# Species, trophic, and functional diversity in marine protected and non-protected areas

Adriana Villamor [1], Mikel A. Becerro *

Center for Advanced Studies of Blanes (CEAB-CSIC), Accés a la Cala Sant Francesc 14, 17300 Blanes, Spain

ABSTRACT

The number of Marine Protected Areas (MPAs) has grown exponentially in the last decades as marine environments steadily deteriorate. The success of MPAs stems from the overall positive benefits attributed to the "reserve effect," the totality of the consequences of protecting marine systems. The reserve effect includes but is beyond the goal of protecting particular species or areas with economical or cultural value. However, most data on the effects of MPAs focus on target species and there is limited evidence for the consequences of protection at larger levels of organization. Quantitative information on the reserve effect remains elusive partly because of its complex nature. Data on biodiversity can be used to quantify the reserve effect if not restricted to specific taxonomic groups. In our study, we quantified species diversity, trophic diversity, and an approach to functional diversity in five MPAs and adjacent non-protected areas along the Mediterranean coast of Spain. Our three measures of diversity were based on the abundance of algae, fish, sessile and mobile invertebrates in shallow water rocky communities and could be used to estimate the reserve effect based on species, trophic levels, or functional roles. We tested the hypothesis that species, trophic, and functional diversity were higher in protected areas than in adjacent non-protected areas. Species diversity varied with geographic area but not with protection status. However, we found higher functional diversity inside MPAs. Also, the effect of protection on functional diversity varied as a function of the geographic area. Our results support the uniqueness of MPAs at a species level and the universality of the reserve effect at the level of the trophic groups' composition. This type of comprehensive ecological approach may broaden our understanding of MPAs and their efficiency as management tools.

© 2012 Elsevier B.V. All rights reserved.

## 1. Introduction

Ever since the beginning of conservation biology as a science in the 70s, biodiversity has been considered one of the most important indicators of ecological value and a main target for protection (Chapin et al., 2000). Biodiversity studies are challenged not only by the complexity of natural systems but also by the concept of biodiversity itself. Measuring biodiversity includes quantification of the number of entities (e.g., species richness), their abundances (e.g., evenness), dissimilarities (e.g., genetic divergence or morphological disparity), and functional roles in the communities they inhabit (Magurran, 2004; Sala and Knowlton, 2006). These four pillars of biodiversity complement each other and should all be taken into account specially when making decisions on protection policies.

Protected Areas are a widespread tool for resource management and biodiversity protection and excel among the numerous lines of actions to preserve biodiversity (Bruner et al., 2001). Protected areas provide multiple conservation benefits and their numbers have increased to cover over 12% of the Earth's land surface (Jenkins and Joppa, 2009). However Marine Protected Areas (MPAs) lag far behind their terrestrial counterparts. For example, the World Database on Protected Areas (www.wdpa.org) includes 362 MPAs in the Mediterranean but they only cover a 3% of its surface. MPAs vary tremendously in conservation benefits (Barrett et al., 2007) partially due to intrinsic differences between MPAs, levels of enforcement, and variety of goals among other factors (Guidetti et al., 2008; McClanahan and Arthur, 2001). Regardless of the specific goals of MPAs, the "reserve effect" is an inherent goal to any type of protected area. It is known as the totality of consequences in the marine environment of the protection measures on benthic and demersal organisms (Bell and Harmelin-Vivien, 1983; Francour, 1994). This reserve effect includes all levels of the community, beyond target species or direct effects of protection, and it remains to be accurately documented.

Many ecological and biological data often refer to one species or group of species that are somehow emblematic and reports of increased abundance and larger size of target species are common in the literature (Barrett et al., 2007; Claudet et al., 2006; Lasiak, 2006;

* Corresponding author at: Natural Products and Agrobiology Institute (IPNA-CSIC), Avda. Astrofísico Francisco Sánchez 3, 38206 La Laguna, Tenerife, Spain. Tel.: +34 922 256 847; fax: +34 922 260 135.
E-mail addresses: adriana.villamor@unibo.it (A. Villamor), m.becerro@csic.es (M.A. Becerro).
[1] Present address: Laboratorio di Ecologia Sperimentale. Università degli Studi di Bologna, Via S. Alberto 163, 48100 Ravenna, Italia.

Pederson and Johnson, 2006; Tuya et al., 2006, among others). This approach is understandable since resource extraction of particular species is a major interaction with the environment and many MPAs aim to preserve these types of resources (Forcada et al., 2008, 2009; Harmelin-Vivien et al., 2008; Ordines et al., 2005). Whether single species conservation can preserve biodiversity or communities as a whole is repeatedly questioned and depends largely in the target species life habits. Single species approach to protection has been and still is widely used for its simplicity and flagship value when facing the public opinion (e.g., panda bear, lynx, monk seal). However, single species conservation has numerous limitations (Lister, 1998; Simberloff, 1998). Comparatively, we barely know the effect of protection on non-target species let alone on whole communities. Studies reporting effects of MPAs on biodiversity often focus on a specific group of species such as algae (McClanahan, 1997; Sala et al., 1998a), molluscs (Lasiak, 2006), macrofauna (Sala et al., 1998b) or fish (Guidetti and Sala, 2007; Guidetti et al., 2005) rather than on biodiversity at the whole community level (Edgar and Barrett, 1999; Guidetti, 2006; Micheli et al., 2005). This is probably due to the inherent difficulty to assign causality to changes in diversity and identity of species (Benedetti-Cecchi, 2004).

Moreover, species inventories are of limited use to set conservation priorities. Many ecosystem processes and services depend more on functional diversity than species diversity *per se* (Nyström, 2006). Understanding the significance of species in their natural habitats is therefore critical in conservation. However, less than 1% of species described have been studied beyond merely noting their existence (Wilson, 2000), so our understanding of their role in nature is limited. We must preserve functional diversity to assure ecosystem functionality. The majority of information on how MPAs affect functional diversity refers to shifts in the abundance of trophic levels mediated by consumer–prey interactions, i.e., trophic cascades (Pace et al., 1999; Paine, 1980). Because fishing typically follows a top-down approach (Friedlander and DeMartini, 2002; Pauly et al., 1998), MPAs provide unique systems to assess the role of trophic cascades in marine environments (McClanahan et al., 1996; Pinnegar et al., 2000; Sala et al., 1998a). Indeed, available evidence supports the contention that the strongest response to protection corresponds to species that are at top trophic levels (Micheli et al., 2004). Beyond the trophic approach, a better understanding of the effect of protection on functional diversity would benefit MPA research tremendously.

