Seasonal variability of rocky reef fish assemblages: Detecting functional and structural changes due to fishing effects

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1. Introduction

Temperate rocky reefs are characterized by a large biological diversity which depends on the interaction of physical and biological factors that can cause strong fluctuations in the distribution and abundance patterns of marine assemblages (Holbrook et al., 1994; Rubal et al., 2011). Habitat complexity and exposure (e.g. Friedlander and Parrish, 1998; Friedlander et al., 2003; García-Charton and Pérez-Ruzafa, 2001; La Mesa et al., 2011b; Lara and Gonzalez, 1998), seasonal variability (e.g. Beldade et al., 2006; Friedlander and Parrish, 1998; Holbrook et al., 1994; Magill and Sayer, 2002) and inter-annual climatic shifts (e.g.Henriques et al., 2007; Holbrook et al., 1994), are some of the main factors affecting the persistence of species in reefs depending on their ecological requirements, interspecific relationships, life-cycle and mobility patterns. Consequently, they can buffer the effects of anthropogenic pressures or lead to misinterpretation of changes in marine communities (Holbrook et al., 1994).

In recent years, short-term indicators of anthropogenic effects on marine assemblages have become an important issue in applied ecology and implementation of international policies, such as the Marine Strategy Framework Directive (Directive, 2008/56/CE). The usefulness of any “state” indicator will depend on how well it is able to distinguish anthropogenic from natural variability (Niemi and McDonald, 2004). Thus, the analysis of any indicator should take into consideration the above-mentioned factors in order to not only understand stress–response relationships, but also to select the most suitable indicators.

Marine Protected Areas (MPA) are broadly used in marine conservation, aiming to restore and protect the structure and function of marine ecosystems (Micheli et al., 2004). By limiting or forbidding fishing activities in some areas (e.g. no-take zones), MPAs are the best case studies to analyze the effects of fishing on multispecies assemblages as well as their recovery trajectories (Micheli et al., 2004). Although the differences between protected and fished areas depend upon the age of reserves, several other factors could contribute to re-serve effectiveness, namely law enforcement, species home-range, fishing effort outside the reserve, reserve size, species recruitment patterns and connectivity between habitats (Claudet et al., 2006; García-Charton et al., 2008; Guidetti et al., 2008). Moreover, these factors could lead to differences between geographical areas in what regards the effects of fishing, making them less predictable.

In general, MPAs are expected to increase the density and biomass of fish assemblages, especially of target and large-bodied species, nevertheless, several studies report complex top-down and bottom-up changes due to habitat quality improvement, competition and predator–prey interactions, which can lead to changes on non-target species depending upon their role in the ecosystem (e.g. Guidetti and Sala, 2007; Micheli...
et al., 2004; Pennigar et al., 2000; Ruitton et al., 2000; Willis and Anderson, 2003). In Europe, MPA effects are well known for some geographical areas like the Mediterranean Sea (see Fenberg et al., 2012; García-Charton et al., 2008 for a brief review), but are poorly studied in the north-eastern Atlantic. Although several fish-based metrics related with the abundance/biomass of trophic groups (e.g. piscivores and macrocarnivores), fish size, high commercial value and indicator species have been successfully used as indicators of fishing pressure within MPAs (e.g. Claudet et al., 2006; Guidetti and Sala, 2007), there is a considerable lack of knowledge about the consequences of seasonal variability on those metrics, since in this case the studies have been focused on seasonal fluctuations of species (e.g. Beldade et al., 2006; Friedlander and Parrish, 1998; Holbrook et al., 1994; Magill and Sayer, 2002).

It is recognized that the use of functional guilds to assess anthropogenic impacts has several advantages as they tend to be more resilient to natural variation and respond more predictably to stress (Elliott et al., 2007; Henriques et al., 2008; Pais et al., 2012). Furthermore, since guilds group species with some degree of functional overlap in the ecosystem they could be easily applicable to other regions (Elliott et al., 2007; Noble et al., 2007). In this context, based on several fish-based metrics (guild approach), the present study aimed to assess structural and functional changes in fish assemblages due to fishing pressure and to understand the influence of seasonal variability on those metrics as well as their ability to detect changes.

