td>
<td>459.47 4.89f</td>
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<td>964.27 12.35e</td>
<td>78.09 0.55ns</td>
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<td>7.43 0.50ns</td>
<td>14.76 7.55d</td>
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<td><em>Caulerpa racemosa</em></td>
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Time 2

<table>
<thead>
<tr>
<th>Source</th>
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<th>Sites(L(P)) = S(L(P))</th>
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<tr>
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<td>43.30 1.70ns</td>
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<td>8052.40 13.29ns</td>
<td>606.04 2.08ns</td>
<td>290.87 1.54ns</td>
</tr>
<tr>
<td>Dark filamentous algae</td>
<td>229.63 0.32ns</td>
<td>711.75 3.77ns</td>
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</tr>
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<td>Sponges</td>
<td>0.02 0.00ns</td>
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<td>5.31 6.10f</td>
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<tr>
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<td>7.01 0.03ns</td>
<td>253.74 2.11ms</td>
<td>120.34 3.11e</td>
</tr>
</tbody>
</table>

ns: not significant.

* Data transformed to remove heterogeneity of variance (as indicated by Cochran’s C test).
* Variances were heterogeneous and could not be stabilised by transformations.
* Tested against the pooled term: L(P) + S(L(P)) (df = 10).
* \( p < 0.1. \)
* \( p < 0.01. \)
* \( p < 0.001. \)
should be?, where they have to be located especially in relation to propagule dispersal distance? have not been considered (Grantham, Eckert, & Shanks, 2003; Shanks, Grantham, & Carr, 2003). The possibility of designing networks of marine reserves to represent regional diversity has been recently pointed out elsewhere in the world (Sala et al., 2002), but this issue is still not debated in the Mediterranean area.

The MPA of Torre Guaceto comprises a set of very different habitats (from bioconstructions to seagrass meadows) characterized by complex spatial patterns. The lack of adequate knowledge of biodiversity distribution before the institution of the MPA prevented appropriate decision about reserve boundaries, with the consequence that habitats such as biogenetic formations are not included in the A zones. Only a very small portion of the meadows of the seagrass *P. oceanica* is represented

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**Fig. 6.** Mean percentage cover (±SE, n = 10) of most abundant taxa in the algal-dominated assemblages in the shallow subtidal. Data are shown for each of the three sites in the two A zones (black bars) and in controls (white bars) in the two sampling times (T1, T2).
inside the A zones, in spite of their biological and ecological importance (Boudouresque, Meinesz, Fresi, & Gravez, 1989). Integral reserve areas, the A zones of Italian MPAs, should incorporate the range of habitats present within MPA boundaries or, at least, the most valuable ones.

In this work, the use of two assemblages described by variables, such as the structure of the assemblage, and the mean abundance of common taxa, should have been able to test protection efficacy against different forms of impact, from exploitation to trampling and diving activities. In general, effects of protection can consist of differences from an increased number of taxa inside the reserve to different pattern of spatial heterogeneity between protected and unprotected areas. However, sometimes removal of fishing pressure may cause a decline in species richness because of unpredictable ecosystem changes (Edgar & Barrett, 1999). Our results showed that few patterns were consistent with the predicted effect of protection, with both the use of univariate and multivariate approaches. There can be several reasons for the general lack of differences between protected and reference areas. We can exclude anthropogenic disturbance in the form of exploitation (e.g., gathering of the bivalves *Mytilus* sp. and/or *L. lithophaga*, diving activities) within the A zones, due to actual protection enforcement. Conversely, it is possible that reference areas were not subjected to ecologically detectable human impact before MPAs were established. The absence of pre-protection data makes it hard to achieve evidence of protection and of selection of proper controls.

Most taxa showed no significant difference between the MPA and the controls. Sponges living under the *Cystoseira* canopy and in the shallow subtidal habitat, however, were found more abundant inside the integral reserve areas than in controls. Sponges are long-lived and slow-growing species. Their higher abundance in the A zones with respect to controls, could indicate an effect of human exclusion on this group. It has been demonstrated that trampling, for instance, in the form of a press disturbance can have appreciable effects on assemblages of shallow rocky shores, leading to differences between disturbed and undisturbed areas (Keough & Queen, 1998). Conversely, encrusting coralline red algae were found more abundant in controls than inside the integral reserve areas. Encrusting coralline red algae along with sea urchin are the dominant components of rocky barrens. Even though mapping confirmed that barrens were substantially absent within the reserve, result of univariate analyses should be considered with caution since sampling resolution is too low to distinguish between barren habitat from small patches of encrusting corallines produced by other processes. Barren habitat has been intensively investigated over the last decades, and is currently considered as a degenerative state of the sublittoral rocky-reef ecosystem. Sea urchin overgrazing, among other possible reasons, might be linked to lack of sea urchin predators (Sala et al., 1998) or to proper conditions for sea urchin development after the destructive fishery of the date mussel, *Lithophaga lithophaga*, which is known to lead to barrens (Fanelli, Piraino, Belmonte, Geraci, & Boero, 1994; Guidetti, Fraschetti, Terlizzi, & Boero, 2003). The higher extent of encrusting coralline red algae outside the reserve could be a sign of indirect effects suggesting a predatory pressure on sea urchin due to higher abundance of fish inside the integral reserve areas than in reference areas, and/or a better
protection from damage caused by date mussel fisheries. More quantitative data have to be appropriately collected to adequately address this issue.

