Detection of protection benefits on predatory fishes depends on the census methodology adopted

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Abstract

1. Marine Protected Areas (MPAs) are used as fisheries management and conservation tools. The establishment of well-enforced no-take zones allows the rebuilding of natural populations of exploited species, although there is still controversy on the role of buffer zones.
2. MPA effectiveness could be underestimated since fish population assessments depend largely on traditional methodologies, which may have difficulties to deal with predatory fish detection due to fish behaviour.
3. Here, we compared the performance of different census methods in assessing protection benefits on large predatory fishes under different protection levels (i.e. no-take and buffer zones) in five Mediterranean MPAs. Specifically, we compared conventional strip transects (CST, 50 x 5 m^2) and tracked roaming transects combined with distance sampling (TRT+DS, variable lengths), including a series of TRT-derived estimators, with variable transect lengths and fixed widths of 20, 10 and 6 meters (TRT20, TRT10 and TRT6, respectively).
4. We found that the transects covering larger areas (i.e. TRT+DS and TRT20) allowed the detection of a greater number of species and yielded more accurate estimates of density and biomass than transects of narrower fixed widths, particularly the CSTs, which were associated with the lowest richness detection capability, accuracy and precision. On average, both no-take zones and buffer zones appeared effective for the conservation of large predatory fishes, indicating that multiple protection areas were ecologically effective. However, differences between MPAs were also observed, probably due to both local environmental and management factors.

5. We suggest the implementation of methodologies with larger transects for the study of MPA effects on large predatory fish populations, in order to avoid bias in fish population assessments.

Keywords

Buffer zone, distance sampling, high-trophic level predators, multiple protection areas, no-take zone, underwater visual census.

1. Introduction

High-trophic level predatory fishes (i.e. groupers, snappers, jacks, etc.) play a key role in marine ecosystems (Baum & Worm, 2009). These species have been historically targeted by fisheries and are currently depleted or even completely absent in coastal ecosystems (Jackson, 2008; Piroddi et al., 2017).

Marine Protected Areas (MPAs) are recognised as effective tools for the conservation of the biodiversity and fisheries management (Pérez-Ruzafa, García-Charton & Marcos, 2017). When effectively designed and managed, MPAs show positive effects on the recovery of density and biomass of exploited fishes, especially those with large sizes, located at higher trophic positions, and having commercial importance (Mosquera et al., 2000; Côté, Mosquera & Reynolds, 2001; Micheli, Halpern & Botsford, 2004; Guidetti & Sala, 2007; Claudet et al., 2008; Guidetti et al., 2008; Sciberras et al., 2013; Edgar et al., 2014; Guidetti et al., 2014; Hackradt et al., 2014; Rojo et al., 2019).

Ecological monitoring is essential to understand the functioning of MPAs (Roberts et al., 2018). Worldwide, underwater visual censuses (hereafter UVCs) have been adopted as monitoring tools to assess fish assemblages in MPAs since they are non-destructive, cost-efficient and allow the detection of a high number of species (García-Charton et al., 2000; Edgar, Barret & Morton, 2004; Harmelin-Vivien & Harmelin, 2013; Prato et al., 2017). Traditionally, UVCs have been mostly performed by using conventional strip (belt) transects (hereafter, CST; e.g. Harmelin, 1987; García-Rubies & Zabala, 1990; García-Charton et al., 2004; Hackradt et al., 2014), in which sampling area is delimited both in length and width. CSTs assume that all fishes present within the surveyed surface will be detected by the observer (i.e. probability of detection = 1; Bukland et al., 2001). However, very often this assumption is not fulfilled, and CSTs generate biased estimates of the descriptors of fish assemblages (Kulbicki, 1998; MacNeil et al., 2008; Bukland et al., 2001; Bozec et al., 2011; Goetze et al., 2017). Many attempts have been made to adapt the methodologies to focus on particular groups of fishes (i.e. large predatory fish) and to maximize species detectability, by increasing the number of observers (Issaris et al., 2012; Bernard et al., 2013), sampling replicates (MacNeil et al., 2008), or varying the size of the transects (Harmelin-Vivien et al., 2015; Prato et al., 2017). Also, sampling techniques have been further developed in order to increase the accuracy of data (Katsanevakis et al., 2012), such as the combination of UVC with mark and recapture methods (Hackradt, 2012), repeated presence-absence surveys for occupancy models (Issaris et al., 2012), removal methods (Söffker et al., 2015), distance sampling (Kulbicki & Sarramégna, 1991; Irigoyen et al., 2018) and GPS-tracked roaming transects (Beck et al., 2014; Lynch, Green & Davies, 2015; Irigoyen et al., 2018; Wong et al., 2018).

Particularly, GPS-tracked roaming transects increase the efficiency of UVC by covering 33–75% more area than CST for a comparable diving time because there is no diving time investment on rolling the census tape and there is not transect length limitation (Beck et al., 2014; Lynch, Green & Davies, 2015; Irigoyen et al., 2018). This method is especially useful as it allows sampling very sparse populations that might otherwise require much more effort to get a record and even remain undetected. Additionally, using wider transects and especially distance sampling methods, in which perpendicular distance from the transect line is estimated on large census area widths (Kulbiki & Sarramégna, 1991; Buckland et al., 2001; see details below), would lead to more accurate and precise estimates when compared to CSTs. This is critical when sampling species that may react to the presence of divers (either attractive or elusive behaviour), and hence when comparing among sites with different levels of human disturbance (e.g. zones under different levels of protection within multiple-use MPAs).

