Assessing the potential of marine Natura 2000 sites to produce ecosystem-wide effects in rocky reefs: A case study from Sardinia Island (Italy)

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Abstract

Marine biodiversity and the related ecosystem goods and services are declining in many regions of the world. A number of policy measures and tools have been adopted to cope with the current degradation of marine ecosystems. Marine Protected Areas (MPAs) make part of them. In the last decades MPAs – considering all types of MPAs – have dramatically increased worldwide, including in EU waters. Natura 2000 sites are the core of the biodiversity conservation strategy of the EU. To date, more than 25 000 Natura 2000 sites, covering >350 000 km^2 at sea, have been declared. They form the most important coordinated system of protected areas in the world. However, there are more and more critical voices questioning their effectiveness and complementarity with other national (e.g. nationally established MPAs), EU (e.g. the Marine Strategy Framework Directive, the Common Fishery Policy) and other international initiatives (e.g. the Ecosystem-Based Approach of the CBD). Using a largely employed indicator of marine coastal ecosystem health, i.e. the fish biomass, we assessed here the ecological effectiveness of Natura 2000 sites in Sardinia Island (Italy), used here as a case study area. We compared fish biomass (total fish biomass and that of selected fish) assessed using visual census in rocky reefs. The assessment was performed at 18 protected sites (i.e. 6 fully protected zones within nationally established MPAs and 12 Natura 2000 sites) and in 18 unprotected control sites open to fishing and adjacent to the protected ones. Results show that the highest fish biomass (total values and those related to commercially and ecologically relevant fishes) is by far the one associated to fully protected MPAs, while the average values observed in Natura 2000 sites do not or slightly differ from those observed in control sites. This study shows that Natura 2000 sites do not presently contribute to the ecosystem-wide management and that declaring Natura 2000 sites is a necessary but not sufficient condition to achieve significant ecological benefits. Re-thinking and widening the scope of Natura 2000 sites in EU waters, providing sound management plans and implementing appropriate conservation measures becomes more and more urgent to make it possible for Natura 2000 sites to provide significant ecological and socio-economic benefits.

Keywords: marine protected areas, site of community importance, ecological effectiveness, implementation, management, Mediterranean Sea, EU policy

Introduction

A number of papers and reports published in the last decades have reported an alarming decline of marine biodiversity worldwide (MEA, 2005; Worms et al., 2006). Future scenarios appear, indeed, quite negative as a consequence of multiple and unsustainable human activities coupled with several additional sources of stress, which are responsible for current ocean degradation, especially in coastal areas (Halpern et al., 2008; Micheli et al., 2013; Worms et al., 2006; Bopp et al., 2013). Countries throughout the world seem to be increasingly aware of that, but also of the fact that the mankind holds the power to reverse this negative trend (Guidetti and Danovaro, 2018).

Multiple scale actions as well as inter-sectoral and international cooperation, accompanied by the adoption of an ecosystem approach, are thus more and more recommended (Douvere and Ehler, 2009; Guidetti and Danovaro, 2018). The logics to pair large-scale initiatives (e.g. the transnational implementation of SDG, Sustainable Development Goals, targets; see https://oceanconference.un.org/callforaction) and regional-local actions (e.g. the creation of effective Marine Protected Areas networks) seems to be the most effective strategy to reverse the ongoing ocean health decline.

Marine Protected Areas (MPAs) have been proven to be a valuable tool capable of alleviating the impact of a number of anthropogenic stresses at sea. They can be effective at local and large scales (in the case MPAs are structured in effective networks), and capable of producing many ecological effects and socio-economic benefits (Gaines et al., 2010; Sumaila et al., 2000; Sala et al., 2013; Giakoumi et al., 2017).

In the last decades MPAs – considering all types of MPAs – have dramatically increased worldwide (Grorud-Colvert and Lubchenco, 2015). In the EU waters, Natura 2000 sites (Nat2000) are the core of the biodiversity conservation strategy of the EU (Evans, 2012). Based on two EU directives (the Habitats and Birds Directives; EC, 1992; EC, 2009), they do not usually include strictly protected zones (e.g. no-take areas), being their main aim to regulate and manage human activities in order to protect core breeding and resting sites for rare and threatened species, and some specific and fragile habitat types (http://ec.europa.eu/environment/nature/natura2000/index_en.htm). Using the same legislative tool, therefore, the 28 EU state members have until now declared >25 000 Nat2000 sites (terrestrial and marine), covering >350 000 km^2 at sea (EU, 2017). On the whole, the Nat2000 sites represent the largest coordinated system of PAs in the world.