A lack of statistical power of tests possibly prevented the detection of ecologically relevant effects of protection. The attempts to increase the power of the test by pooling supported the possibility that in some cases (i.e. dark filamentous algae in the shallow subtidal) there might be signs of a “reserve effect”. However, they were never consistent in time, with the consequences that it is hard to interpret their higher abundance as a consequence of human exclusion. Differences in the outcomes for structure of the assemblage and for some taxa between the two times suggest that, with more resources, it would have been feasible to test for differences between protected vs. unprotected locations in spatio-temporal variation of populations and assemblages. On this basis, optimization of the experimental design defining the optimal replication level in space and time should be considered in further studies. Only long-term biodiversity monitoring will allow a more precise evaluation of the efficacy of protection, teasing apart the effects of human exclusion from other sources of variability.

As already stressed in Section 1, protection effectiveness has mostly been demonstrated on species of commercial importance. Very few studies (Benedetti-Cecchi et al., 2003; Edgar & Barrett, 1999; Lasiak & Field, 1995) tried to assess the potential effects of MPAs on several biological attributes of benthic assemblages. In general, results for invertebrates are less clear than those obtained from fish and the effects of reserve protection seem to depend in part on the exploitation level of the invertebrates and in their position in the food chain (Halpern, 2003). Lasiak & Field (1995) exploring the effects of a press disturbance in the form of shellfish gathering between exploited and non-exploited rocky intertidal macrofaunal assemblages in Transkei (South Africa) found contrasting evidence according to the employed approaches. Univariate analyses did not evidence any significant difference between impacted and non-impacted sites, whereas these were sharply separated by multivariate analyses. Evident signs of protection were not detected by Benedetti-Cecchi et al. (2003) on assemblages of algae and invertebrates of shallow rocky coasts in the northwest Mediterranean and the conclusion was that neither the full range of assemblages nor the relevant scales of variation were properly represented within the MPA.

Edgar & Barrett (1999) found significant increases in the number and density of fish, invertebrates and algal species in the largest reserves studied, whereas changes in the same variables were not detected in the smallest reserves, thus showing that effectiveness of marine reserve was directly proportional to their size.

The comparison of impacted sites with suites of non-impacted sites is a powerful tool to detect the result of intense influences on environmental integrity, and is widely employed to show the effect of negative human activities (Underwood, 1994). These experimental designs have been seldom used to show evidence of protection between reserve areas and controls, considering protection as a “positive” impact. As in cases of environmental impact assessment, the efficacy of protection might prove difficult to test since the uniqueness of protected sites makes difficult the choice of proper controls. Inadequate controls will lead to confound effects of protection from intrinsic differences between protected and unprotected sites.
Another result is the observed spreading of *Caulerpa racemosa*. This species has been classically considered as a Lessepsian migrant introduced via the Suez Canal into the Mediterranean Sea (Aleem, 1948), where it colonized several areas of the southern and eastern basin (Buia, Gambi, Terlizzi, & Mazzella, 2001; Piazzii, Balestri, Magri, & Cinelli, 1997), rapidly expanding westwards along the north-western coasts. This invasive species can affect native ecosystem by decreasing biodiversity (Ceccherelli, Piazzii, & Balata, 2002). Its appearance in the second sampling time apparently did not affect diversity patterns, but this phenomenon merits attention since turf habitats, widely dominating the reserve, are conducive to the spreading of this alien species (Ceccherelli et al., 2002). From this perspective, a longer monitoring within MPAs will be essential not only for the understanding of the effects of protection on resident assemblages but also to provide guidelines for more comprehensive strategies of marine environment protection in case of sudden modifications such as the arrival of alien species and their interactions with resident populations (Edgar, Moverley, Barrett, Peters, & Reed, 1997).

The main outcomes of this paper are that the design of MPAs should be based on scientific criteria, preferably with the goal of covering the main components of regional diversity. Habitats occur as a mosaic of interconnected units and this distribution pattern must be considered in conservation policies (Gray, 1997). In addition, the efficacy of protection might prove difficult to test even with sound experimental designs, if the goals of each MPA have not been clearly stated before.

**Acknowledgements**

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**References**