On the other hand, it is essential to assess which MPA features favour the recovery of this group of fish species (Rojo et al., 2019). One of the most debated attributes of MPAs is the adequacy or not to implement partially protected areas - or buffer zones, allowing extractive activities to different degrees (Sala & Giakoumi, 2017). While the efficacy of fully protected areas - or no-take zones as conservation measures has been widely accepted (Claudet et al., 2008; Edgar et al., 2014; Friedlander et al., 2017), there is still controversy on the role of buffer zones, which are expected to be less effective than fully protected areas (Lester & Halpern, 2008; Lester et al., 2009; Sciberras et al., 2013; Giakoumi et al., 2017; Sala & Giakoumi, 2017), or even exert a negative effect on conservation purposes (Claudet et al., 2008; Sciberras et al., 2013). However, it has been recently found that buffer zones may produce considerable ecological benefits (Hackradt et al., 2014; Zupan et al., 2018), thus understanding the effects of both protection levels is crucial for management purposes.

The aim of the present study was to compare the performance of different visual census methodologies in evaluating the effects of protection in five multiple-use MPAs (i.e. including no-take and buffer zones) in the Mediterranean Sea. Specifically, we compared estimates of predatory fish species richness, density and biomass gathered through traditional CSTs and GPS-tracked roaming transects combined with distance sampling (hereafter TRT+DS; *sensu* Irigoyen et al., 2018), including a set of fixed-width TRT-derived transects. Based on the previous literature, we hypothesise that longer and wider transects will result in more accurate estimates of the descriptors of fish assemblages. Moreover, we expect no-take zones to exert a stronger protection effect than buffer zones. Our results will help to identify the most accurate and realistic method for the study of predatory fishes and consequently to provide better assessments of MPA effects on this trophic group.

2. Methods

2.1. Study areas and sampling design

The study was carried out during the summer of 2016 and 2017 in five MPAs located in the Western Mediterranean Sea, from south to north: Cabo de Gata-Níjar marine reserve (hereafter Cabo de Gata), Cabo de Palos-Islas Hormigas marine reserve (Cabo de Palos), Isla de Tabarca marine reserve (Tabarca), Es Freus d'Eivissa i Formentera marine reserve (Es Freus), and North of Menorca marine reserve (Menorca) (Fig. 1, Appendix A). Each MPA consists of one or several no-take zones, where all activities are banned except scientific research (subject to administrative authorization), and a buffer zone, where certain human activities (e.g. small-scale fishing and recreational diving) are allowed but strongly regulated. In addition, adjacent unprotected areas with habitat characteristics comparable to their corresponding MPA were included in the study.

Within each protection level (no-take zone, buffer zone, unprotected areas) of each location (every MPA and its adjacent unprotected area) a number of sites were randomly selected separated by hundreds to thousands of meters, so that the number of sites selected in each protection level was proportional to the surface of available habitat. Sites were characterised by being rocky reefs located both in coastal cliff areas and on islands or rocky outcrops that did not reach the surface. In general, the sampling design consisted of three sites within each no-take zone, six inside each buffer zone and nine in the unprotected areas. As exceptions, only six unprotected sites were sampled in Tabarca and Es Freus due to the lack of suitable habitats outside these MPAs. In each site, three haphazardly distributed replicates were performed with each sampling technique, separated at least 20 m in order to avoid spatial dependence. Replicates consisted on fish UVCs using two different methodologies: CST and TRT+DS (see below), for which only predatory large-sized economically important fish species occupying high trophic levels in Mediterranean coastal ecosystems were considered (Appendix B). The procedure for randomly placing sample replicates was constrained to include comparable habitats, consisting as much as possible of steep rocky bottoms with small and medium sized boulders interspersed in some sites with patches of *Posidonia oceanica*, surrounded by soft bottoms and seagrass meadows at 25-35 m depth. In general, the replicates were conducted at depths between 13 and 18 m, with the exception of some sites in Tabarca and Cabo de Gata MPAs and their respective unprotected areas, in which rocky reefs deeper than 6-10 m depth are very scarce. Visual censuses were carried out by experienced divers at daytime between 10 a.m. and 3 p.m., when light and water conditions were optimal.

2.2. Sampling methods

In the case of CST, transects of 50 m length (measured by a tape) and 5 m width (2.5 m at each side of the diver, estimated visually; i.e. covering a surface of 250 m^2) were performed. Predatory fish in the sampled area were recorded, annotating the name of the species, and the number and size of the individuals observed. Abundance was assigned to one of nine predetermined abundance classes for which the limits coincide with the terms of a base ~ 2 geometric series (1, 2–5, 6–10, 11–30, 31–50, 51–100, 101–200, 201–500, > 500; Harmelin, 1987) (see García-Charton et al., 2004, for further details of the sampling protocol used).

For the TRT+DS method, two divers performed transects of variable lengths following an imaginary line at a constant depth contour while pulling a GPS located on a body-board on the surface. The tracks were recorded by the GPS, and the distance covered along each transect was extracted by geo-referencing start/end census points with a synchronized clock. Transects lasted 8 minutes, which gave a mean transect length of 110 m (± 2 m SE) in an equivalent diving time to CST. Each diver considered 20 m of width at her/his respective side from the imaginary line; thus, for a total of 40-m width, transects surveyed an average surface of 4400 m^2. Similarly to the case of CST, for each observation the name of the species was noted, the number of individuals was counted and first-sight perpendicular distance in 1-m intervals between every fish observed and the imaginary line was estimated, either for single individuals or groups. Fish moving from one side to the other were noted and cross-checked between the two divers after the sampling in order to avoid double counts of individuals (see Irigoyen et al., 2018, for further details of the sampling protocol used).