2. Material and methods

2.1. Study area

The Arrábida MPA (mainland Portugal; NE Atlantic) was formally established in 2005 but the regulatory measures were gradually implemented over the following four years (see Sousa, 2011 for details of implementation process). The before MPA covers about 53 km² which includes a total protection zone (TPZ) of 4 km², four partial protection zones (PPZ) and three complementary protection zones (CPZ) covering 21 km² and 28 km², respectively (Fig. 1). Regarding the commercial fishing inside this MPA, only licensed vessels under 7 m in length are allowed to fish, but with several restrictions: In the CPZ, fishing activities with traps, jiggering, longline and handline are allowed in all areas, whereas nets are permitted only farther than 1/4 NM from shore line; In the PPZ, only traps, jiggering and handline are allowed farther than 200 m from shore line; The TPZ is a “no-take” area; In the whole MPA, trawling, dredges and handcatching commercial fishing are forbidden. Finally, recreational angling is only permitted in the CPZ while spearfishing is forbidden in all zones.

At the time of this study, about 73 vessels operated inside the MPA, but the number of angling fishermen is unknown since anyone with a recreational fishing license can fish in the CPZ. Fishing effort is high in all fished zones, being concentrated in the CPZ near the Sesimbra village and in the PPZ surrounding the TPZ (Cabral et al., 2008). According to Cabral et al. (2008), traps are one of the most commonly used gears within the MPA, while gill and trammel nets concentrated in the CPZ near the exit of the Sesimbra port (>1/4 NM) as well as vessels fishing with jigs, longlines and handlines that normally operate less than 200 m from shoreline (see Fig. 1 for fishing pressure details). It is important to note that the values of fishing pressure in Fig. 1 correspond to a normal fishing day and were estimated between September 2007 and February 2008, just before the end of fishing allowance in the TPZ. This data represents the best available information to date about the fishing effort within the MPA.

This MPA faces south and is therefore protected from the prevailing north and northwest winds and waves (Gonçalves et al., 2002; Henriques et al., 2007). Subtidal rocky habitats are highly heterogeneous, resulting from the disintegration of calcareous cliffs that border the coastline and extend to tens of meters (Gonçalves et al., 2002). These rocky areas are composed of mixed patches of sand, gravel, cobble, random-sized blocks and bedrock. Due to their heterogeneity and topographic complexity, they support a large number of fish species (Beldade et al., 2006; Gonçalves et al., 2002).

To analyze the effects of fishing on rocky reef fish assemblages, three zones were selected inside the MPA, one at each protection level (TPZ, PPZ, and CPZ) (Fig. 1). These zones were selected based on their habitat complexity of the sampled sites is showed in the graphs. Fishing pressure values were based on the previous study of Cabral et al. (2008).
on their habitat characteristics which are of similar complexity. The Combined Topography Index (CTI), the percent cover of rock, cobble and sand, the cover of algae by structural groups (i.e. creeping, encrusting, tufts, sheet and filamentous) and the presence/absence of invertebrate groups (i.e. sponges, anemones, hydrozoans, gorgonians, polychaetes, gastropods, crustaceans, sea urchins, star-fish, sea cucumbers and ascidians) were used to characterize habitat complexity. Habitat sampling was performed by depth strata, by deploying 12 quadrats (50 × 50 cm) to estimate algal cover and the presence of invertebrates, and 8 replicates of the “chain-and-tape” method for the remaining measures, by using a 25 m leaded rope as a “chain” and a 25 m measuring tape to calculate the linear distance traveled by contouring the “chain” over the substrate and to estimate the percent cover of different substrates (see Pais et al., 2013 for details).

Since topography remains similar year round, only quadrant sampling was repeated seasonally. CTI was estimated for each “chain-and-tape” replicate through the formula \( \text{CTI} = \left( \frac{1}{2} \times \text{SR} \right) + \frac{\text{NC}}{25} + \frac{\text{MVR}}{25} \), where \( \text{SR} \) is the substrate rugosity index, \( \text{NC} \) the number of corrugations and \( \text{MVR} \) the maximum vertical relief in meters (see Pais et al., 2013). The average value of the CTI among replicates of both depth strata was used to characterize each sampled zone.