The scarcity of information on the effect of protection on species and functional diversity at a community level contrasts with the worldwide reliance on MPAs to preserve biodiversity and the services it provides. We need empirical evidence to support or refute the capacity of MPAs to preserve species, trophic, and functional diversity. In this study we focused on the overall reserve effect at a community level, testing whether MPA-driven changes in marine communities cause shifts in the ecological organization and functioning of the ecosystem (García-Chartón et al., 2008). Protection may increase both the number and abundance of species locally absent in a particular area, contributing to increase species richness and evenness after protection. We therefore expect higher species diversity, higher trophic levels, and higher functional diversity in protected as compared to non-protected areas. Specifically, we tested whether the Shannon diversity index calculated with i) species, ii) trophic groups, and iii) functional groups differ between protected and non-protected areas. To categorize species, we used the six widespread trophic groups modified from McClanahan and Sala (1997) and a functional grouping that join species life history traits and taxonomic groups (Villamor and Becerro, 2010). Since the magnitude of the reserve effect may vary with the geographic location, the species present, the design of the MPA, and type of community (García-Chartón et al., 2008), replication is mandatory to overcome the confounding effects of treatments with those of uncontrolled natural factors (García-Chartón and Pérez-Ruzafa, 1999). Although spatial and temporal replication before and after MPA implementation is the ideal sampling design to test for the effect of reserves (Guidetti, 2002), most studies seldom satisfy this design. In our study, we identified and quantified the abundance of algae, sessile invertebrates, echinoderms, and fish fauna in multiple locations of five well-established MPAs and nearby unprotected areas distributed along the Spanish Mediterranean coast. Because our surveys took place at least 10 years after the creation of the MPA, our data represent an estimate of the effect of protection on the biodiversity and functional diversity of Mediterranean shallow communities.

## 2. Materials and methods

### 2.1. Field survey

We surveyed five MPAs distributed along the Spanish Mediterranean coast in August 2008. Two MPAs are located in the North of Spain (Cap Creus and Medes Islands, Girona), at about 30 km of distance from each other (Fig. 1). The others three MPAs are located in the Southeast of Spain (Tabarca, Alicante; Cabo Palos-Islas Hormigas, Murcia; and Cabo de Gata-Níjar, Almeria), and are more distant from each other (60 and 160 km respectively, Fig. 1). Each MPA has its own particularities (Table 1) but they all represent typical Mediterranean habitats including shallow rocky bottoms, deeper coralligenous bottoms and *Posidonia oceanica* beds. In order to minimize the effect of different protection regimes we aimed to sample in the no-take areas defined in each of them. To target equivalent communities in each MPA we investigated rocky bottoms between 4 and 11 m deep. Shallow water habitats are highly affected by many human activities so we expected these communities to obtain the largest benefits from protection.

We surveyed three sites inside each MPA and three in unprotected areas nearby. Average distance between protected and unprotected sites was approximately 4 km in Medes Islands and Tabarca MPAs, 7 km in Cabo Palos-Islas Hormigas, 10 km in Cabo de Gata-Níjar and 12 km in Cap Creus, depending mainly on the MPA area and shape. Precise coordinates of each sampling site are shown in Table 1. In each of these sites we quantified fish, sessile benthos, sea urchins, and sea stars. In each site we used underwater visual census (UVC) to quantify fish fauna by family and size along a 50 m long transect line in a 5 m wide strip. Size was categorized as small, medium, and large. For each fish family we defined the size categories according to the most common species in the area (Table 2). We used this same 50 m long transect to quantify benthic composition using the point-intercept method: every 0.5 m, we identified the organism underneath the transect line to the lowest taxonomic level possible. Sea urchin and sea star abundance was quantified along 50 m$^2$, in a one meter wide strip centered in the transect line. Only sea urchins over 2.5 cm of diameter were quantified (Edgar et al., 2011).



**Fig. 1.** Situation map of the five MPAs studied on the east coast of Spain, western Mediterranean: from north to south Cap de Creus, Medes Islands, Tabarca island, Cabo Palos—Islas Hormigas, and Cabo de Gata.

## 2.2. Data treatment

We assigned species to six simple but universal trophic groups: top predators, carnivores, herbivores, filter feeders, erect producers, and barren ground producers (modified from McClanahan and Sala, 1997, for the same geographic area). Abundances of species belonging to the same trophic group were pooled together. Herbivore data were previously standardized because herbivore fish and sea urchins were quantified in different units. Resulting trophic groups were again standardized for the same reason.

We also grouped species into categories that represent ecologically distinct and relevant groups suitable to examine overall changes in the benthic community (Britton-Simmons, 2006; Perner and Voigt, 2007; Villamor and Becerro, 2010). The categories applied merge taxonomy with physical structure and life history traits, preventing overrating species over their function in the community. We classified algal abundance data into seven widely used groups modified from Steneck and Watling (1982), i.e. filamentous, foliose, corticated, leathery, calcareous, crustose coralline algae, and phanerogams (mainly *P. oceanica*). Invertebrates were grouped as: sea urchins, sea stars, sciaphilic sponges (those living away from direct light, as caves, crevices or overhangs), photophilic sponges (those living exposed to direct light in open areas), Hydrozoa, Actiniaria, Madreporaria, Alcyonacea, Gorgonacea, Ascidiacea, Bryozoa, and Polychaeta. We grouped fish according to their taxonomical family (mainly Sparidae, Serranidae, and Labridae, but also some Sciaenidae) and body size, so each family is divided in three groups, small, medium and large. *Sarpa salpa* was quantified in separate groups, apart from the rest of the Sparidae family to which it belongs, as this species is herbivorous in contrast with most of the family species, which are carnivorous or omnivorous. The aim of this classification is to propose an intermediate way of organization, not as rough as trophic groups neither as redundant as species. It cannot be considered a functional classification as we maintain a certain taxonomic grouping; however we divide taxonomic units into subgroups, not only for their feeding mode but also for their habitat use and life history traits (Table 2).

Three diversity indexes were calculated using the classic Shannon *H'* diversity index on the abundance of species, trophic levels, and our functional groups, providing a single value for each of the three classifications of our multivariate data. Since *H'* values can be affected by sampling size (Ramezani and Holm, 2011), the functionality of this index as a standard measure of biodiversity is limited. However, the method used provides identical sample size among all sites so, within the scope of our study, *H'* values are comparable and hence can be used to quantify differences in biodiversity between geographic areas and protection status (Garrabou et al., 2002; Wlodarska-Kowalczuk et al., 2005).

## 2.3. Data analysis

We tested for differences in species, trophic, and functional diversity between geographic areas and protection status with a two-way mixed model analysis of variance. Geographic area was the random factor and included all protected and unprotected sites located in and around each particular MPA (6 replicates for each geographic area). Protection status was the fixed factor, which referred to the protected and unprotected sites regardless of geographic location (15 replicates for each protection status). This analysis allowed testing for a significant interaction between geographic area and protection status, i.e. for the variable effect that protection may have on diversity as a function of geographic area.