Both in CST and TRT+DS fish lengths were estimated in 5-cm size classes (Harmelin-Vivien et al., 1985).

2.3. Response variables and estimators

The predatory fish assemblage was described by the response variables species richness (number of species), density (number of individuals · 100 m^{-2}) and biomass (in g · 100 m^{-2}). The latter was calculated through adequate length-weight relationships from local studies when available (Morey et al., 2003), otherwise data obtained from FishBase (Froese & Pauly, 2017) were used.

2.3.1. Conventional Strip transects (CST)

To calculate density in each CST, geometric means of fish abundance classes were used as class marks (García-Charton et al., 2000; García-Charton et al., 2004). Thus, for each species in each transect, the resulting density is the sum of the geometric means of the abundance class of each observation (individual or group) in a transect of 250 m^2.

2.3.2. Tracked Roaming Transects with Distance Sampling (TRT+DS)

Distance sampling theory (DS) allows the estimation of density of biological populations based on the recorded distances from each individual to a randomly placed line. The detection function g(*y*) is the probability of detecting an object located at a perpendicular distance (*y)* from the line (Buckland et al., 2001). The g(*y*) decreases as *y* increases, always being $0 \leq g(y) \leq 1$, and assuming that g(0) = 1 (i.e. the probability of detecting an object right on the line is 1). Thus, although a large proportion of objects may be undetected, by adjusting the sightings to the g(*y*) detection function it is possible to calculate reliable estimates of the true density (Burnham, Anderson & Laake, 1980; Buckland et al., 2001). DS theory requires at least 30 records to calculate trustworthy densities (Buckland et al., 2001; Buckland et al., 2004). Most transects, however, had less than 30 records for each species (especially in unprotected areas). As a consequence, in order to graphically represent the densities taking into account the detection probability function, the calculations were made considering the most abundant species pooled (see Appendix B), so that only one density value has been provided for each MPA and protection level. Calculations were made through the free software DISTANCE 6.2 (Thomas et al., 2010).

For statistical modelling purposes, densities were calculated using a series of estimators derived from the data taken with the TRT+DS method, but without taking into account the detection function g(*y*), as detailed below.

Firstly, an encounter rate (*ER*) was calculated in each transect i as the number of individuals of all preselected species recorded (n_i) per meter of transect length (L_i) as measured with the GPS track (i.e. $ER_i = n_i/L_i$). This estimator has been widely used as an appraisal of distance-calculated densities in terrestrial ecology when DS theory does not allow to make calculations at the transect level due to a too low abundance of the recorded species (Burgi et al., 2011; Nabte et al., 2013).

Secondly, a value of density was calculated through the average distance estimator (*AD*). This estimator considers the distance at which individuals were recorded but, unlike DS theory, no function is applied to the histograms of sighting distances. Instead, it calculates mean distances and refers the abundance to the area in which widths are calculated as average distance as follows: $AD_i = [n_i/(2Ld_i)]$, with $d_i = \left({}^{1}/_{n_i}\right)\sum_{j=1}^{n_i} d_{ij}$ (Kulbicki & Sarramégna, 1999). In order to be able to compute the formulae, the minimum width ($2d_i$) at which each observation has been recorded is 1 m, even in the cases of those fishes that were seen directly on the imaginary line (i.e. at distance 0).

Finally, Tracked Roaming Transects of fixed widths were considered by including the fishes seen within distances ≤ 3, 5, and 10 on each side of the imaginary line over which the census was made. Fixed width transects larger than 20 m (10 m from each side of the line) were not included because the probability of detection dropped sharply after that distance. A similar decline in the probability of detection has been previously found (Bozec et al., 2012) and is the maximum width adopted in similar studies (Prato et al., 2017). Densities D_i were calculated as $D_i = n_i / (L_i \times W)$, with L_i being the transect length (i.e. the GPS track), W the widths of 6, 10 and 20 (called TRT6, TRT10 and TRT20, respectively), and n_i the number of individuals of the selected species sighted in each case.

For each response variable, different methods and estimators were taken into account. Richness and biomass data were analysed through TRT20, TRT10, TRT6 and CST. For density data, though, formal analyses were done through the *ER*, *AD*, TRT20, TRT10, TRT6 and CST.

2.4. Data analysis

Firstly, in order to understand how the limitation in transect widths may affect the detection by each of the estimators, mean detection distances were calculated for each of the species in the study in the different protection levels for all MPAs pooled.

Then, generalized linear mixed models (GLMMs; Zuur et al., 2009) were applied to explore the factors affecting the richness, density and biomass of predatory fish in Mediterranean MPAs. Different analyses were performed for each of the methodologies or estimators and response variables, as estimators within a response variable were not independent among them. For the density and biomass analyses, species that appeared in at least 10% of the total transects performed including the two methodologies (Appendix A) were retained and evaluated pooled together. This allowed us to assess differences among MPAs and protection levels without having the effect of rare species. The species included were *Epinephelus marginatus, Epinephelus costae*, *Mycteroperca rubra*, *Dentex dentex*, *Sphyraena viridensis*, *Sciaena umbra* and *Muraena helena* (Appendix B); the latter was excluded for the biomass analyses because it appeared mostly in cracks in the rocks, so it was not possible to estimate individual's lengths.