Besides the formal framework, nevertheless, Nat2000 sites are more and more frequently the object of critical voices that 1) question their actual role and effectiveness in protecting marine biodiversity, and 2) suggest the need for a proper integration into the wider conservation and environmental EU policy. Meinesz and Blanfuné (2015), for instance, stated that Nat2000 sites along the Mediterranean French coasts do not include any regulation of fishing activities potentially impacting marine coastal biodiversity, or any specific regulation regarding the protection of a species or biotope, with the exception of the seagrass *Posidonia oceanica*. This latter species, however, is already and may be better protected thanks to a national law, both within and outside Nat2000 sites. Recently, Mazaris et al. (2017) reported that the Nat2000 system fails to meet several CBD (Convention on Biological Diversity, 2011) targets: the relative % of marine surface covered is extremely variable among member states, deep/offshore marine ecosystems are underrepresented, and connectivity is not guaranteed at all. In addition, less than 40% of Nat2000 sites have a management plan and shared Nat2000 sites between member states are limited (Mazaris et al., 2018). Finally, in spite of the evident implications related to the implementation of the Nat2000 sites for fisheries (Pedersen et al., 2009), the initiatives to develop fisheries management measures in Nat2000 sites are extremely limited. These elements are in clear contrast with the more and more evident ambition of the Commission for larger scopes of the Nat2000 system, going beyond the Birds and Habitats Directives (see Fock, 2011).

Nowadays, for the reasons exposed above, Nat2000 sites do not seem to be capable of effectively contributing to the ecosystem-wide marine protection policy of the EU or to properly integrate the Marine Strategy Framework Directive (MSFD) and Common Fishery Policy (CFP) objectives, with some studies that have been published stressing the serious risk that specific fishing activities could impede the attainment of the conservation objectives of the Nat2000 sites (Pedersen et al., 2009).

While several features of Nat2000 system (*e.g.*, the spatial properties; Mazaris et al. 2018) have been studied both for the terrestrial and (to a lesser extent) the marine counterpart, their effectiveness in preserving and/or restoring marine biodiversity has never been investigated. In order to eventually re-think and widen their role into the wider and evolving conservation EU policy framework, it is crucial and timely to improve the body of evidence about whether or not Nat2000 sites can contribute to ecosystem-wide conservation.

Fish assemblages are largely used for evaluating the effectiveness of any type of MPA, for a number of reasons: i) fish can be easily assessed using non-destructive methods (Harmelin-Vivien et al., 1985; Caldwell et al., 2016); ii) fish clearly respond to the implementation of protection/management measures (Guidetti et al., 2008; Graham et al., 2014; Guidetti et al., 2014; Giakoumi et al., 2017); iii) fish are effective indicators of socio-economic MPA benefits, e.g. those related to fisheries and tourism (Kerwath et al., 2013; Di Franco et al., 2016; Sala et al., 2016; Gill et al., 2017); iv) fish are commonly used as indicators of ecosystem health and are linked to the provision of crucial ecosystem goods and services (Pauly et al., 1998; Micheli et al., 2004; Leenhardt et al., 2015).

Being the EU waters subjected to multiple anthropogenic sources of stress and impacts capable of producing community- and ecosystem-wide alterations (Coll et al., 2012; Fenberg et al., 2012; Micheli et al., 2013; Katsanevakis et al., 2015), it becomes urgent to know whether Nat2000 sites, in combination with other EU or national initiatives (e.g. MSFD, CFP, nationally established MPAs), have the potential to provide an adequate protection to natural marine assemblages and ecosystems, while safeguarding the sustainability of fisheries and other human activities.

The present study aims, therefore, to evaluate the effectiveness of Nat2000 along the coasts of Sardinia Island (Mediterranean Sea, Italy), used here as a case study area, by assessing and comparing coastal fish assemblages sampled in Nat2000 sites, in fully protected (i.e. no-take) MPAs and in adjacent control sites.

Materials and methods

Sampling area and methods

Fish assemblages were assessed at 18 locations situated along the coasts of Sardinia Island (Italy; Mediterranean Sea). Six fully protected (i.e. no-take) locations within nationally established MPAs (FP-MPA: “Tavolara-Punta Coda Cavallo”, “Capo Carbonara”, “Penisola del Sinis-Isola di Mal di Ventre”, “Capo Caccia-Isola Piana”, “Isola dell’Asinara” and “Parco Nazionale dell’Arcipelago di Maddalena”) and 12 Nat2000 sites (“Capo Figari ed Isola Figarolo”, “Berchida e Bidderosa”, “Golfo di Orosei”, “Capo di Pula”, “Promontorio, dune e zona umida di Porto Pino”, “Isola di San Pietro”, “Costa di Nebida”, “Stagno di Putzu Idu”, “Entroterra e zona costiera tra Bosa, Capo Marangiu e Porto Tangone”, “Coste e Isolette a Nord-Ovest della Sardegna", "Monte Russu" and "Capo Testa") were sampled, along with adjacent sites open to fishing (regulated by national/regional laws) and used as controls (Fig. 1). With "Nat2000" we mean here Nat2000 sites that do not overlap with other MPA types. Two 'protected' and two 'unprotected' stations were sampled at each of the 18 sampling locations. Three fish visual assessments were performed underwater on rocky reefs at 5-12 m depth, along 3 replicate strip transects of 25×5 m at each station, for a total of 216 visual census (i.e. replicates).