2.2. Fish assemblages

Fishes were seasonally sampled at each site from May 2010 to February 2011 using underwater visual census methods. Based on a pilot study (see Henriques et al., 2013), 50 m long strip-transects were randomly placed parallel to the coastline at two depth strata (0–5 m and 5–10 m). Each transect was inspected twice, first pass for demersal species (50 × 2 m) and the second for cryptobenthic species (50 × 1 m). On the cryptobenthic pass only the families Gobiidae, Bleniidae, Congridae, Gadidae (subfamilies Phycinae and Lutinae), Gobiosocidae, Gobiidae, Muraenidae, Scorpaenidae, Scophthalmidae, Soleidae, Synagmidae, Tripterygiidae and the species Centrolabrus rupestris and Labrus mixtus, as well as Symphodus spp. with less than 5 cm total length, were counted (Henriques et al., 2013). A total of 144 transects were performed, corresponding to six replicates per zone and per season. Each replicate included observations for both depth strata pooled together, i.e. one transect at a 0–5 m depth range and another at a 5–10 m range, performed in the same dive (~80 min). A total of two replicates were done per day. Transects were performed with a minimum visibility of 5 m. In all transects, the abundance and total length of fish were recorded by the same divers (S Henriques or MP Pais) in order to minimize observer effects.

All fish species were allocated to their ecological and functional guilds based on the previous classification by Henriques et al. (2008) and updated with available literature and FishBase online database (Froese and Pauly, 2012) (Supplementary material). Species were considered “invertebrate feeders” when they feed mostly on non-planktonic invertebrates, otherwise being considered “zooplanktivores”. “Macrocarnivores” feed both on macroinvertebrates and vertebrates (mostly fish). “Herbivores” feed predominantly on macroalgae, macrophytes, phytoplankton and microphytobenthos and “omnivores” feed on detritus, filamentous algae, macrophytes, epifauna and infauna. The concepts of habitat association and functional guilds were preserved since no pair was found with a Spearman correlation coefficient higher than 0.85.

2.3. Data analysis

Multivariate analysis of variance using permutations (PERMANOVA) tests the effect of one or more factors on one or more variables on the basis of any distance or dissimilarity measure of choice and does not assume normality of errors since the p-values are obtained by permutations (Anderson et al., 2008). Nevertheless, PERMANOVA is sensitive to differences in dispersion among groups, and therefore homogeneity of multivariate dispersions was tested using a PERMDISP routine before running the PERMANOVA tests (Anderson et al., 2008).

The similarity of habitat complexity among zones was tested using two-way PERMANOVA analyses for biotic cover (functional groups of algae and presence/absence of invertebrate groups) and one-way PERMANOVA analysis for habitat structure (CTI and the percent cover of rock, cobbles and sand). When significant values of biotic cover were found for factor “zone”, univariate PERMANOVA analyses were performed on each variable individually, in order to find those responsible for the differences. The effects of different protection levels and seasonality on fish-based metrics were analyzed both through a multivariate (all metrics) and a univariate (each metric individually) perspective using 2-way (PERMANOVA; Anderson et al., 2008). With the exception of habitat structure, all analyses were performed with both factors zone (3 levels) and season (4 levels) treated as fixed. Only the factor zone was tested for habitat structure since it is not expected to change seasonally. When significant differences were detected, factors were investigated through post-hoc pair-wise comparisons.

In order to visualize multivariate patterns of fish-based metrics without constraints, Principal Coordinates Analysis was used (PCO; Anderson et al., 2008). In addition, Canonical Analysis of Principal Coordinates (CAP; Anderson and Willis, 2003) was also performed with the purpose of uncovering patterns that could be masked by unconstrained analysis, by finding axes through the multivariate cloud that best discriminate between different zones and seasons. Furthermore, Spearman correlation coefficients of metric values with PCO and CAP axes were calculated and the most correlated metrics (\( r > 0.5 \)) supported the discussion of the observed patterns.

All the analyses performed with fish-based metrics and habitat structure variables were based on Euclidean distance metrics, constructed after normalizing each variable by subtracting the mean and dividing by the standard deviation, in order to place all variables on a comparable scale. For algae functional groups, the percentage of cover was fourth-root transformed and the Bray–Curtis similarity index used to construct the resemblance matrix, while for the presence/absence of invertebrate groups the resemblance matrix was calculated using the Jaccard Index. All the above-mentioned analyses were performed using PRIMER 6 with PERMANOVA + software package. P-values were calculated using 9999 permutations and the level of statistical significance adopted was 0.05. After running the analyses, redundancy between metrics was checked and all of them were preserved since no pair was found with a Spearman correlation coefficient higher than 0.85.
Finally, the size structure of the most abundant species with high commercial value (*Diploplus vulgaris* and *Diploplus sargus*) was plotted per zone and season in order to better understand the effects of fishing and seasonal variability. Size structure was plotted according to the following size classes: early juveniles (below 10 cm), juveniles (between 10 cm and the size at first maturity) and adults (above the size at first maturity).