GLMMs models included the factors Location [fixed, with five levels (the different MPAs and their unprotected counterparts); Protection level [fixed, with three levels (no-take, buffer and unprotected)]; the interaction among Zone and Protection Level; and Site (random, with a variable number of levels depending on the location). Location has been considered a fixed factor because we hypothesize that there is a gradient of primary production, being maximum in the Alboran Sea and decreasing northwards and offshore from the mainland to the north-eastern Balearic Islands (García-Charton et al., 2004). Indeed, the Alboran Sea has the greatest levels of primary productivity due to phytoplankton uptake of nutrients upwelled at Gibraltar and the cyclonic circulation cells (Lazzari et al., 2012; García-Martínez et al., 2019). Moreover, there is a coastal-island gradient due to permanent frontal structures combined with river runoff and upwellings created by submarine canyons (Estrada et al., 1996). Menorca island has been found to be one of the most oligotrophic areas in the western Mediterranean Sea (Cardona et al., 2007) due to phosphorus limitations because of the occurrence of oceanographic gyres (García-Martínez et al., 2019). Arguably, primary production is the most important environmental factor determining the production of marine fishes (Pauly & Christensen, 1995; Watson, Zeller & Pauly, 2013; Piroddi et al., 2017). Thus, accounting for this hypothesis, the factor Location would represent discrete steps along a gradient of primary production following the path Cabo de Gata > Cabo de Palos > Tabarca > Es Freus > Menorca. Consequently, and because all factors are categorical, reference values were set for the Cabo de Gata location (i.e. the location where the highest values of primary productivity occur) and the unprotected protection level (the level for which the lowest values of richness, density and biomass are expected to occur), thus analyses are made according to them.

For species richness, Poisson error distributions and log-link functions were used. The glm() and glmer() functions of the packages stats (R Core Team, 2017) and lme4 (Bates et al., 2015) were applied, respectively. For the density and biomass response variables, the glmmTMB() function from the glmmTMB package was applied (Brooks et al., 2017) in which models are fitted using maximum likelihood estimation and random effects are integrated using the Laplace approximation. This package has been recently developed and is more flexible than other packages to deal with zero-inflation and overdispersion on the non-zero data (Brooks et al., 2017). Raw abundance and biomass per transect were modelled using negative binomial distributions and log-link functions, and the area covered by each transect was included as an offset parameter. Model selection was based on Akaike's information criteria (AIC). The model with the lowest AIC was selected, and when Δ AIC was ≤ 2 the simplest model was selected (Burnham & Anderson, 2002). Significant terms were considered when $p \leq 0.05$. Model validation was done through visual inspection of residual plots and Shapiro tests for deviations from homoscedasticity and normality, respectively. The performance of the final models was evaluated by means of the marginal R^2, which measures the variability accounted by the fixed terms of the model (Nakagawa & Schielzeth, 2013). The function r2() from the sjstats package (Lüdecke, 2018) was used. All analyses were performed in R (R Core Team, 2017).

3. Results

3.1. Diversity of predatory fish assemblage

A total of 252 transects with each methodology were performed along the five MPAs. The complete set of predatory species recorded in the study includes *Caranx crysos*, *Dentex dentex*, *Dicentrachus labrax*, *Epinephelus marginatus*, *Epinephelus caninus*, *Epinephelus costae*, *Euthynnus alletteratus*, *Muraena helena*, *Mycteroperca rubra*, *Pagrus pagrus*, *Sarda sarda*, *Sciaena umbra*, *Scorpaena scrofa*, *Seriola dumerili*, *Sparus aurata*, *Sphyraena* spp., *Myliobatis aquila* and *Torpedo marmorata* (Appendix

B). The number of species recorded by the CST, the TRT+DS and their derived estimators differed according to the MPA and the protection level considered, as did the values obtained for the density and biomass (Fig. 2).

3.2. Average detection distance

The average detection distance of individuals using the TRT+DS method was similar among the species considered, and values varied between 1 and 6 m from the observation line (Fig. 3). However, this parameter tended to increase as the level of anthropogenic disturbance augmented (i.e. from no-take zones to unprotected areas; no-take = 3.12 ± 1.11; buffer = 3.20 ± 0.39; unprotected = 3.91 ± 0.31 m; mean values across species ± SE), what was more evident for the species *E. costae* (no-take = 2.79 ± 0.92; buffer = 3.72 ± 0.36; unprotected = 4.5 ± 0.29 m; mean ± SE) and *M. rubra* (no-take = 3.03 ± 0.90; buffer = 3.48 ± 0.39; unprotected = 6.00 ± 0.42 m; mean ± SE).

3.3. Methods and estimators performance in detecting MPA effects

3.3.1. Richness of predatory fish

For any location and protection level considered, the methods and estimators covering greater areas per transect tended to yield higher values of richness (i.e. more species detected with the estimator; TRT20 > TRT10 > TRT6 > CST; Fig. 2a). AIC did not differ among the GLM and GLMM models for richness, indicating the absence of effect of the factor Site. The results of GLM applied to the richness of predatory fish species measured by the CST method showed that the response of this assemblage to protection level varied depending on the location considered (as shown by the significant interaction between particular Locations and Protection levels, while it was non-significant when measured through the TRT6 method). The TRT20 and TRT10 models included the interaction but none of the specific terms was statistically significant

(Table 1). Regarding the interaction, CST showed that richness was higher in the buffer and no-take zone of Cabo de Palos, as well as in the buffer zone of Menorca and Tabarca (Table 1). Moreover, regarding the main effects, data obtained with methods TRT20 and TRT10 showed that species richness was significantly greater in no-take zones, while the data obtained with TRT6 detected significantly higher values of species richness in both no-take and buffer zones (Table 1). Additionally, Cabo de Palos and Es Freus harboured higher species richness than the other locations (positive and statistically significant results, all protection levels considered together, Table 1) with all census methods, apart from the case of the data obtained with CST. The R^2 of the final models were around 50% for all the estimators considered (Table 1).