Most of sampling sites were sampled between mid-June and mid-August 2016. Data from the Maddalena, Capo Caccia and Asinara MPA were gathered in August-September 2011, 2015 and 2017, respectively.

Visual censuses were performed on rocky substrates where other substrate types, like sand or seagrasses, represented less than 15% in cover (both within and around transects). Along each transect, the diver swam one way at constant speed (approximately 4 meters/min.), identifying and recording the number and size of each fish encountered. Fish density was estimated by counting single specimens to a maximum of ten individuals, whereas classes of abundance (11–30, 31–50, 51–100, 101–200, 201–500, >500 individuals) were used for larger schools. Fish size (total length: TL) was recorded within 2-cm size classes for most of the species, and within 5-cm size classes for large-sized species such as *Epinephelus marginatus*. Fish wet mass (hereafter called biomass) was estimated from size data by means of length-weight relationships from the available literature (Froese and Pauly, 2012).

We focused on biomass data of fish associated with rocky reefs because: (1) fish biomass is recognized as the most responsive indicator of the conservation status of fish assemblages as it inherently integrates both density and size (Sandin et al. 2008; Guidetti et al., 2014); (2) rocky reefs are the most common habitat protected within coastal MPAs in the Mediterranean Sea; (3) previous studies showed that rocky reefs host the most of fish targeted by fishing and therefore these fish assemblages more clearly respond to protection from fishing than others (see Guidetti et al., 2008 and references therein).

Data analyses

The effects of different protection levels on fish biomass variables were analyzed using univariate techniques. 'Protection' (PR) was considered as a fixed factor (3 levels: FP-MPA, Nat2000, unprotected control), and 'Station' (ST) was a random factor (2 levels) nested in each level of PR. The 6 variables taken into consideration are: total fish biomass, that of most relevant categories (High and Low-Null Commercial Importance fishes; indicated hereinafter as H CI and L-N CI) and that of some fish species ecologically important and targeted by commercial and recreational fishing (the dusky grouper *Epinephelus marginatus*, the brown meager *Sciaena umbra*, the sea breams *Diplodus sargus* and *D. vulgaris*; these latter fishes have been pooled and named hereinafter as *Diplodus* spp.). This selection of relevant variables is in agreement with the available literature suggesting that fishery targeted fish have the potential to respond more clearly to the effectiveness of management measures (Guidetti et al., 2014).

Univariate PERMANOVA based on Euclidean distance measure (Terlizzi et al., 2007) was used in order to avoid any assumption about the distribution of the variables (Anderson et al., 2001). In this analysis P-values associated with F statistics are obtained by permutation. The PRIMER 6 and Permanova + B20 package (Plymouth Marine Laboratory) was used to perform the analyses (Clarke and Gorley, 2006).

Methods derived from meta-analysis were used to examine and summarize the general response of fish to protection. As visual censuses were done at several protected (FP-MPA and Nat2000) and unprotected (control) stations, mean values were used to approximate average conditions in space (see Guidetti and Sala, 2007). We examined the response to protection on the 6 fish biomass variables mentioned above. We quantified the effects of protection *versus* control conditions as the natural logarithm of the ratio between the values of each response variable (i.e. total fish biomass) in protected and control conditions as response ratios (lnRR; Hedges and Olkin, 1985; Micheli et al., 2004). Data were thus normalized and the response to protection examined independently of the absolute biomasses at each location. As estimations of average values can be affected by sampling effort, we calculated weighted means using the natural logarithm of the total area covered by the censuses from which the estimates were obtained (Mosquera et al., 2000). Positive RRs indicate greater biomass in protected than in control conditions, whereas negative values indicate greater values in control conditions compared to protected ones. A ratio of zero, instead, means that the biomass is similar between protected and control conditions. Averages of the mean RRs were considered significantly different from zero (i.e. there is a significant protection effect) when the 95% confidence limits around the mean do not overlap with zero (Micheli, 1999 and references therein).