We also ran two factor PERMANOVAs (Anderson, 2001) on the species, trophic groups, and functional groups multivariate data with geographic area as random and protection status as fixed factors, using Bray–Curtis as similarity measure. Similarity percentage analysis provided the main trophic and functional groups that differed between protected and unprotected areas. The significance of the differences found among groups between protected and unprotected sites was evaluated with univariate analysis of variance with protection status as a fixed factor. Post-hoc multiple comparisons were performed when the interaction term resulted significant. This analytical strategy

**Table 1**

Information on the five MPAs investigated in this study ordered from north to south, with exact coordinates of our sampling points, protection status, year of declaration, protected area (in Ha), some examples of studies carried out in the same MPAs, and a link to obtain further information.

| Geographic area | Protection status | Sampling sites coordinates | Year of declaration | Area | Recent MPA research | Web site |
|---|---|---|---|---|---|---|
| 1. Cap de Creus | Terrestrial and marine natural park | 42° 17′ 4 ″ N/3° 17′ 54″ E<br>42° 14′ 56″ N/3° 13′ 39″ E<br>42° 19′ 22″ N/3° 18′ 29″ E | 1998 | 3064 | Rossi et al. 2008<br>Lloret and Riera 2008 | www.gencat.cat/parcs/ |
| | Unprotected areas | 42° 6′ 50″ N/3° 10′ 1″ E<br>42° 25′ 35″ N/3° 9′ 53″ E<br>42° 21′ 6″ N/3° 11′ 9″ E | | | | |
| 2. Medas Islands | Natural reserve | 42° 2′ 31″ N/3° 13′ 34″ E<br>42° 3′ 0.1″ N/3° 13′ 28″ E<br>42° 2′ 50″ N/3° 13′ 33″ E | 1983 | 511 | Francour et al. 2001<br>Lopez-Sanz et al. 2009<br>Sala et al., 1998a, 1998b | www.gencat.cat/parcs/ |
| | Unprotected areas | 42° 3′ 39″ N/3° 12′ 49″ E<br>42° 4′ 1″ N/3° 12′ 39″ E<br>42° 4′ 23″ N/3° 12′ 20″ E | | | | |
| 3. Tabarca Island | Marine reserve | 38° 9′ 41″ N/0° 27′ 30″ W<br>38° 9′ 22″ N/0° 28′ 11″ W<br>38° 10′ 8″ N/0° 29′ 28″ W | 1986 | 1400 | Francour et al. 2001<br>Forcada et al., 2008;<br>Forcada et al., 2009 | www.alicante-ayto.es |
| | Unprotected areas | 38° 10′ 35″ N/0° 29′ 9″ W<br>38° 10′ 3″ N/0° 27′ 8″ W<br>38° 11′ 37″ N/0° 31′ 1″ W | | | | |
| 4. Cabo Palos-Is. Hormigas | Marine reserve | 37° 39′ 21″ N/0° 39′ 0″ W<br>37° 39′ 9″ N/0° 39′ 12″ W | 1995 | 1931 | García-Chartón &<br>Pérez-Ruzafa 2001<br>García-Chartón et al. 2004 | www.magrama.es |
| | Unprotected areas | 37° 38′ 5″ N/0° 41′ 42″ W<br>37° 38′ 13″ N/0° 41′ 19″ W<br>37° 37′ 26″ N/0° 42′ 9″ O | | | | |
| 5. Cabo Gata-Níjar | Protected landscape and seascape | 36° 56′ 21″ N/1° 54′ 4″ W<br>36° 49′ 51″ N/2° 0′ 26″ W<br>36° 43′ 15″ N/2° 11′ 33″ W | 1995 | 4613 | Figueroa et al. 2002 | www.magrama.es |
| | Unprotected areas | 37° 15′ 13″ N/1° 45′ ′ 49″ W<br>37° 1′ 4″ N/1° 52′ 40″ W<br>36° 59′ 30″ N/1° 53′ 12″ W | | | | |

takes full advantage of our multivariate data, provides detailed information on the specific groups that are responsible for differences between protected and unprotected sites, and can be used to check the adequacy of single diversity values to estimate reserve effect.

## 3. Results

Species diversity varied significantly between geographic areas but not between protected and unprotected sites (Table 3, Fig. 2a). We also found significant differences in species composition between geographic areas (PERMANOVA, $p = 0.001$, Table 4, Fig. 3a).

Differences in trophic diversity between protected and unprotected sites varied with geographic area (significant interaction term, Table 3, Fig. 2b). Only Tabarca and Cabo de Palos showed significant differences between protected and unprotected trophic diversity ($F$-ratio = 48.39 and 42.83; $p = 0.006$ and 0.002 respectively). We found no differences in trophic diversity associated with geographic area or protection status alone. Multivariate analysis of trophic groups composition showed significant differences associated exclusively with protection status (PERMANOVA, $p = 0.027$, Table 4, Fig. 3b). Top predators and herbivores contributed the most to the dissimilarity between protected and unprotected sites (26.34% and 21.46% respectively, Fig. 4). Top predators were significantly more abundant inside than outside MPAs (ANOVA, $p = 0.021$, Fig. 4) but the higher abundance of herbivores outside MPAs was not statistically different (ANOVA, $p = 0.271$). We also found significantly larger abundances of carnivores and filter feeders ($p = 0.022$ and $p = 0.029$ respectively) inside MPAs (Fig. 4).

We found higher functional diversity (*sensu* the present approach) in protected than in unprotected areas (Table 3, Fig. 2c). Functional diversity also varied between geographic areas (Table 3). We found differences in functional groups composition between geographic areas (PERMANOVA, $p = 0.001$, Table 4). Differences in functional groups between protected and unprotected sites might vary as a function of geographic area (interaction term nearly significant, $p = 0.072$, Table 4). Several functional groups consistently showed different abundances between protected and unprotected areas across all geographic areas: fish functional groups were generally more abundant inside MPAs, with

the exception of *S. salpa*, which was more abundant in unprotected sites, although only small labrids and serranids showed significant coefficients ($p = 0.007$ and $p = 0.033$ respectively). Two invertebrate groups, Madreporaria and Bryozoa, were also more abundant inside than outside MPAs ($p = 0.046$ and $p = 0.05$). On top of these broad scale differences, each geographic area showed particular differences between protected and unprotected sites. Filamentous algae were more abundant inside than outside Cap de Creus MPA ($p = 0.002$). Foliose algae were more abundant and corticated algae less abundant inside than outside Cabo Palos MPA ($p = 0.032$ and $p = 0.021$ respectively). Calcareous algae and sciaphilic sponges were more abundant inside than outside Tabarca MPA ($p = 0.013$ and $p = 0.012$ respectively). We found no significant differences in benthic functional composition between protected and unprotected sites neither in Medas nor in Cabo de Gata geographic areas.

## 4. Discussion

Our study aimed to test for community-level differences in marine protected and unprotected areas as an attempt to quantify the so called "reserve effect," i.e., the consequence of protection on the system rather than on specific target species (Bell and Harmelin-Vivien, 1983; Francour, 1994). We took a comprehensive approach to quantify the abundance, trophic levels, and functional groups of the species inhabiting shallow rocky communities inside and outside five Mediterranean MPAs. Our data also allowed us to calculate species, trophic, and functional diversity, reducing our multivariate data into single diversity values. We found higher functional diversity in MPAs than in adjacent unprotected areas. Species diversity differed between the localities studied but not between protected and unprotected sites while differences in trophic diversity between protected and unprotected sites varied as a function of the locality. Beyond these differences in diversity values, we also found that species and functional groups composition varied with locality while trophic structure differed between protected and unprotected areas. Overall, our results suggest that the effect of protection may be more pronounced in the functional traits of the ecosystem than in species composition and abundance.