### 3. Results

The analysis of habitat variables showed no significant differences for habitat structure among zones (Pseudo-F = 1.96 p > 0.05), as well as for both the factor “zone” and the interaction between factors “zone × season” in the case of algae cover (Pseudo-F = 2.02 p > 0.05 and Pseudo-F = 1.60 p > 0.05, respectively). Regarding the presence of invertebrate groups, the overall multivariate PERMANOVA results revealed significant differences for the factors “zone” and “season” (Pseudo-F = 5.17 p < 0.05 and Pseudo-F = 1.60 p < 0.05, respectively) but, more importantly, no significant differences for the interaction of both factors (Pseudo-F = 1.02 p > 0.05). Pair-wise comparisons for the factor “zone” in the PERMANOVA analyses performed on each invertebrate group individually showed that only the hydrozoans, anemones, gorgonians and ascidians were significantly different among zones (Pseudo-F = 5.94 p < 0.05, Pseudo-F = 11.04 p < 0.05, Pseudo-F = 10.76 p < 0.05 and Pseudo-F = 4.50 p < 0.05, respectively). With the exception of gorgonians, which differ in the TPZ when compared to the remaining zones, hydrozoans and anemones differ in the CPZ when compared to the PPZ and the TPZ, while ascidians only differ between the CPZ and the TPZ. Habitat structure (substrate and topography) features are shown in Fig. 1, while the algae cover and the frequency of occurrence of invertebrates are presented in Table 1.

In the present study, a total of 47 fish species belonging to 20 families were counted in the Arrábida MPA. Sparidae and Labridae were the most represented families in terms of number of species (12 and 9 species, respectively) and abundance (95% of the total abundance, on average). PERMANOVA for the fish-based metrics showed a significant overall multivariate effect of both season and zone (Pseudo-F = 7.47 p < 0.05 and Pseudo-F = 5.68 p < 0.05, respectively) as well a significant interaction effect (Pseudo-F = 1.51 p < 0.05). Additionally, no significant differences in multivariate dispersions was found by the PERMDISP routine (F = 0.23 p > 0.05). Pair-wise comparisons showed significant differences among all zones and seasons (p < 0.05), except between winter and spring (p > 0.05), while no consistent patterns were found for the interaction between both factors. Actually, significant differences between all zones were obtained in spring and an opposite effect in winter, no differences were detected between the TPZ and the CPZ in summer and between TPZ and the PPZ in autumn (see pair-wise comparisons in Table 2).

The PCO plots show a strong effect of season when compared to the effect of zone, since all seasons, particularly autumn, are clearly separated in the multivariate data cloud. No patterns of zones were globally detected if seasons were not taken into account (Fig. 3A and B). The discriminant CAP analysis, however, was able to find axes to separate zones (Fig. 3C), with a squared canonical correlation of δ2 = 0.523 (p < 0.05). The first canonical axis clearly separated the fish-based metrics in the CPZ from the TPZ and PPZ, and the second canonical axis separated the TPZ from the PPZ (Fig. 3C). Vectors representing Spearman correlations with CAP axes (r > [0.5]) showed that some metrics are apparently associated with the different levels of protection. The density of generalist individuals is higher in zones where fishing is permitted (CPZ), the individuals with high commercial value tends to have higher values in protected zones (TPZ and PPZ) and the large individuals with medium to high commercial value is associated with the TPZ. The CAP analysis performed to discriminate seasons, as expected, was able to find axes that maximize seasonal variation with a square canonical correlation of δ2 = 0.743 (p < 0.05) (Fig. 3D). The first canonical axis clearly separated the fish-based metrics in autumn and summer from spring and winter, with winter and spring seeming to cluster. In this case, the density of invertebrate feeders was associated with summer and autumn, while the juveniles and omnivores seems to be more related with autumn (r > [0.5]) (Fig. 3D).