Results

The visual inspection of the graphs reporting the average values of fish biomass reveals a common general pattern: total fish biomass, that of H CI and L-N CI fishes, and that of relevant species (*E. marginatus*, *S. umbra*, *Diplodus* spp.) are generally highest in FP-MPAs, followed by Nat2000 sites, and lowest at controls (Fig. 2).

Statistical analyses (univariate PERMANOVAs) show that none of the 6 fish biomass variables considered in this study varied significantly at the spatial scale of stations (Tab. 1). Total fish biomass, that of H CI fish, and that of *E. marginatus* and *Diplodus* spp. significantly changed in relation to the protection level, with the highest average values observed in FP-MPAs. Pair-wise post-hoc tests showed that total fish biomass and that of *E. marginatus* were statistically highest at FP-MPAs, followed by Nat2000, with the lowest average values observed at control sites. Average values of H CI and *Diplodus* spp. were significantly higher at FP-MPAs than at Nat2000 and control sites, with no statistical difference between Nat2000 sites and controls. Conversely, biomass of L-N CI fish and *S. umbra* did not change with the protection level (Tab. 1).

In terms of RR, one important point to stress is the non-negligible variability observed among FP-MPAs and among Nat2000 sites. Just as an example, lnRRs of the total fish biomass greatly varied among FP-MPAs, with one value that was clearly negative, while lnRRs calculated for Nat2000 sites are approximately equally distributed from one side to the other of the zero value (Fig. 3).

Across all FP-MPAs combined, average RRs concerning all 6 fish biomass variables taken into account in the present study showed positive values (with lnRR ranging from 0.6 to 4.8) (Fig. 4). For the 6 variables the confidence intervals (95% CI) did not overlap the zero value, which means that differences are statistically significant.

Across all Nat2000 sites combined, instead, all 6 variables but the biomass of *E. marginatus* did not show any significant pattern, although a general tendency seems to emerge, showing larger values in Nat2000 sites than in control sites. As far as Nat2000 sites are concerned, only the biomass of *E. marginatus* was significantly higher in Nat2000 compared to control sites (lnRR=2.19±1.76; 95% CI). For the 5 other fish biomass variables, conversely, the confidence intervals (95% CI) overlap the zero value, which means that differences are not statistically significant.

Discussion and conclusions

This study, in a nutshell, shows that FP-MPAs preserve more effectively fish assemblages than Nat2000 sites. This result comes out by comparing the biomass of i) whole fish assemblages, ii) fish of high commercial importance and iii) a few relevant species (the dusky grouper *E. marginatus* and the sea breams *Diplodus sargus* and *D. vulgaris*) in FP-MPAs, Nat 2000 sites and control sites. The sole status of Nat2000 site, therefore, does not seems to guarantee an effective management and any significant ecological effect. The tendency for slightly higher values in Nat2000 sites compared to controls (expect for *E. marginatus*) is more likely to attribute, in agreement with the available literature (Friedlander et al., 2013; Edgar et al., 2014), to the isolation and distance of some Nat2000 from fishing ports and villages than to an effect of conservation measures. From this point of view, the choice of the “Regione Sardinia” (the public institution responsible for the management of Nat2000 sites) to establish some Nat2000 marine sites within the borders of previously established MPAs seem to be a solution that guarantees an effective management of these Nat2000 sites. Conversely, the mere declaration of a Nat2000 sites does not seem to ensure any significant effect.

Another aspect coming out from these data is the ample variability of the results within each level of protection, in particular considering the results of FP-MPAs. Such a variability in terms of ecological effectiveness of FP-MPAs can be the result of multiple factors acting locally, such as the design, the organization, the management, the rule enforcement, etc., which may vary among MPAs, something which well known in the Mediterranean context (Guidetti et al., 2008; Sala et al., 2012; Giakoumi et al., 2017).

As it is the case of Nat2000 sampled in Sardinia in this study, Nat2000 sites do not usually include fully protected (i.e. no-take) zones. Consequently, the fact fish biomass (especially that of fish species targeted by fishing) is higher in FP-MPAs is an expected outcome. Conversely, the very small or inexistent differences between Nat2000 and controls deserves major attention. The point is that some fish are key species playing pivotal roles at community level. The dusky grouper is one of the largest predator in coastal Mediterranean ecosystems (Sala, 2004; Guidetti and Micheli, 2011; Condini et al., 2017). This species is plays a major community-wide ecological role, as it is usually the case for large predators in nature (Ray et al., 2005). However, except for limited areas benefiting from effective conservation measures (like the well managed and enforced FP-