Species composition varied between localities but not between protected and unprotected sites. The value of species diversity showed the same pattern. Besides supporting the role of geography in the abundance and distribution of species, these results suggest that we need to increase the number of geographic areas that are protected to increase biodiversity protection. Barrett et al. (2009) detected quite stable species richness of benthic invertebrates and algae on four adjacent Tasmanian MPAs regardless of protection status, while Alexander et al. (2009) even found lower species richness inside than outside one of these Tasmanian MPAs. They concluded that species abundance was correlated with structural features rather than protection status. Although species can clearly respond to protection, species diversity fails to account for species identity so i)

**Table 2**
Trophic groups, categories, sizes, and example species used to calculate the three diversity measures studied.

| Trophic groups | Categories | Species (examples) |
|---|---|---|
| Top predators | Serranidae L | *Epinephelus marginatus* |
| | Sparidae L | *Dentex dentex* |
| Carnivores | Labridae S, M, L | *Coris julis, Thalassoma pavo, Symphodus tinca* |
| | Sparidae S, M | *Oblada melanura, Diplodus sargo* |
| | Serranidae S, M | *Serranus cabrilla, Mycteroperca* sp. |
| | Sea stars | *Marthasterias glacialis* |
| Herbivores | Sarpa salpa S, M, L | *Sarpa salpa* |
| | Sea urchins | *Paracentrotus lividus* |
| Filter feeders | Sciaphilic sponges | *Phorbas* sp. |
| | Photophilic sponges | *Ircinia fasciculata* |
| | Hydrozoa | *Aglaophenia pluma* |
| | Madreporaria | *Cladocora caespitosa* |
| | Actiniaria | *Anemonia sulcata* |
| | Alcyonacea | *Alcyonum palmatum* |
| | Gorgonacea | *Eunicella singularis* |
| | Ascidiacea | *Didemnum* sp. |
| | Bryozoa | *Schizobrachiella* sp. |
| | Polychaeta | *Serpula vermicularis* |
| Erect producers | Filamentous | *Cladophora* sp. |
| | Corticated | *Cladostephus hirsutus* |
| | Calcareous | *Jania rubens* |
| | Foliose | *Udotea petiolata* |
| | Leathery | *Cystoseira* sp. |
| | Phanerogams | *Posidonia oceanica* |
| Barren ground producers | Crustose coralline algae | *Mesophyllum* sp. |

**Table 3**
Two-way Anova on species diversity, trophic diversity, and functional diversity as a function of geographic area (random factor) and protection status (fixed factor). $p$-Values under 0.05 are considered significant.

| Measure | Effect | df | Mean squares | $F$-ratio | $p$-Value |
|---|---|---|---|---|---|
| Species diversity | Geographic area | 4 | 0.718 | 6.322 | 0.003 |
| | Protection status | 1 | 0.243 | 4.492 | 0.101 |
| | Interaction | 4 | 0.054 | 0.478 | 0.751 |
| | Error | 17 | 0.114 | | |
| Trophic diversity | Geographic area | 4 | 0.022 | 0.960 | 0.454 |
| | Protection status | 1 | 0.195 | 1.826 | 0.248 |
| | Interaction | 4 | 0.107 | 4.599 | 0.011 |
| | Error | 17 | 0.023 | | |
| Functional diversity | Geographic area | 4 | 0.504 | 7.686 | 0.001 |
| | Protection status | 1 | 0.340 | 10.113 | 0.033 |
| | Interaction | 4 | 0.033 | 0.513 | 0.726 |
| | Error | 17 | 0.065 | | |

changes in species identity may alter the functioning of the ecosystem without changing species diversity values and ii) low diversity values may correspond with high quality environments such as those dominated by meadows of the seagrass *P. oceanica* (Pergent-Martini and Pergent, 2000; Romero et al., 2007). As explained before, in the present study fish were not treated as species but as families and sizes, that in the area of study might mean an average of 5 different species in each of the categories used (Ojeda-Martinez et al., 2007). Studies focused on fish populations have clearly demonstrated that diversity is higher inside protected areas (Barrett et al., 2007; Claudet et al., 2006). When included as a part of the whole community those differences may weaken unless the remaining organisms follow the same trend.

Links between biodiversity and ecosystem processes are debateable (Duarte, 2000; Duffy, 2002). Given that higher level consumers are less diverse, less abundant, and under stronger anthropogenic pressure, it
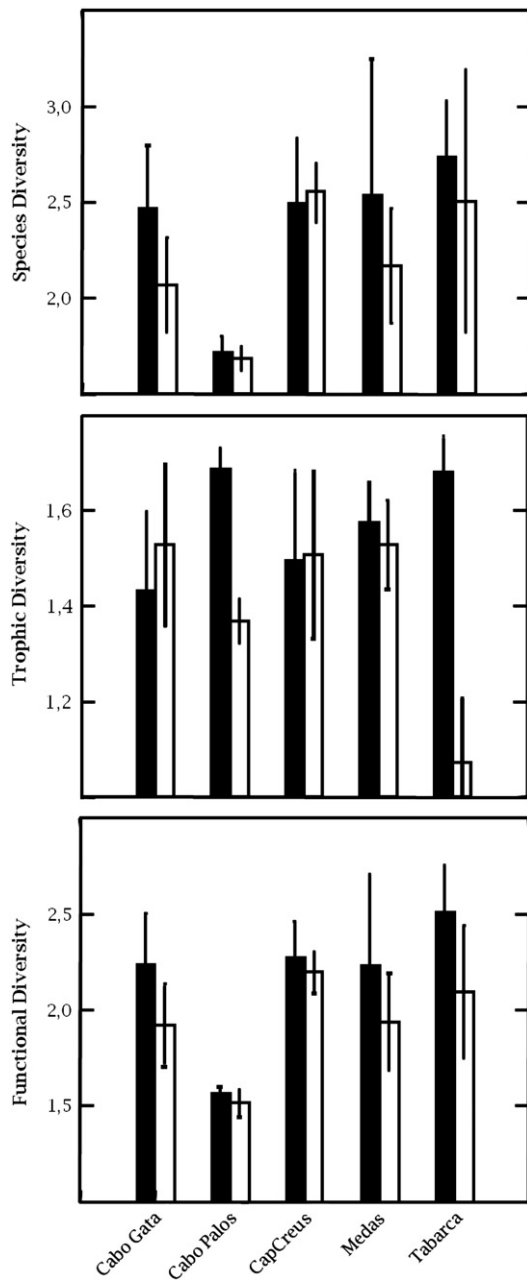
**Table 4**
PERMANOVA on the abundance species, trophic groups, and functional groups as a function of geographic area (random factor) and protection status (fixed factor). *p*-Values under 0.05 are considered significant.