Permutational univariate ANOVAs on fish-based metrics revealed a significant effect of season in the density of omnivores and juveniles, while significant differences were found among zones for herbi-vores and the individuals with high commercial value (Table 3). Both factors had significant effects on the density of generalist individuals, invertebrate feeders and adults with high commercial value, while no effects were obtained for the macrocarnivores and the large individuals with medium to high commercial value (Table 3). Additionally, no significant differences among all zones and seasons (p < 0.05), except between winter and spring (p > 0.05), while no consistent patterns were found for the interaction between both factors. Actually, significant differences between all zones were obtained in spring and an opposite effect in winter, no differences were detected between the TPZ and the CPZ in summer and between TPZ and the PPZ in autumn (see pair-wise comparisons in Table 2).
the interaction between factors “zone” and “season” showed a significant effect in the density of invertebrate feeders, density of generalist individuals and density of individuals with high commercial value (Table 3). Pair-wise comparisons of metrics with significant results for factor “season” showed that, in general, the majority of them were significantly different between autumn and other seasons (Table 3). Furthermore, some metrics were not significantly different between successive seasons as well as between summer and winter (Table 3). Regarding the effects of zone, only the density of generalists showed significant differences among all zones, while the density of invertebrate feeders and the density of individuals with high commercial value differed significantly between the CPZ and the zones with higher protection status (TPZ and PPZ) (Table 3). However, this effect of zone was not consistent among all seasons (see pair-wise comparisons for the interaction of both factors in Table 3). The density of generalist individuals was consistently different among all zones in all seasons with exception of summer. For the remaining fish-based metrics, no differences between protection zones was detected in winter. In spring, the density of generalists was significantly different between the TPZ and the CPZ. On the other hand, in autumn, all metrics were different between the CPZ and the remaining zones and no differences in the density of individuals with high commercial value and invertebrate feeders were detected between the TPZ and the PPZ.

Finally, plots with size distribution of the most representative species with high commercial value (D. vulgaris and D. sargus) showed differences among seasons (Fig. 2). Abundance of juveniles of D. vulgaris peaked in summer and no strong peak was identified for early juveniles of D. sargus. In general, mean number of adults of D. vulgaris was higher
in autumn and when higher differences between protection zones were observed, especially between the TPZ and the PPZ in comparison with the CPZ. In the case of *D. sargus*, the mean number of juveniles and adults was higher in winter and spring and the juveniles class was the most represented in all seasons.

### 4. Discussion

The present study highlights the influence of seasonal variability in fish assemblage patterns and stresses the importance of taking seasonality into account when assessing changes due to anthropogenic disturbance. A marked degree of seasonal variability of the fish-based metrics was evident in both PCO and CAP analyses, which was less pronounced between winter and spring and more in the autumn. These trends were consistent with PERMANOVA results, where significant differences were obtained among all seasons except between winter and spring, matching the results from previous studies that analyzed seasonal patterns of rocky fish species (e.g. Beldade et al., 2006; Friedlander and Parrish, 1998; Holbrook et al., 1994; Magill and Sayer, 2002). Seasonal changes in fish assemblage composition and abundance have been broadly attributed to the input of recruits and to spawning and feeding migrations (Aburto-Oropeza and Balart, 2001; Friedlander and Parrish, 1998; Holbrook et al., 1994). Indeed, a general increasing trend in the density of juveniles, the omnivores and the invertebrate feeders was associated with summer and autumn samples.

In a study about the very-near-shore fish larvae distribution at Arrábida MPA, Borges et al. (2007) obtained higher diversity and abundance of larvae from May to July followed by a strong decrease in August. This period corresponds to the spawning season of most of the rock-associated species in this area (Almada et al., 1999; Borges et al., 2007; Gonçalves et al., 2002). These results suggest that in this area recruitment processes can occur in late summer. This pattern is also confirmed by Garcia-Rubies and Macpherson (1995) which observed an increase of recruits (smaller than 1.5 cm length) from July to September with a very similar fish assemblage in the NW Mediterranean, although some variances in the starting and range of recruitment pattern of certain species could occur due to temperature differences between Atlantic and Mediterranean waters. It is important to note that the metric density of juveniles includes all individuals bellow the size at first maturity and not merely early juveniles. Consequently, despite the density of juveniles increasing in summer (July), the highest densities were found in autumn (October) as a cumulative result of recruitment processes of various species.

Although the increase in juveniles could partly explain the increased values for the trophic structure metrics that they represent (e.g. omnivores and invertebrate feeders), other factors like spawning migrations or feeding activity might be important (e.g. Friedlander and Parrish, 1998; Harmelin et al., 1995).

In temperate rocky reefs, two main spawning strategies occur: (1) demersal spawners, species producing eggs that become attached...
Table 3
PERMANOVA results for the effects of seasons, zones and their interaction on each metric individually (*p-values < 0.05). Shaded areas denote significant results for pair-wise tests (p-values < 0.05). Seasons: wi — winter; au — autumn; su — summer; sp — spring. Protection zones: TPZ — total; PPZ — partial; CPZ — complementary.