MPAs; Giakoumi et al., 2017), this species displays a progressive decline through time in the Mediterranean due to the impact of fishing (Guidetti and Micheli, 2011). Similarly, the ecological role of *Diplodus* fish has been demonstrated to be crucial for the preservation of macroalgal beds in Mediterranean rocky reefs (Sala et al., 1998). Via their predation upon sea urchins, *Diplodus* fish significantly contribute to control sea urchin populations and their (over)grazing upon macroalgae, thus preventing the formation of the so-called barrens (i.e. bare rocks; Sala et al., 1998; Guidetti, 2006). The recovery of *Diplodus* fishes within effective MPAs can trigger a cascading effect: more abundant and larger *Diplodus* populations reduce sea urchin populations abundance and their grazing rate, which allows the recovery of macroalgal forests (Guidetti, 2006), which are, on their turn, crucial habitats for many juvenile coastal fishes (Thiriet et al., 2016). The results of this study, therefore, go well beyond the results about having more or less fish, but have community- and ecosystem-wide implications.

Although the main objective of Nat2000 sites is to protect the habitats and species included in the EU Birds and Habitats Directives, it becomes urgent to integrate this objective in other more recent EU initiatives, principally the Marine Strategy Framework Directive (MSFD) and the Common Fisheries Policy (CFP). The MSFD, whose initial implementation phase started in 2012, has the main aim to achieve the so-called “Good Environmental Status” (GES) in EU marine waters by 2020. The CFP is a reform launched by the EU Commission in 2013 aiming at achieving a good status for all commercial stocks exploited in EU waters by 2020. Both MSFD and CFP aim at contributing to achieve GES and fisheries sustainability via an ecosystem-based approach (Garcia et al., 2003) where MPAs (all types, Nat2000 sites included) are seen as pivotal tools (Fenberg et al., 2012). Until now any consideration about fishing in Nat2000 has been done considering its potentials impacts on the species and habitats included e.g. in the Habitat Directive. It is time to change this perspective, making possible for marine Nat2000 sites to contribute more significantly to the ecosystem-wide conservation policies in EU waters.

Re-thinking and widening the role of Nat2000 is vitally important, but to do that properly it would be desirable that at EU level the site selection, the organization, the management and monitoring of Nat2000 sites would be harmonized and standardized. This is a crucial step to avoid, for instance, what happens to the nationally established MPAs in EU waters, where the different countries have created MPAs very different in terms of design, mission, goals, management, infrastructures, staffs, funding, regulations and zoning, enforcement, monitoring system, etc. (Scianna et al., submitted). This situation represents the major limitation to the development of a coherent network of MPAs. Strictly concerning Nat2000 sites, the lack of a systematic planning process, the fact that in most cases the Nat2000 sites covering marine surfaces are mere extensions of terrestrial sites into the sea, the scarce consideration of specific marine conservation needs, the lack of management plans for most cases, and the general lack of political will of member states towards the real protection of EU marine waters (the mere declaration of protected surfaces is useless if management and enforcement remain poor if not inexistent) make these tools until now poorly effective (Meinesz and Blanfuné, 2015; PISCO and UNS, 2016; Mazaris et al., 2017). These elements justify to some extent the diffuse opinion that in the EU (and Mediterranean) context, i) we are far from building an actually coherent, connected and effective network of MPAs, and that ii) there is a urgent need for a major harmonization and standardization of the available conservation tools (Mazaris et al., 2018; Scianna et al., submitted).

In consideration of all this, it appears clear that, if the establishment of (M)PA networks is a crucial step in the path towards the conservation of ecological mechanisms and the support of ecosystem functions, focusing solely on coverage targets (e.g. protecting 10% of marine waters by 2020) is likely to be get a substantial failure. Nat2000 marine sites should thus evolve and meet not merely extension criteria but also the key features and high quality criteria constituting the solid base for an effective networks of MPAs. To achieve effective conservation, policy and decision makers should chiefly guarantee the effective management of the currently designated Nat2000 sites by integrating them in the wider EU policy, rather than keep enlarging the declared “protected” surfaces. Once this perspective change is accepted, then, management measures in Nat2000 sites could be extended to fisheries regulations and to other human activities representing a potential risk to marine ecosystems. Even though Nat2000 sites, as any other MPA type, cannot be a panacea against any form of marine community alteration, they could play a role, aside other MPA types, e.g. in limiting the spread of invasive species in the era of climate change (Gallardo et al. 2017), provided they are effectively managed.

These issues do corroborate the increasing need to integrate the Habitat and Bird Directive objectives within other more recent EU initiatives, principally the Marine Strategy Framework Directive (MSFD) and the Common Fisheries Policy (CFP), which, altogether could increase the chances to achieve a Good Environmental and Fishery status in EU waters.