| Measure | Effect | df | Mean squares | Pseudo-*F* | P-PERMANOVA |
|---|---|---|---|---|---|
| Species | Geographic area | 4 | 2601.8 | 2.9218 | 0.001 |
| | Protection status | 1 | 1640.5 | 1.2512 | 0.286 |
| | Interaction | 4 | 1314.9 | 1.4767 | 0.052 |
| | Residuals | 17 | 890.48 | | |
| Trophic groups | Geographic area | 4 | 791.5 | 0.9731 | 0.493 |
| | Protection status | 1 | 4438.7 | 3.8971 | 0.027 |
| | Interaction | 4 | 1141.8 | 1.4037 | 0.187 |
| | Residuals | 17 | 813,43 | | |
| Functional groups | Geographic area | 4 | 1164.7 | 2.2869 | 0.001 |
| | Protection status | 1 | 1084.4 | 1.4572 | 0.217 |
| | Interaction | 4 | 746.3 | 1.4653 | 0.072 |
| | Residuals | 17 | 509.29 | | |

seems reasonable to assume that top predator species are likely to become locally extinct even in areas with moderate anthropogenic pressure like those investigated in our study area. We found evidence that trophic and functional diversity could respond to protection more than species diversity. Similarly, Libralato et al. (2010) studied trophic
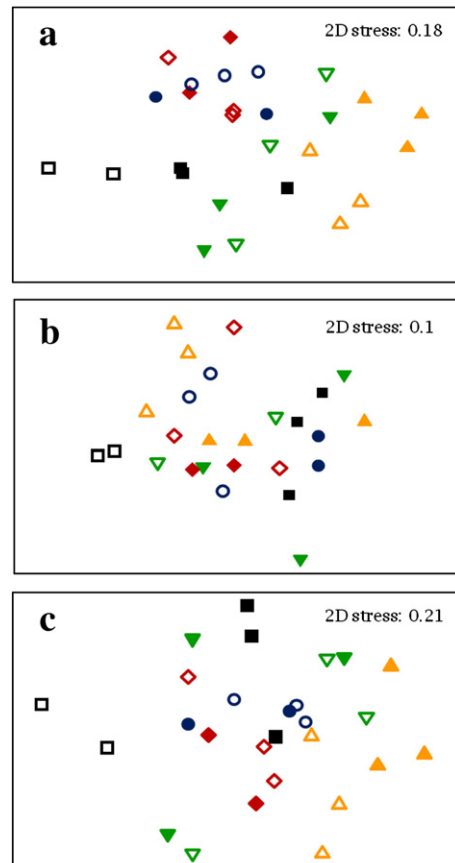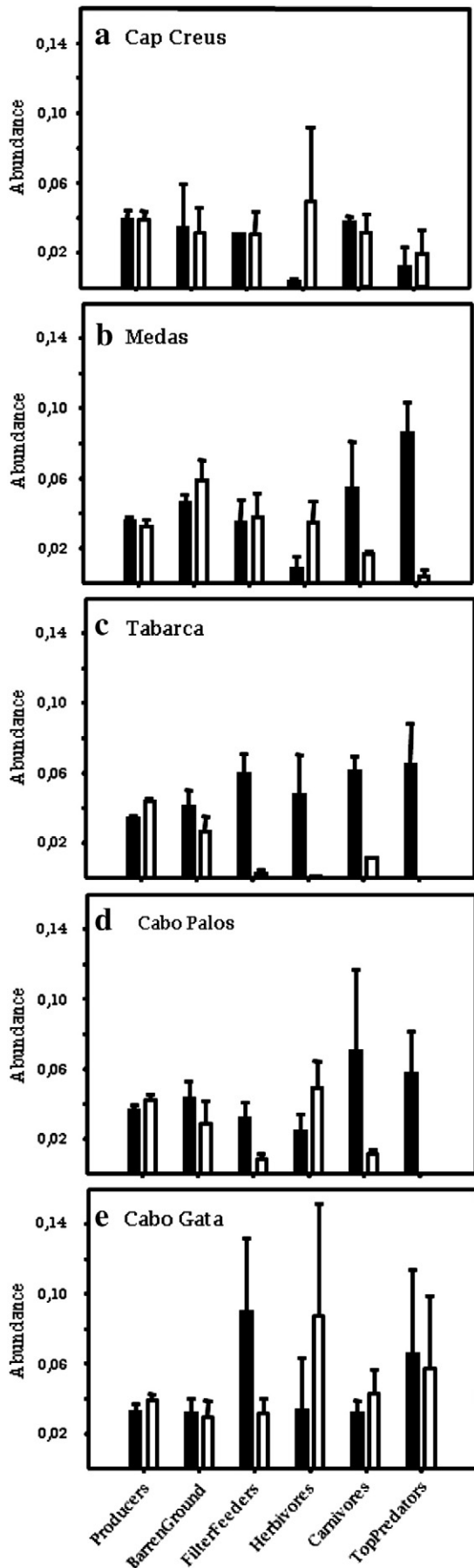
**Fig. 2.** Bar graphs of the species diversity, trophic diversity, and functional diversity on the five MPAs studied. Significance values of protection, geographic area, and the interaction of both factors are listed in Table 2.

**Fig. 3.** Multi dimensional scaling of a. species composition, b. trophic groups, and c. functional groups inside (solid symbols) and outside (empty symbols) the five MPAs: Cap Creus (red diamonds), Medas Islands (blue circles), Tabarca (black squares), Cabo Palos (orange triangles) and Cabo Gata (green triangles). *p* Values of protection status effect and geographic area are given in Table 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Fig. 4.** Bar graphs of the abundance (mean ± st. error) of each trophic group for each geographic area and protection status (black bars represent protected sites and white bars represent the unprotected sites). Significant parameters are detailed in the text.

levels in several Mediterranean areas and found that inside protected areas there are higher mean trophic levels. Our data showed that top predators were much more abundant inside than outside MPAs, opposite to herbivores that were more abundant outside than inside protected areas. This shift on the trophic interactions after protection of over exploited species has been reported in many MPAs around the world (Friedlander and DeMartini, 2002; Pace et al., 1999; Pinnegar et al., 2000) from Australian temperate MPAs (Barrett et al., 2009; Shears and Babcock, 2003) to the Mediterranean Sea (Hereu et al., 2008; Pinnegar et al., 2000), and according to our results these changes in the ecosystem trophic structure are general for every MPA studied regardless their geographic location.

The significant change in the abundance of filter feeders inside protected areas is also noteworthy. This might be due to other processes interacting with the top-down control dominating on temperate MPAs (Shears and Babcock, 2002). Bottom-up processes related with a myriad of environmental variables, water quality for instance, could be modulating the net outcome of the trophic shift (Freidenburg et al., 2007) by enhancing competitive exclusion for space between colonial filter feeders and algae (Cebrian and Uriz, 2006; Rützler, 1970; Schmidt and Warner, 1986). Ward et al. (1999) concluded that not only fish but also invertebrate assemblages are good surrogates of species diversity, so taking into account not only target species but every trophic level of the community, might uncover other aspects of the complex reserve effect.