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to the substrate or in nests, normally with males or females displaying parental care, for example the genus Sympodus, and (2) pelagic spawners, species producing planktonic eggs with high dispersive capabilities, for example the families Sparidae, Serranidae and Mugilidae (Almada et al., 1999; Borges et al., 2007). In both cases, females and/or males must travel long distances in search for nests/partners (demersal spawners) or migrate to spawning sites like bays, deep or shallow waters (pelagic spawners) (Almada et al., 1999). These migrations could lead to changes on adult’s abundance in the assemblages, either by their departure from reefs or by their aggregation, which is expected to happen at higher intensity in the spawning season from mid-spring to early summer (May–July). In agreement, despite not being initially included in the metrics list, since it had previously shown weak response to anthropogenic pressures (Henriques et al., 2013), the density of individuals over the size at first maturity showed strong seasonal variation (PERMANOVA, $F = 4.307 \ p < 0.05$ for factor season, $F = 1.43 \ p > 0.05$ for factor zone and $F = 1.28 \ p > 0.05$ for the interaction of both factors), with significant differences found between summer (July) and spring and autumn (April and October, respectively). Notwithstanding the above-mentioned results, some species exhibited an extended spawning strategy with several peaks yearlong, in order to reduce inter-specific competition as they share similar habitat requirements with other species (García-Rubies and Macpherson, 1995). Thus, other seasonal differences in spawning and recruitment patterns could be observed for some species as they have several recruitment and adult migration peaks yearlong (e.g. Diplofus spp.).

Finally, seasonal changes on feeding activity could also contribute for the observed patterns during summer and autumn, specially the density of invertebrate feeders and density of omnivores. Benthic invertebrates, which are known to increase with proliferation of macroalgae canopy (e.g. fleshy erect algae) during spring and summer (Sala, 1997), constitute the prevailing preys of most of the observed fish species (Almada et al., 1999). In accordance, the frequent occurrence of several invertebrate groups (e.g. gastropods, crustaceans, bivalves) started in spring and persisted through autumn, which could be related with seasonal fluctuations of macroalgae cover that showed an increase in algae tufts during spring and summer, as observed by Rubal et al. (2011) in tidepool macroalgal assemblages (north of Portugal). An increment of prey availability during summer and autumn might attract invertebrate feeders and omnivores that forage on rocky substrates, being easier to observe them. However, because these results were based on presence/absence data of major invertebrate groups (frequencies of occurrence), further research about invertebrate’s abundance at lower taxonomic levels is desirable to fully understand seasonal changes in fish assemblages. This could prove to be particularly important in the assessment of anthropogenic pressures that also affect invertebrate assemblages.

Despite the fact that some year-to-year variation on the global values of species abundance is expected due to inter-annual biological and environmental conditions (e.g. oceanographic features, recruitment patterns, larval mortality), coastal fish assemblages display a relatively stable and predictable response to seasonality for both juveniles and adults, with higher abundance in summer and autumn (Beldade et al., 2006; Harmelin et al., 1995; Holbrook et al., 1994; Magill and Sayer, 2002) and peak larval density in Spring and early summer (Borges, 2006).

It is expected that environmental conditions on each season affect all zones in a similar manner. Therefore, and taking into account the similarity between habitats, if differences in fish-based metrics were detected among zones within seasons they are likely to be due to the effects of protection. In fact, non-significant differences were found among zones for the habitat structure and algae cover as well as in the interaction “zone x season” of biotic cover (algae and invertebrates). Only the sessile ascidians and gorgonians (higher in TPZ) and the hydrozoans and anemones (lower in CPZ) showed significant differences among zones. However, it is unlikely that these differences are responsible for the differences found for fish-based metrics, considering that most of the observed fish species that feed on invertebrates exhibit some flexibility in their diets, with a preference for molluscs (gastropods and bivalves), crustaceans (isopods, amphipods and decapods) and polychaetes, which did not differ among zones (see Stergiou and Karpouzi, 2002 for feeding habits details).

Moreover, there are two additional facts that also suggest the lack of response of fish assemblages to these particular invertebrate groups: (1) the metric density of invertebrate feeders had higher values outside the reserve (CPZ), where a lower frequency of occurrence of those sessile invertebrates was observed; (2) the interaction of both factors (season and zone) was non-significant for these sessile invertebrates due to large differences between samples, while for the fish-based metrics that responded to protection, significant differences were found.