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Figures

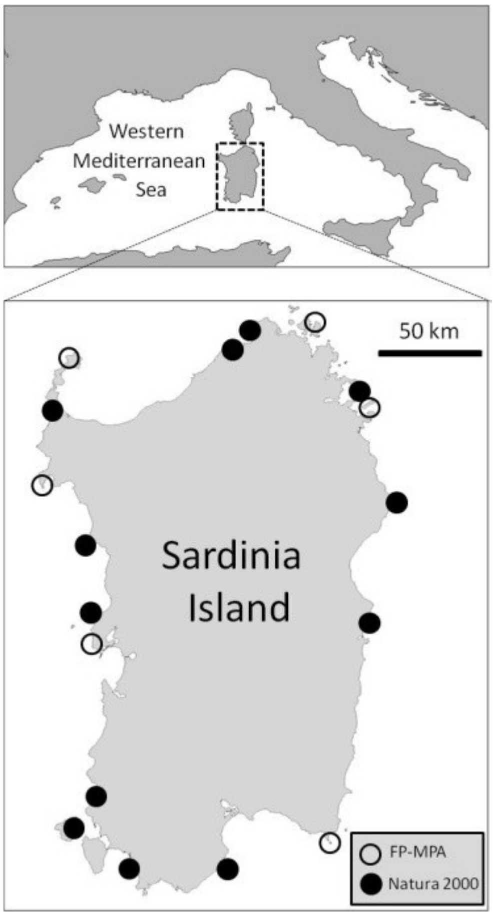

Fig. 1 – Sampling locations around Sardinia Island: FP-MPA=fully protected Marine Protected Area; Natura 2000=Natura 2000 site.

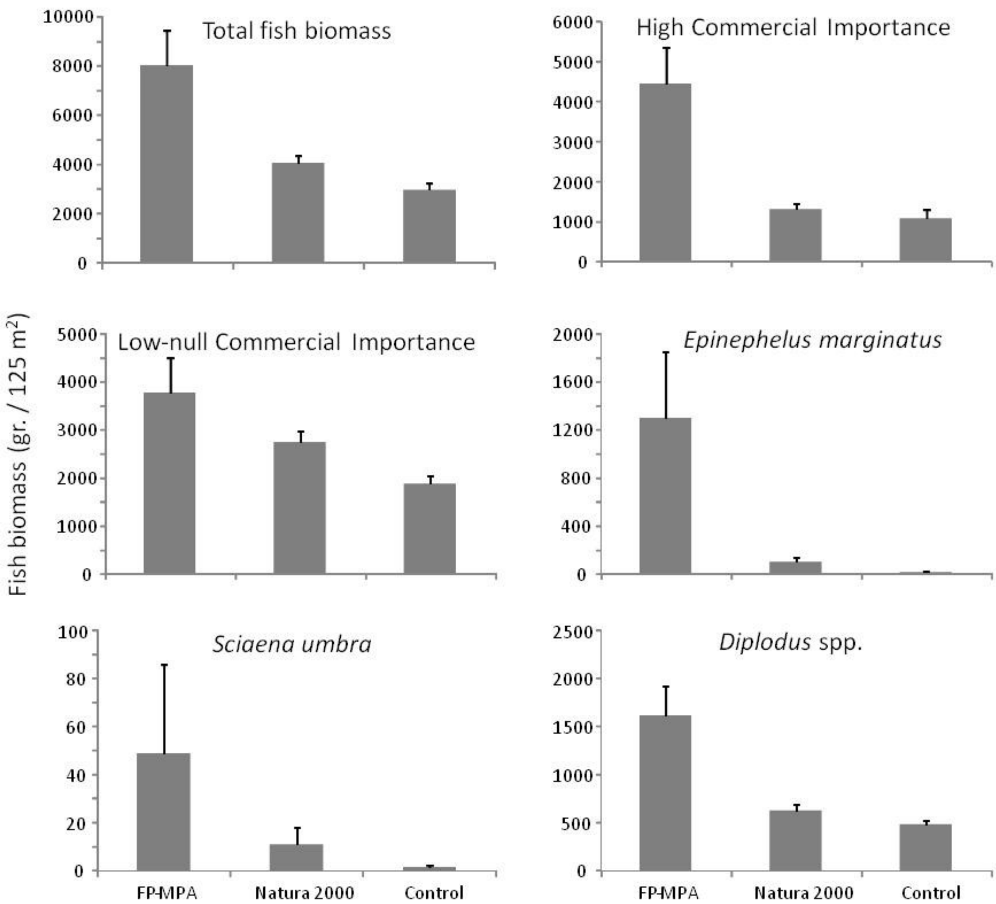

Fig. 2 – Fish biomass (mean±SE) assessed at the sampling locations under different conditions of protection: FP-MPA=fully protected Marine Protected Areas; Natura 2000=Natura 2000 sites; Control=areas open to fishing according to national/regional laws.