3.3.2. Density of predatory fish

Density of predatory fish varied according to the Protection Level and the Location considered for all the studied methods and estimators. Density was significantly lower in Menorca than the reference level for all methodologies, as did the *AD* estimator for the Es Freus and Tabarca MPAs. Moreover, *ER*, TRT20 and TRT10 found that Cabo de Palos MPA harboured statistically significant higher density, while CST found the opposite pattern for the same MPA (Table 3). According to the protection levels, *AD*, *ER*, TRT20 and TRT10 showed that generally no-take zones harboured greater density of predatory fish (Table 2, Fig. 2b, 4).

However, when looking at the interactions, *ER*, TRT20, TRT10, TRT6 and CST found that there was significantly greater density in buffer zones of the Cabo de Palos, Es Freus, Menorca and Tabarca MPAs when compared to unprotected areas (except Es Freus for the CST; Table 2). With *AD*, though, we found a greater density both in the no-take and buffer zones of Es Freus, as well as CST did for the no-take of Cabo de

Palos MPA (Table 2). The dispersion model showed greater overdispersion in all MPAs and the no-take zone with all the estimators, except the Es Freus MPA when the *ER* was considered (Table 2). However, the zero-inflation model did not vary according to the factors studied, which means that the proportion of zeros is equivalent among Locations and Protection Levels. Marginal R^2 were around 39 – 43 % for all the estimators considered.

3.3.3. Biomass of predatory fish

Biomass of predatory fish was significantly greater in no-take zones than in unprotected areas for the TRT20, TRT10 and TRT6 estimators. Moreover, the TRT20 found that Cabo de Palos MPA had greater biomass of predators while Menorca had the opposite trend (Table 3, Fig. 2c).

Furthermore, TRT20, TRT10 and TRT6 found a positive and significant interaction between Cabo de Palos, Es Freus and Tabarca and the buffer protection level, while CST found positive and significant values of biomass in the no-take and buffer zones of Cabo de Palos, and the no-take of Es Freus and Menorca MPAs (Table 3). In this case, the zero-inflation model varied according to the location and protection level factors, and it was significantly lower for the Cabo de Palos and Es Freus MPAs and both the no-take and buffer zones for most estimators. The dispersion models were only significant for the no-take zone when the TRT10 was considered. Marginal R^2 were between 61 – 65 % for all estimators (Table 3).

4. Discussion

Understanding the best methodological approaches to evaluate MPA effectiveness is of great interest for a correct assessment of the ecological effects of protection, particularly when focusing on vulnerable and valuable species. Moreover, determining the comparative performance of both no-take zones and buffer zones in MPAs is essential to evaluate the potential conservation usefulness of multiple-use areas while allowing certain human activities.

Effects of the method and estimator applied on detecting MPA effectiveness

The average detection distance (as estimated by the TRT+DS method) was greater as the level of protection was lower (i.e., the level of human disturbance increased), especially for some species, such as *E. costae* and *M. rubra*. In fact, regardless of the protection level, the average distances were always greater than 2.5 m (the maximum width taken into account in the CST). These findings compromise the reliability of the density and biomass estimates made with CST, particularly in unprotected sites. The influence of the behaviour of medium and large commercial fish species has been previously signalled (Kulbicki & Sarramégna, 1991; Irigoyen et al., 2018). Therefore, selecting a method to visually census reef fishes that best allows the detection of predatory species in multiple-use MPAs (i.e. including zones where different human uses occur, from no-use to regulated, compatible uses) is essential to correctly assess MPA effectiveness.

Our results showed that mean values of species richness, density and biomass differed among methods and estimators. Those census methods covering larger areas yielded higher values of species richness for all locations and protection levels, and the values diminished as the censuses covered smaller areas. Similar results have been previously found when comparing methodologies, as significantly higher mean richness of reef fishes were measured when sampling was done through line transects combined with DS than CST (Thanopoulou et al., 2012). This is in concordance with the species-area relationship (SAR), which states that an increase in the area sampled must be accompanied by a greater species richness because of an increase in the habitat heterogeneity (Kallimanis et al., 2008). However, the method *per se* may be accounting for the increased species richness, as methods that use “first contact” are more likely to record fish that may respond to the presence of divers than belt transects (Beck et al., 2014). Moreover, for the same area covered by the transects, increasing their length allows to detect a greater number of species than increasing their width (Loiseau et al., 2016), since the detectability of species is complete on the line that marks the center of the transect while it decreases rapidly as the fish is further away from that line (Kulbicki et al., 2010; Bozec et al., 2011; Loiseau et al., 2016).