The community organization, which we approached as diversity and composition of functional groups, seems to be particular of each area. Even when grouping species into functional groups, the effect of protection on the relationships among functional groups varies with geographic area. Some functional groups seem to be more relevant for this differentiation than others (urchins, fishes…), but no clear pattern of functional organization is detected according solely to protection status in this type of community. Graphically, this is quite clear in the MDS of Fig. 3c, where protected and unprotected sites appear spatially separated but failed to follow a common pattern across MPAs. It is noteworthy that northern sites, protected and unprotected localities of Cap de Creus and Medas Islands geographic areas, appear mixed with no clear boundary separating protected and unprotected sites, as opposed to the other three MPAs. The proximity of both MPAs to densely populated areas together with a permissive policy of permitted uses makes us think that the two northern MPAs might be less effective than their southern counterparts.

Vegetal functional groups seem to be the most affected by the protection, although in a different way in each geographic area. Differences reflect the specific characteristics of the geographic area. Fish seem to play a major role as well in the protection status differences, although these differences detected were not fully significant. This lack of significance could be due to several and opposing explanations: one answer would be that the protection is so effective that spillover is enhancing fish populations in nearby unprotected sites. This enhancement has already been noted (Goñi et al., 2008; Harmelin-Vivien et al., 2008) in many Mediterranean MPAs, including three that were also investigated in our study. The opposite explanation would be that protection is not effective and illegal fishing prevents differences between protected and unprotected areas. This is more unlikely given that all 5 MPAs are provided with comprehensive surveillance.

The dynamics of Mediterranean communities are well documented with two opposite stages separated by two distinct phases (Ballesteros, 1991). After a diversification phase, benthic communities reach a stage with maximum levels of spatial diversity (sensu Pielou, 1966), followed by a high production phase that leads to well-developed communities (Ballesteros, 1991). Although species abundance and distribution may

change seasonally and with other parameters not investigated in our study, species richness and diversity show no distinct trend between the developed and diversified stages (Ballesteros, 1991). Given the age of the MPAs investigated in our study, certain patterns should arise beyond the natural seasonal variation in species. This has been shown mainly for fish species (Anticamara et al., 2010; Myers et al., 2011) and also for invertebrates (Bertocci et al., 2012). For the algal communities, seasonal variation is also assumed; however the categories we used as surrogates of diversity have been proved to be consistent in space and time (Smale, 2010).

In our opinion, species diversity shows weaknesses to measure the effectiveness of protection on MPAs, as it is driven by many factors including biogeography. Our data showed that the effect of protection on trophic structure is more general and independent of geography. This simple trophic division allowed us to find an effect of protection common to all the studied MPAs. As expected, the most affected trophic groups were top predators and carnivores, the usual target species, but herbivores and filter feeders also showed some differences related to protection. Producers were not quantitatively affected, but the effect of protection on the composition of functional groups varied with geographic areas. Our study showed evidence for an effect of protection on community organization and trophic structure, supporting the concept of reserve effect beyond target species. Our data also showed that the effect of protection varied as a function of geographic location even between localities a few hundred kilometers apart. These considerations make us suggest that the creation of more MPAs (even in nearby areas) seems a reasonable practical consequence of our study. By protecting more areas we will increase our protection on biodiversity and the ecosystem services it provides.

## Acknowledgments

## References

Alexander, T.J., Barrett, N., Haddon, M., Edgar, G., 2009. Relationships between mobile macroinvertebrates and reef structure in a temperate marine reserve. Marine Ecology Progress Series 389, 31–44.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26, 32–46.

Anticamara, J.A., Zeller, D., Vincent, A.C.J., 2010. Spatial and temporal variation of abundance, biomass and diversity within marine reserves in the Philippines. Diversity and Distributions 16, 529–536.

Ballesteros, E., 1991. Structure and dynamics of north-Western Mediterranean phytobenthic communities: a conceptual model. Oecologia Aquatica 10, 223–242.

Barrett, N.S., Edgar, G.J., Buxton, C.D., Haddon, M., 2007. Changes in fish assemblages following 10 years of protection in Tasmanian marine protected areas. Journal of Experimental Marine Biology and Ecology 345, 141–157.

Barrett, N.S., Buxton, C.D., Edgar, G.J., 2009. Changes in invertebrate and macroalgal populations in Tasmanian marine reserves in the decade following protection. Journal of Experimental Marine Biology and Ecology 370, 104–119.

Bell, J.D., Harmelin-Vivien, M.L., 1983. Fish fauna of french Mediterranean *Posidonia oceanica* seagrass meadows. 2: Feeding habits. Tethys 11, 1–14.

Benedetti-Cecchi, L., 2004. Increasing accuracy of causal inference in experimental analyses of biodiversity. Functional Ecology 18, 761–768.

Bertocci, I., Dominguez, R., Freitas, C., Sousa-Pinto, I., 2012. Patterns of variation of intertidal species of commercial interest in the Parque Litoral Norte (north Portugal) MPA: comparison with three reference shores. Marine Environmental Research 77, 60–70.

Britton-Simmons, K.H., 2006. Functional group diversity, resource preemption and the genesis of invasion resistance in a community of marine algae. Oikos 113, 395–401.

Bruner, A.G., Gullison, R.E., Rice, R.E., da Fonseca, G.A.B., 2001. The effectiveness of parks: response. Science 293, U2–U3.

Cebrian, E., Uriz, M.J., 2006. Grazing on fleshy seaweeds by sea urchins facilitates sponge *Cliona viridis* growth. Marine Ecology Progress Series 323, 83–89.

Chapin, F.S., Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E., Mack, M.C., Diaz, S., 2000. Consequences of changing biodiversity. Nature 405, 234–242.

Claudet, J., Pelletier, D., Jouvenel, J.Y., Bachet, F., Galzin, R., 2006. Assessing the effects of marine protected area (MPA) on a reef fish assemblage in a northwestern Mediterranean marine reserve: identifying community-based indicators. Biological Conservation 130, 349–369.

Duarte, C.M., 2000. Marine biodiversity and ecosystem services: an elusive link. Journal of Experimental Marine Biology and Ecology 250, 117–131.

Duffy, J.E., 2002. Biodiversity and ecosystem function: the consumer connection. Oikos 99, 201–219.

Edgar, G.J., Barrett, N.S., 1999. Effects of the declaration of marine reserves on Tasmanian reef fishes, invertebrates and plants. Journal of Experimental Marine Biology and Ecology 249, 107–144.

Edgar, G.J., Banks, S.A., Bessudo, S., Cortes, et al., 2011. Variation in reef fish and invertebrate communities with level of protection from fishing across the Eastern Tropical Pacific seascape. Global Ecology and Biogeography 20, 730–743.

Figueroa, F.L., Jimenez, C., Vinegla, B., Perez-Rodriguez, E., Aguilera, K., Flores-Moya, A., Altamirano, M., Lebert, M., Hader, D.P., 2002. Effects of solar UV radiation on photosynthesis of the marine angiosperm Posidonia oceanica from southern Spain. Marine Ecology Progress Series 230, 59–70.