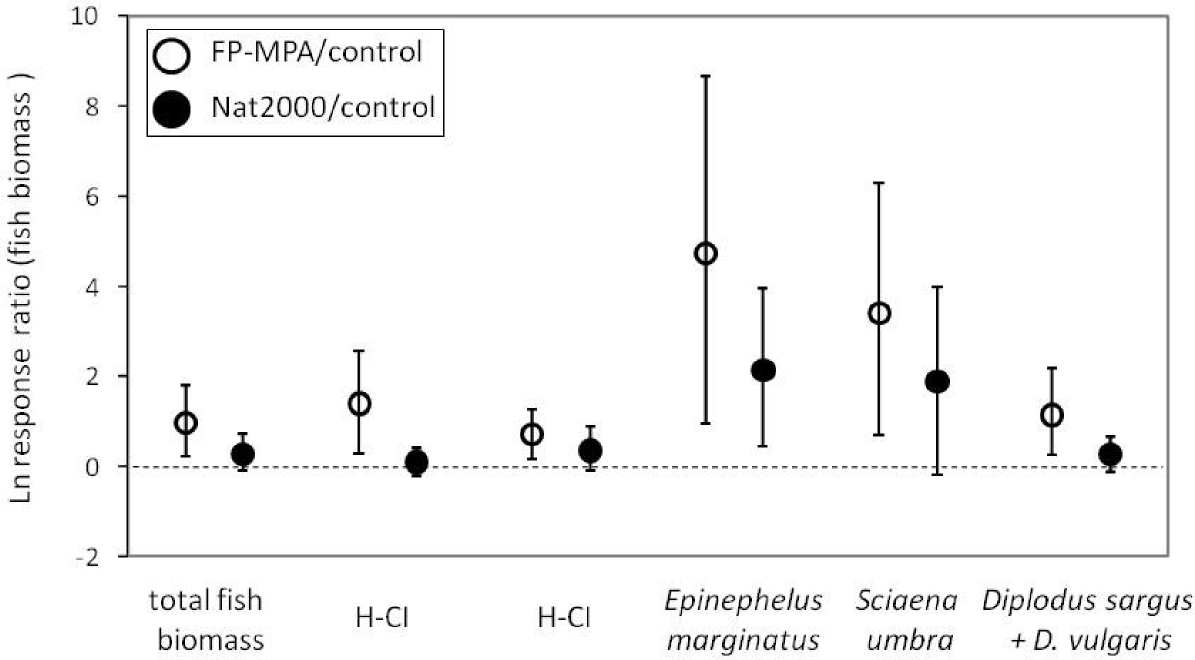

Fig. 3 – Fish response to protection of the 6 variables related to fish biomass, measured as the natural log ratio, observed in the 6 FP-MPAs (fully protected Marine Protected Areas) and 12 Nat2000 (Natura 2000) sites considered in the present study, compared to Control sites (i.e. areas open to fishing according to national/regional laws). Bars indicate 95% confidence intervals. See methods for more details.

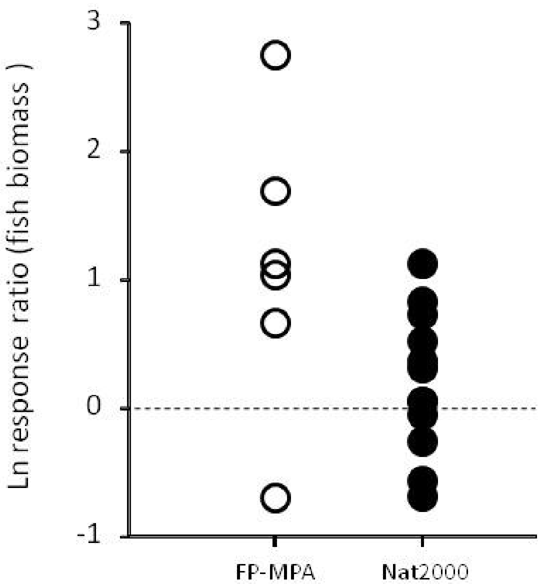

Fig. 4 – Variability in the response of total fish biomass (measured as the natural log ratio) observed in the 6 FP-MPAs (fully protected Marine Protected Areas) and 12 Nat2000 (Natura 2000) sites considered in the present study, compared to Control sites (i.e. areas open to fishing according to national/regional laws).