For their part, the values of density and biomass in our study increased as the area covered by the methods or estimators decreased, being much greater for the *AD* and CST and lower for the DS and TRT20. This observation is contrary to what is indicated by previous studies (Kulbicki & Sarramégna, 1999; Thanopoulou et al., 2012) which found that density estimates obtained with line transects combined with DS were higher than those obtained with CST, because DS takes into account both the detectability of the species and their behavior (including particularly shy species). The fact that our density estimates made with CST were higher than those obtained by using DS or TRT methods may be due to several reasons. Firstly, CST counts may be biased to overestimate abundances of highly mobile or aggregated species. For instance, Ward-Paige, Flemming & Lotze (2010), through a simulation model, found that counts go overestimated when non-instantaneous census are performed (i.e. when counting animals that enter the transect after the survey has started), and showed how biases increased with fish speed, although slow moving fish were also overestimated. In fact, calculations applied by Ward-Paige, Flemming & Lotze (2010) to the results of Sandin et al., (2008), which supported the idea of the inverted trophic pyramid in Kingman and

Palmyra, were down-sized by two orders of magnitude when correcting for the detected bias; in addition, this overestimation was empirically demonstrated by Bradley et al., (2017) to be one order of magnitude lower through mark-recapture and acoustic-tracking methods. Secondly, increasing transect areas may have reduced the density estimates as a "dilution effect" (i.e. area increasing more than the number of individuals recorded, which leads to lower density estimates). Thus, if the purpose of the study is to have more accurate estimates of density and biomass, methods surveying a larger area should be preferred (Prato et al., 2017; Irigoyen et al., 2018). Thirdly, the lower density found with the estimators covering larger areas may also be partially explained by a "habitat effect" (García-Charton et al., 2004), derived from the complex and steep topography of the sites in our study. Wider UVCs include sightings of fish from a wide depth range because it incorporates shallow areas as well as deeper ones due to the narrow and cliffy nature of the rocky reefs, and frequently the deeper areas included other habitat types, such as soft bottoms and seagrass meadows (García-Charton & Pérez-Ruzafa, 1998; García-Charton & Pérez-Ruzafa 2001). This has direct consequences on fish counts, as the abundance of rocky dweller predatory fish species like groupers is generally lower in both abovementioned habitats.

Therefore, in order to bring the most accurate estimates of fish richness, density and biomass of predatory species, it is advisable to choose underwater visual census methods that maximize the area covered by enlarging the length and width of the transects. To achieve this, the DS is the most reliable method, as additionally it takes the detectability of the species into account when calculating fish densities. However, for modelling purposes, we have ascertained the difficulty of reaching 30 records per transect for the species studied even in some no-take zones (e.g. Menorca). Thus, wide

TRT, and especially the TRT20, which allows the detection of most of the species, will be a solution for increasing the area sampled and performing calculations easily,

MPA effectiveness for the protection of predatory fish

Our results showed some differences in the magnitude of the effect of MPAs on predatory fish assemblage according to the method and estimator considered, although altogether they indicate that all the studied MPAs, with the notable exception of Menorca, are being ecologically effective for this important group.

The local primary productivity, as an environmental factor added to the fishing pressure to explain the observed fish biomass both within and outside MPAs (Piroddi et al., 2017), has not fully accounted for the patterns found. Although Menorca was the location performing the worst in terms of conserving predatory fish, coinciding with the lowest level of primary productivity, Cabo de Palos and Es Freus were the locations where the highest values of density and biomass were found, which are not located at any end of the gradient of primary productivity. Therefore, other MPA features and environmental conditions must concurrently allow for an explanation of the patterns observed.

Many factors have been found to affect the effectiveness of MPAs for the conservation of fish, such as the time elapsed since the protection has been in force, the area of the no-take and the buffer zones, and the level of enforcement. All MPAs included in this study have been protected for long periods, from 17 (Menorca) to 31 years (Tabarca). The number of years necessary to detect a response to protection varies with the species considered, and for high level predators these effects are expected to be noticed in the long term (from 5 to 20 years; Guidetti & Sala, 2007; Claudet et al., 2008; Edgar et al., 2014; Friedlander et al., 2017). Thus, all MPAs in the study are susceptible to have reached the age to show comparable results. It was previously thought that bigger no-take zones would produce greater benefits (Claudet et al., 2008; Claudet et al., 2010; Edgar et al., 2014; Friedlander et al., 2017), though it has been recently found that even small but well enforced MPAs are effective for some commercial fish species and the whole community in the Mediterranean Sea (Giakoumi et al., 2017) and for piscivore populations worldwide (Rojo et al., 2019). In fact, enforcement has been found as a critical factor driving ecological effectiveness (Guidetti et al., 2008; Edgar et al., 2014; Giakoumi et al., 2017: Rojo et al., 2019). In our study, no-take zones ranged between 78 (Tabarca) and 2310 (Cabo de Gata) hectares. Cabo de Palos, followed by Tabarca and Es Freus were the MPAs in which there were higher levels of enforcement, measured as the total number of days per year with effective surveillance and no major episodes of poaching (following Guidetti et al., 2008). In fact, Menorca was found to have few patrols, thus the poor level of enforcement may explain, at least partially, the lack of ecological effects of protection in this MPA.

Importantly, our results show that, when looking at specific MPAs, buffer zones support higher density and biomass of predatory fish compared to unprotected areas. Moreover, mean values appeared to be similar between no-take and buffer zones (even greater, as is the case of Menorca), which may indicate that this partially protected areas are being effective for the conservation of predatory fish. Buffer zones are traditionally seen as less beneficial for the species than no-take zones (Lester & Halpern, 2008; Sciberras et al., 2013; Giakoumi et al., 2017; Sala & Giakoumi, 2017), especially when the species being harvested are the same that are protected in no-take areas (Sciberras et al., 2013). Some authors even consider that they might reduce the effectiveness of the entire MPA when their sizes are excessively large, as these areas are attractive for local artisanal fishers, thus reducing MPA efficacy (Claudet et al., 2008). However, it has been recently found that buffer zones can be ecologically effective when they are well regulated and located adjacent to fully protected areas (Abecasis et al., 2015; Zupan et al., 2018), as is the case of the MPAs in our study. Buffer zones benefit from the presence of adjacent no-take zones, either through juveniles and adult spillover or by larval supply. The fact that they may enhance ecological benefits is especially important in areas where traditionally many livelihoods are linked to the sea (Di Franco et al., 2016). The activities allowed in buffer areas are usually small-scale fisheries and recreational diving, the first representing around 80% of the fishing boats in the Mediterranean Sea, hence being an activity of enormous socio-economic importance for the local communities (Maynou et al., 2013). Thus, if buffer zones are ecologically effective, they may help to achieve a win-win scenario in which both fish and fishermen would benefit from protection measures (Dalton, 2010).