Forcada, A., Bayle-Sempere, J.T., Valle, C., Sanchez-Jerez, P., 2008. Habitat continuity effects on gradients of fish biomass across marine protected area boundaries. Marine Environmental Research 66, 536–547.

Forcada, A., Valle, C., Bonhomme, P., Criquet, G., Cadiou, G., Lenfant, P., Sanchez-Lizaso, J.L., 2009. Effects of habitat on spillover from marine protected areas to artisanal fisheries. Marine Ecology Progress Series 379, 197–211.

Francour, P., 1994. Pluriannual analysis of the reserve effect on ichthyofauna in the Scandola natural reserve Corsica, Northwestern Mediterranean. Oceanologica Acta 17, 309–317.

Francour, P., Harmelin, J.G., Pollard, D., Sartoretto, S., 2001. A review of marine protected areas in the northwestern Mediterranean region: siting, usage, zonation and management. Aquatic Conservation: Marine and Freshwater Ecosystems 11, 155–188.

Freidenburg, T.L., Menge, B.A., Halpin, P.M., Webster, M., Sutton-Grier, A., 2007. Cross-scale variation in top-down and bottom-up control of algal abundance. Journal of Experimental Marine Biology and Ecology 347, 8–29.

Friedlander, A.M., DeMartini, E.E., 2002. Contrasts in density, size, and biomass of reef fishes between the northwestern and the main Hawaiian islands: the effects of fishing down apex predators. Marine Ecology Progress Series 230, 253–264.

García-Chartón, J.A., Pérez-Ruzafa, A., 1999. Ecological heterogeneity and the evaluation of the effects of marine reserves. Fisheries Research 42, 1–20.

García-Chartón, J.A., Pérez-Ruzafa, A., 2001. Spatial pattern and the habitat of a SW Mediterranean reef fish assemblage. Marine Biology 138, 917–934.

García-Chartón, J.A., Pérez-Ruzafa, A., Sánchez-Jerez, P., Bayle-Sempere, J.T., Reñones, O., Moreno-Lampreave, D., 2004. Multi-scale spatial heterogeneity, habitat structure, and the effect of marine reserves on Western Mediterranean rocky reef fish assemblages. Marine Biology 144, 161–182.

García-Chartón, J.A., Pérez-Ruzafa, A., Marcos, C., Claudet, J., Badalamenti, F., Benedetti-Cecchi, L., Falcón, J.M., Milazzo, M., Schembri, P.J., Stobart, B., Vandeperre, F., Brito, A., Chemello, R., Dimech, M., Domenici, P., Guala, I., Le Diréach, L, Maggi, E., Planes, S., 2008. Effectiveness of European Atlanto-Mediterranean MPAs: do they accomplish the expected effects on populations, communities and ecosystems? Journal of Nature Conservation 16, 193–221.

Garrabou, J., Ballesteros, E., Zabala, M., 2002. Structure and dynamics of north-western Mediterranean rocky benthic communities along a depth gradient. Estuarine, Coastal and Shelf Science 55 (3), 493–508.

Goñi, R., Adlerstein, S., Alvarez-Berastegui, D., Forcada, A., Renones, O., Criquet, G., Polti, S., Cadiou, G., Valle, C., Lenfant, P., Bonhomme, P., Pérez-Ruzafa, A., Sánchez-Lizaso, J.L., García-Chartón, J.A., Bernard, G., Stelzenmuller, V., Planes, S., 2008. Spillover from six western Mediterranean marine protected areas: evidence from artisanal fisheries. Marine Ecology Progress Series 366, 159–174.

Guidetti, P., 2002. The importance of experimental design in detecting the effects of protection measures on fish in Mediterranean MPAs. Aquatic Conservation 12, 619–634.

Guidetti, P., 2006. Marine reserves reestablish lost predatory interactions and cause community changes in rocky reefs. Ecological Applications 16, 963–976.

Guidetti, P., Sala, E., 2007. Community-wide effects of marine reserves in the Mediterranean Sea. Marine Ecology Progress Series 335, 43–56.

Guidetti, P., Verginella, L., Viva, C., Odorico, R., Boero, F., 2005. Protection effects on fish assemblages, and comparison of two visual-census techniques in shallow artificial rocky habitats in the northern Adriatic Sea. Journal of the Marine Biological Association of the UK 85, 247–255.

Guidetti, P., Milazzo, M., Bussotti, S., Molinari, A., Murenu, M., Pais, A., Spano, N., Balzano, R., Agardy, T., Boero, F., Carrada, G., Cattaneo-Vietti, R., Cau, A., Chemello, R., Greco, S., Manganaro, A., di Sciara, G.N., Russo, G.F., Tunesi, L., 2008. Italian marine reserve effectiveness: does enforcement matter? Biological Conservation 141, 699–709.

Harmelin-Vivien, M., Le Diréach, L., Bayle-Sempere, J., Charbonnel, E., García-Chartón, J.A., Ody, D., Pérez-Ruzafa, A., Renones, O., Sánchez-Jerez, P., Valle, C., 2008. Gradients of abundance and biomass across reserve boundaries in six Mediterranean marine protected areas: evidence of fish spillover? Biological Conservation 141, 1829–1839.

Hereu, B., Zabala, M., Sala, E., 2008. Multiple controls of community structure and dynamics in a sublittoral marine environment. Ecology 89 (12), 3423–3435.

Jenkins, C.N., Joppa, L., 2009. Expansion of the global terrestrial protected area system. Biological Conservation 142, 2166–2174.

Lasiak, T., 2006. Spatial variation in density and biomass of patellid limpets inside and outside a marine protected area. Journal of Molluscan Studies 72, 137–142.

Libralato, S., Coll, M., Tempesta, M., Santojanni, A., Spoto, M., Palomera, I., Arneri, E., Solidoro, C., 2010. Food-web traits of protected and exploited areas of the Adriatic Sea. Biological Conservation 143, 2182–2194.

Lister, N.M.E., 1998. A systems approach to biodiversity conservation planning. Environmental Monitoring and Assessment 49, 123–155.

Lloret, J., Riera, V., 2008. Evolution of a Mediterranean coastal zone: human impacts on the marine environment of Cape Creus. Environmental Management 42, 977–988.

Lopez-Sanz, A., Vert, N., Zabala, M., Sabates, A., 2009. Small-scales distribution of fish larvae around the Medes Islands marine protected area (NW Mediterranean). Journal of Plankton Research 31, 763–775.

Magurran, A.E., 2004. Measuring Biological Diversity. Blackwell.

McClanahan, T.R., 1997. Primary succession of coral-reef algae: differing patterns on fished versus unfished reefs. Journal of Experimental Marine Biology and Ecology 218, 77–102.

McClanahan, T.R., Arthur, R., 2001. The effect of marine reserves and habitat on populations of east African coral reef fishes. Ecological Applications 11, 559–569.