Tables

Tab. 1 - Summaries of PERMANOVAs and pair-wise analyses testing for differences among protection levels (PR=Protection, 3 levels: MPA=fully protected MPA; N2000=Natura 2000 site; C=control open to fishing) and over the spatial scale of stations (ST=Station, 2 levels). P-values calculated using Montecarlo permutations. NA: not applicable.

Variable	PR	ST(PR)	Pair-wise tests (PR)
Total Biomass	**0,004**	0,572	FP-MPA>N2000>C
High Commercial Importance	**0,000**	0,800	FP-MPA>N2000=C
Low-Null Commercial Importance	0,091	0,394	NA
Epinephelus marginatus	**0,000**	0,985	FP-MPA>N2000>C
Sciaena umbra	0,278	0,159	NA
Diplodus spp.	**0,002**	0,620	FP-MPA>N2000=C

Supplementary material

S1 - Detailed results of two-way PERMANOVAs (and related pair-wise tests, when appropriate, on the levels of the factor PR) examining (1) total fish biomass; (2) biomass of High Commercial Important species; (3) biomass of Low-Null Commercial Important species; (4) biomass of *Epinephelus marginatus*; (5) biomass of *Sciaena umbra*; (6) biomass of *Diplodus* spp., among the 3 levels of protection (FP-MPAs=fully protected Marine Protected Areas; Nat2000=Natura 2000 sites; C=controls, i.e. areas open to fishing according to national/regional laws), and between 2 stations within location. Factors: PR=protection; ST=station. Significant P-values indicated in bold. See methods for more details.

1) Total fish biomass.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
PR	2	7,45E+08	3,73E+08	29,984	0,0318	90	**0,0043**
ST(PR)	3	3,59E+07	1,20E+07	0,67625	0,5786	9942	0,5717
Res	210	3,71E+09	1,77E+07				
Total	215	4,49E+09					

Groups	t	P(perm)	Unique perms	P(MC)
FP-MPA, C	6,2234	0,1645	6	**0,0031**
MPA, Nat2000	4,9716	0,1712	6	**0,0148**
C, Nat2000	4,2756	0,3338	6	**0,0322**

2) biomass of High Commercial Important species.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
PR	2	3,40E+08	1,70E+08	77,668	0,052	90	**0,0001**
ST(PR)	3	5,64E+06	1,88E+06	0,33903	0,8025	9939	0,7999
Res	210	1,16E+09	5,55E+06				
Total	215	1,51E+09					

Groups	t	P(perm)	Unique perms	P(MC)
FP-MPA, C	9,5236	0,166	6	**0,0001**
MPA, Nat2000	8,1369	0,165	6	**0,0004**
C, Nat2000	3,1761	0,168	6	**0,0738**

3) biomass of Low-Null Commercial Important species.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
PR	2	8,42E+07	4,21E+07	5,125	0,0347	90	0,0911
ST(PR)	3	2,47E+07	8,22E+06	1,019	0,3935	9932	0,3937
Res	210	1,69E+09	8,07E+06				
Total	215	1,80E+09					

4) biomass of biomass of *Epinephelus marginatus.*

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
PR	2	4,73E+07	2,36E+07	100,28	0,2012	90	**0,0001**
ST(PR)	3	2,78E+05	92804	0,051314	0,9835	9948	0,9847
Res	210	3,80E+08	1,81E+06				
Total	215	4,27E+08					

Groups	t	P(perm)	Unique perms	P(MC)
FP-MPA, C	7,6463	0,3361	6	**0,0001**
MPA, Nat2000	8,0881	0,3346	6	**0,0001**
C, Nat2000	4,6589	0,1716	6	**0,0276**

5) biomass of *Sciaena umbra*.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
PR	2	61165	30583	1,9006	0,2222	90	0,2779
ST(PR)	3	50029	16676	1,7283	0,123	9946	0,1591
Res	210	2,03E+06	9649				
Total	215	2,14E+06					

6) biomass of *Diplodus* spp.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
PR	2	3,60E+07	1,80E+07	39,586	0,0824	90	**0,0018**
ST(PR)	3	1,29E+06	4,30E+05	0,59464	0,6288	9953	0,6202
Res	210	1,52E+08	7,23E+05				
Total	215	1,89E+08					

Groups	t	P(perm)	Unique perms	P(MC)
FP-MPA, C	7,3872	0,1722	6	**0,0006**
MPA, Nat2000	6,4202	0,1659	6	**0,0041**
C, Nat2000	1,849	0,3294	6	**0,1973**