In conclusion, partially protected areas of the studied MPAs are effective for the conservation of predatory fish species. Thus, well-managed multiple-use MPAs are still to be retained as important tools to conserve marine resources and protect marine biodiversity. Regarding the methodologies applied, most methods or estimators allowed the detection of the effects of protection on fish assemblages, but they yielded different patterns. Those UVC methodologies covering larger areas allowed for a better detection of most of the predatory species studied, regardless of the protection level considered, and yielded more accurate estimates for the response variables. This is particularly important because UVCs are used worldwide for the evaluation of fish populations and MPA performance, and our results show that CSTs may provide biased estimates, particularly when comparing among levels of human disturbance. This is essential because in the future MPAs, and particularly multiple-use MPAs, will tend to increase, thus identifying the best methodology will allow a better assessment of MPA effectiveness. Therefore, we recommend to routinely adopt methodologies that include larger sampled areas (and, more specifically, to use TRT+DS or TRT20) when surveying predatory fish populations, for instance to evaluate MPA performance or to measure demographic parameters of the species.

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7. Tables

Table 1. Results of the GLMs for the estimators applied TRT20, TRT10, TRT6 and CST) showing the variables explaining fish species richness, based on model selection. Models excluded by AIC are not shown in the table.

	TRT20			TRT10			TRT6			CST		
Parameter	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P
Intercept	-0.08	0.20	0.70	-0.25	0.22	0.25	-0.61	0.17	**<0.001**	-0.25	0.22	0.25
LOC CP	1.07	0.23	**<0.001**	1.11	0.25	**<0.001**	1.31	0.17	**<0.001**	-0.97	0.42	**0.02**
LOC EF	0.95	0.25	**<0.001**	1.00	0.27	**<0.001**	1.14	0.18	**<0.001**	0.25	0.32	0.43
LOC MN	-0.17	0.30	0.56	-0.10	0.32	0.75	0.09	0.21	0.68	-0.85	0.40	**0.03**
LOC TA	-0.17	0.33	0.60	-0.34	0.38	0.38	0.25	0.21	0.24	-0.56	0.42	0.18
PL BZ	0.28	0.29	0.34	0.14	0.33	0.69	0.62	0.12	**<0.001**	0.19	0.33	0.55
PL NT	0.82	0.30	**0.01**	0.89	0.33	**0.01**	0.84	0.13	**<0.001**	0.76	0.39	**0.02**
LOCCP: PLBZ	0.10	0.34	0.77	0.32	0.38	0.40				2.29	0.50	**<0.001**
LOCEF: PLBZ	0.17	0.35	0.64	0.42	0.39	0.28				0.13	0.45	0.77
LOCMN: PLBZ	0.69	0.40	0.08	0.66	0.45	0.14				1.19	0.51	**0.02**
LOCTA: PLBZ	0.42	0.44	0.34	0.86	0.50	0.08				1.42	0.51	**0.01**
LOCCP: PLNT	-0.05	0.35	0.90	-0.02	0.38	0.97				1.81	0.52	**<0.001**
LOCEF: PLNT	-0.53	0.39	0.18	-0.46	0.41	0.26				0.09	0.47	0.86
LOCMN: PLNT	-0.13	0.46	0.78	-0.17	0.49	0.73				-0.25	0.65	0.70
LOCTA: PLNT	0.63	0.44	0.16	-0.64	0.50	0.20				0.69	0.55	0.21
AIC	821.34			776.92			729.11			663.70		
Marginal R^2	0.52			0.55			0.54			0.54		

LOC: location. CP: Cabo de Palos. EF: Es Freus. MN: Menorca. TA: Tabarca. PL: protection level. BZ: buffer zone. NT: no-take zone. The final models were: Estimator ~ ZN * PL + ZN + PL. The estimate (including the sign), standard error and p-value of the parameters are shown.

Table 2. Results of the GLMMs for the estimators applied (*ER*, *AD*, TRT20, TRT10, TRT6 and CST) showing the variables explaining the density response variable. Models excluded by AIC are not shown in the table.