McClanahan, T.R., Sala, E., 1997. A Mediterranean rocky-bottom ecosystem fisheries model. Ecological Modelling 104, 145–164.

McClanahan, T.R., Kamukuru, A.T., Muthiga, N.A., Yebio, M.G., Obura, D., 1996. Effect of sea urchin reductions on algae, coral, and fish populations. Conservation Biology 10, 136–154.

Micheli, F., Halpern, B.S., Botsford, L.W., Warner, R.R., 2004. Trajectories and correlates of community change in no-take marine reserves. Ecological Applications 14, 1709–1723.

Micheli, F., Benedetti-Cecchi, L., Gambaccini, S., Bertocci, I., Borsini, C., Osio, G.C., Roman, F., 2005. Cascading human impacts, marine protected areas, and the structure of Mediterranean reef assemblages. Ecological Monographs 75 (1), 81–102.

Myers, M.C., Wagner, J., Vaughan, C., 2011. Long-term comparison of the fish community in a Costa Rican rocky shore marine reserve. Revista De Biologia Tropical 59, 233–246.

Nyström, M., 2006. Redundancy and response diversity of functional groups: implications for the resilience of coral reefs. Ambio 35, 30–35.

Ojeda-Martinez, C., Bayle-Sempere, J.T., Sanchez-Jerez, P., Forcada, A., Valle, C., 2007. Detecting conservation benefits in spatially protected fish populations with meta-analysis of long-term monitoring data. Marine Biology 151, 1153–1161.

Ordines, F., Moranta, J., Palmer, M., Lerycke, A., Suau, A., Morales-Nin, B., Grau, A.M., 2005. Variations in a shallow rocky reef fish community at different spatial scales in the western Mediterranean Sea. Marine Ecology Progress Series 304, 221–233.

Pace, M.L., Cole, J.J., Carpenter, S.R., Kitchell, J.F., 1999. Trophic cascades revealed in diverse ecosystems. Trends in Ecology & Evolution 14, 483–488.

Paine, R.T., 1980. Food webs linkage, interaction strength and community infrastructure. The 3rd Tansley Lecture. The Journal of Animal Ecology 49, 667–685.

Pauly, D., Christensen, V., Dalsgaard, J., Froese, R., Torres, F., 1998. Fishing down marine food webs. Science 279, 860–863.

Pederson, H.G., Johnson, C.R., 2006. Predation of the sea urchin *Heliocidaris erythrogramma* by rock lobsters (*Jasus edwardsii*) in no-take marine reserves. Journal of Experimental Marine Biology and Ecology 336, 120–134.

Pergent-Martini, C., Pergent, G., 2000. Marine phanerogams as a tool in the evaluation of marine trace-metal contamination: an example from the Mediterranean. International Journal of Environment and Pollution 13, 126–147.

Perner, J., Voigt, W., 2007. Measuring the complexity of interaction webs using vertical links between functional groups. Agriculture, Ecosystems & Environment 120, 192–200.

Pielou, E.C., 1966. Species-diversity and pattern-diversity in the study of ecological succession. Journal of Theoretical Biology 10, 370–383.

Pinnegar, J.K., Polunin, N.V.C., Francour, P., Badalamenti, F., Chemello, R., Harmelin-Vivien, M.L., Hereu, B., Milazzo, M., Zabala, M., D'Anna, G., Pipitone, C., 2000. Trophic cascades in benthic marine ecosystems: lessons for fisheries and protected-area management. Environmental Conservation 27, 179–200.

Ramezani, H., Holm, S., 2011. Sample based estimation of landscape metrics; accuracy of line intersect sampling for estimating edge density and Shannon's diversity index. Environmental and Ecological Statistics 18 (1), 109–130.

Romero, J., Martinez-Crego, B., Alcoverro, T., Pérez, M., 2007. A multivariate index based on the seagrass *Posidonia oceanica* (POMI) to assess ecological status of coastal waters under the water framework directive (WFD). Marine Pollution Bulletin 55, 196–204.

Rossi, S., Tsounis, G., Orejas, C., Padron, T., Gili, J.M., Bramanti, L., Teixido, N., Gutt, J., 2008. Survey of deep-dwelling red coral (Corallium rubrum) populations at Cap de Creus (NW Mediterranean). Marine Biology 154, 533–545.

Rützler, K., 1970. Spatial competition among Porifera: solution by epizoism. Oecologia 5, 85–95.

Sala, E., Knowlton, N., 2006. Global marine biodiversity trends. Annual Review of Environment and Resources 31, 93–122.

Sala, E., Boudouresque, C.F., Harmelin-Vivien, M., 1998a. Fishing, trophic cascades, and the structure of algal assemblages: evaluation of an old but untested paradigm. Oikos 82, 425–439.

Sala, E., Ribes, M., Hereu, B., Zabala, M., et al., 1998b. Temporal variability in abundance of the sea urchins *Paracentrotus lividus* and *Arbacia lixula* in the northwestern Mediterranean: comparison between a marine reserve and an unprotected area. Marine Ecology Progress Series 168, 135–145.

Schmidt, G.H., Warner, G.F., 1986. Spatial competition between colonial ascidians: the importance of stand-off. Marine Ecology Progress Series 31, 101–104.

Shears, N.T., Babcock, R.C., 2002. Marine reserves demonstrate top-down control of community structure on temperate reefs. Oecologia 132, 131–142.

Shears, N.T., Babcock, R.C., 2003. Continuing trophic cascade effects after 25 years of no-take marine reserve protection. Marine Ecology Progress Series 246, 1–16.

Simberloff, D., 1998. Flagships, umbrellas, and keystones: is single-species management passe in the landscape era? Biological Conservation 83, 247–257.

Smale, D.A., 2010. Monitoring marine macroalgae: the influence of spatial scale on the usefulness of biodiversity surrogates. Diversity and Distributions 16, 985–995.

Steneck, R.S., Watling, L., 1982. Feeding capabilities and limitation of herbivorous mollusks: a functional-group approach. Marine Biology 68, 299–319.

Tuya, F., Garcia-Diez, C., Espino, F., Haroun, R.J., 2006. Assessment of the effectiveness of two marine reserves in the Canary Islands (eastern Atlantic). Ciencias Marinas 32, 505–522.

Villamor, A., Becerro, M.A., 2010. Matching spatial distributions of the sea star *Echinaster sepositus* and crustose coralline algae in shallow rocky Mediterranean communities. Marine Biology 157, 2241–2251.

Ward, T.J., Vanderklift, M.A., Nicholls, A.O., Kenchington, R.A., 1999. Selecting marine reserves using habitats and species assemblages as surrogates for biological diversity. Ecological Applications 9, 691–698.

Wilson, E.O., 2000. On the future of conservation biology. Conservation Biology 14, 1–3.

Wlodarska-Kowalczuk, M., Pearson, T.H., Kendall, M.A., 2005. Benthic response to chronic natural physical disturbance by glacial sedimentation in an Arctic fjord. Marine Ecology Progress Series 303, 31–40.