Several differences among zones were found in spring, summer and autumn and no differences were found in winter, suggesting that seasonal variability of fish assemblages affects the detection of their response to anthropogenic pressures. Many of the fish species undergo regular migrations during the winter to find shelter or to spawn (e.g. deeper water), probably as a response to unfavorable conditions (e.g. thermoregulation, food availability) which create a general pattern of low abundance in this season (Friedlander and Parrish, 1998; Magill and Sayer, 2002). These regular movements might clarify why no differences were detected among zones during winter in the multivariate approach as well as for the fish-based metrics individually, with the exception of the density of generalists.

The density of generalists was always higher at the zones with the lowest level of protection in all seasons except in summer, where no effects were found. The most abundant generalist Coris julis has a wide habitat range and depth distribution, flexibility of diets and has no commercial value in this area (Harmelin et al., 1995; Henriques et al., 2013). Such flexibility is an advantage in a context of inter-specific competition that is expected to be higher inside protected zones (Mosqueira et al., 2000; Ojeda-Martínez et al., 2007). Coris julis may migrate to shelf waters to spawn during the spring and summer (see results of Borges et al., 2007). Although the density of generalists was varied with fishing pressure, some care is needed in their widespread use because in some geographical areas C. julis has commercial interest (e.g. Mediterranean Sea), prefers deeper habitats (García-Charton and Pérez-Ruzaña, 2001) and there may be other generalist species with higher commercial interest (e.g. Dicentrarchus labrax and Sparus aurata). Furthermore, C. julis is also tolerant to contamination (Fasulo et al., 2010). In this context, the concept of opportunistic species seems to be more adequate and the metric should therefore be changed to density of opportunistic individuals in order to avoid misinterpretations.

Regarding the trophic structure metrics, no pattern of response to the interaction between the level of protection and season was found for the density of invertebrate feeders even though some significant differences were obtained in pair-wise comparisons. In this case, the most probable explanation is related to the functional overlap between species that responded positively or negatively to protection (C. julis vs. other invertebrate feeders). As for the density of herbivores (only with differences between TPZ and PPZ but with no significant results for the interaction between factors), the most probable explanation is related to the migration of schools between feeding grounds as it only comprises the species Sarpa salpa, which has a broad home range (Abecasis et al., 2012; Jadot et al., 2006). These results suggest that these trophic levels were not good indicators of fishing pressure as opposed to macrocarnivores and piscivores (top predators) as the most sensitive groups (e.g. Guidetti and Sala, 2007; Micheli et al., 2004; Villamor and Becerro, 2012). The low density values observed for top predators could be related not only to the early age of the reserve and their high mobility but also to their preference by other types of habitats (e.g. Pogrus puger; García-Charton and Pérez-Ruzaña, 2001).

Concerning the distribution of fishing activities along the MPA (see Section 2.1), greater differences would be expected between
the CPZ and the remaining zones, since no fishing activity is allowed in the TPZ while in the PPZ only traps, jigs and handlines are allowed farther than 200 m from shore line. This pattern was only observed in the autumn and for the metrics density of generalists and density of invertebrate feeders (as explained before), as well as for the density of individuals with high commercial value. In accordance with the expected pattern, results of CAP showed that the density of large individuals with medium to high commercial value (>20 cm) was also associated with the TPZ and the PPZ, however, no significant differences were found, meaning that it was not consistently different. On the other hand, the density of individuals with high commercial value was only significantly different between zones in autumn, following the expected pattern due to fishing effort distribution, which is probably due to a cumulative result of the abundance of juveniles and adults during this season, in accordance with the general seasonal pattern observed. These results suggest that this reserve is still in a trajectory of recovery.

An increase of large-bodied species, especially those targeted by fishing, has been pointed out as one of the main changes in fish assemblages due to protection (e.g. Claudet et al., 2006, 2010; Guidetti and Sala, 2007; Halpern and Warner, 2002; Micheli et al., 2004). Because large-bodied species are many times slow-growing and late-maturing, they will respond slower to protection measures than short-lived and fast-growing species (Halpern and Warner, 2002; Mosqueira et al., 2000). Moreover, the increase in species abundance is extremely dependent on recruitment processes, fishing effort outside and between the different levels of protection, as well as their catchability and migration patterns (Côté et al., 2001; Guidetti and Sala, 2007; Micheli et al., 2004). This means that recovery of fish size and species density depends on the age and size of the reserve (Claudet et al., 2008). For instance, some studies suggest that 1–3 yr appears to be enough to detect significant changes in total density and biomass (see meta-analysis of Halpern and Warner, 2002), while this time period seems to be insufficient to detect differences in fish size or trophic structure (e.g. the abundance of top predators) (e.g. Claudet et al., 2006; Micheli et al., 2004; Russ and Alcala, 2004). In this context, despite the sampled site being under fully protection measures for 2 yr, there was probably not enough time to detect clear changes on fish size, explaining why no consistent effects of zone were observed for the density of adults with high commercial value and the large individuals with medium to high commercial value (>20 cm).

A previous study by Harmelin et al. (1995) showed higher abundances of Diplodus spp. (especially large individuals) within protected zones in all seasons and successive years, though these abundances peaked in late summer (Mediterranean). Therefore, the fact that the density of individuals with high commercial value only responded to the effect of zone in autumn and the lack of response of the density of adults with high commercial value, stress the idea that the MPA is at an early stage. In fact, seasonal variations on size structure of the most abundant species with high commercial value, D. sargus and D. vulgaris were observed. These variations followed the life-cycle features of each species and resulted in clear differences between zones in autumn, probably due to cumulative abundances of both juveniles and adults. Both species have a large spawning period that peaks in winter and results in an extended recruitment (García-Rubies and Macpherson, 1995), but while D. vulgaris travels to shelf waters (Correia et al., 2011), D. sargus congregates in sheltered bays and nearshore waters to spawn (Almada et al., 1999; Veiga et al., 2010). These results are also supported by the research of Horta e Costa et al. (in press) performed in the same study area, during the spring and autumn (pooled together) in two consecutive years (2009–2010), where eight zones were sampled inside TPZ and PPZ (four in each one) and four zones in CPZ (spatial variability). The authors found significant differences in the density of both D. vulgaris (below the legal size of 15 cm) and D. sargus (all sizes) between protected (TPZ and PPZ) and unprotected zones (CPZ) (Horta e Costa et al., in press).

It is important to note that this study was not based on before–after MPA establishment data, so other factors beyond the ones discussed could hinder the detection of significant differences among the different levels of protection: (1) size of the no-take and partially protected areas relative to the target species’ home-range; (2) the ban of spearfishing in the whole MPA area which has been reported to heavily impact target species (e.g. Diplodus spp.) (Coll et al., 2004; La Mesa et al., 2011a; Lloret et al., 2008); (3) the increased surveillance of illegal fishing activity. In this way, further research is needed to follow the reserve effects over time, like changes on size-structure of large-bodied fish (e.g. target species) and increase in the abundance of top predators (macrocarnivores and piscivores). This should also be complemented with the study of complex succesional trajectories of inter-species relationships (e.g. decreases in the abundance of preys due to the increase in top predators) in order to test new metrics. Further studies should include other habitats and depth strata in order to accomplish higher spatial variability.

In conclusion, this study showed that (1) seasonal variability affects the structural and functional features of fish assemblages that can influence the detection of changes as a result of anthropogenic pressures; (2) comparisons between control and impacted sites should only be made using data from similar seasons; (3) it is important to take the species life history and their ecological traits into account to analyze fish-based metrics, especially those represented by one or few species; (4) the metrics density of generalists (i.e. density of opportunistic individuals) and density of individuals with high commercial value seem to be adequate to assess fish assemblages’ response to fishing pressure. Overall, the choice of a particular season to assess impacts on fish assemblages, during warm sea conditions after the spawning period (July–November), can probably give better results while minimizing monitoring costs. This is particularly important in wide-ranging environmental assessments, those required to implement the Marine Strategy Framework Directive.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.seares.2013.02.004.

Acknowledgments

The authors would like to thank all the volunteers for the invaluable aid during field surveys and to Instituto da Conservação da Natureza e Biodiversidade (ICNB) for issuing diving permissions at the Arrábida Marine Protected Area. Host institution was funded with project PEst-OE/MAR/UI0199/2011 and PhD grants attributed to S. Henriques (SFRH/BD/47034/2008) and M.P. Pais (SFRH/BD/46693/2008), both from Fundação para a Ciência e Tecnologia (FCT). The authors wish to thank the anonymous reviewers for providing useful comments on the paper.

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