	ER			*AD*			TRT20			TRT10			TRT6			CST		
Random effects																		
Parameter	Variance	SD		Variance	SD		Variance	SD		Variance	SD		Variance	SD		Variance	SD	
ST	0.19	0.43		0.30	0.53		<0.001	<0.001		0.23	0.48		0.12	0.35		0.33	0.57	
Conditional model																		
Parameter	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P
Intercept	-3.46	0.43	**<0.001**	-3.13	0.51	**<0.001**	-6.39	0.43	**<0.001**	-5.78	0.5	**<0.001**	-5.34	0.48	**<0.001**	-5.10	0.41	**<0.001**
LOC CP	1.51	0.49	**0.00**	0.23	0.59	0.70	1.45	0.47	**0.00**	1.29	0.6	**0.02**	0.87	0.52	0.09	-1.13	0.56	**0.04**
LOC EF	0.47	0.58	0.41	-1.60	0.68	**0.02**	0.24	0.55	0.66	-0.20	0.6	0.75	-0.40	0.58	0.49	0.17	0.62	0.79
LOC MN	-1.40	0.52	**0.01**	-2.76	0.62	**<0.001**	-1.47	0.52	**0.01**	-1.60	0.6	**0.01**	-1.63	0.59	**0.01**	-1.78	0.60	**0.01**
LOC TA	-0.62	0.55	0.26	-1.55	0.65	**0.02**	-0.64	0.53	0.23	-0.75	0.6	0.23	-0.70	0.62	0.26	-1.00	0.65	0.13
PL BZ	-0.40	0.64	0.53	-0.57	0.84	0.50	-0.49	0.65	0.45	-0.73	0.7	0.31	-0.87	0.69	0.20	-0.27	0.60	0.65
PL NT	1.57	0.66	**0.02**	2.07	0.90	**0.02**	1.49	0.64	**0.02**	1.56	0.7	**0.02**	0.89	0.71	0.39	0.83	0.62	0.18
LOCCP: PLBZ	1.46	0.73	**0.05**	1.56	1.09	0.11	1.47	0.73	**0.05**	1.70	0.8	**0.04**	0.61	0.77	**0.01**	3.70	0.78	**<0.001**
LOCEF: PLBZ	1.90	0.83	**0.02**	3.51	0.99	**0.00**	1.93	0.81	**0.02**	2.11	0.9	**0.02**	2.13	0.82	**0.01**	1.32	0.86	0.13
LOCMN: PLBZ	2.20	0.76	**0.00**	1.91	1.00	0.06	2.24	0.79	**0.01**	2.29	0.9	**0.01**	2.10	0.85	**0.01**	2.00	0.83	**0.02**
LOCTA: PLBZ	1.56	0.78	**0.05**	1.66	1.06	0.10	1.52	0.78	**0.05**	1.85	0.9	**0.04**	1.80	0.86	**0.04**	2.57	0.89	**0.01**
LOCCP: PLNT	0.11	0.77	0.89	-0.27	1.19	0.80	0.12	0.71	0.87	0.06	0.8	0.94	1.07	0.80	0.18	3.64	0.83	**<0.001**

LOCEF: PLNT	0.71	0.88	0.42	3.04	1.21	**0.01**	1.57	0.86	0.51	0.02	0.9	0.99	1.25	0.92	0.17	0.75	0.95	0.44
LOCMN: PLNT	-0.53	0.82	0.52	-1.72	1.07	0.11	-0.44	0.79	0.57	-0.47	0.9	0.59	0.14	0.92	0.90	0.55	0.91	0.55
LOCTA: PLNT	0.30	0.82	0.71	-1.30	1.06	0.22	0.32	0.76	0.68	0.01	0.9	0.99	0.75	0.91	0.41	1.54	0.93	0.10
Zero-inflation model																		
Parameter	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P
Intercept	-4.23	1.30	**0.00**	-20.85	3293.04	1.00	-4.46	1.74	**0.01**	-3.78	1.29	**0.00**	-3.31	1.05	**0.00**	-20.58	4664.68	1.00
Dispersion model																		
Parameter	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P
Intercept	-1.20	0.29	**<0.001**	-1.5	0.25	**<0.001**	-1.34	0.27	**<0.001**	-1.47	0.31	**<0.001**	-1.44	0.30	**<0.001**	-0.59	0.45	0.20
LOC CP	1.48	0.34	**<0.001**	1.2	0.29	**<0.001**	1.53	0.33	**<0.001**	1.63	0.37	**<0.001**	0.68	0.38	**<0.001**	0.50	0.52	0.34
LOC EF	0.66	0.37	0.07	0.7	0.31	**0.02**	0.85	0.36	**0.02**	1.04	0.43	**0.02**	1.34	0.46	**0.00**	-0.16	0.49	0.75
LOC MN	1.65	0.58	**0.00**	1.7	0.40	**<0.001**	1.35	0.53	**0.00**	1.46	0.63	**0.02**	1.08	0.52	**0.04**	0.90	0.89	0.31
LOC TA	1.41	0.50	**0.01**	1.8	0.44	**<0.001**	1.41	0.44	**<0.001**	1.39	0.50	**0.01**	1.06	0.51	**0.04**	-0.02	0.52	0.98
PL BZ	0.21	0.32	0.51	-0.2	0.27	0.41	0.06	0.30	0.85	0.30	0.34	0.37	0.27	0.33	0.42	0.31	0.46	0.50
PL NT	0.83	0.36	**0.02**	0.3	0.30	0.37	0.86	0.35	**0.01**	1.28	0.42	**0.00**	0.83	0.40	**0.04**	1.01	0.50	**0.04**
AIC	1540.01			1633.52			1501.11			1382.93			1213.52			1050.44		
R^2 marginal	0.49			0.54			0.47			0.41			0.39			0.54		

LOC: location. CP: Cabo de Palos. EF: Es Freus. MN: Menorca. TA: Tabarca. PL: protection level. BZ: buffer zone. NT: no-take zone. The final models were: Abundance Estimator ~ ZN * PL + ZN + PL + offset(log(area transect) + (1 | ST). The estimate (including the sign), standard error and p-value of the parameters are shown for the conditional, zero-inflated and dispersion models, as well as the variance and standard deviation of the random effects model.

Table 3. Results of the GLMMs for the estimators applied (TRT20, TRT10, TRT6 and CST) showing the variables explaining the biomass response variable. Models excluded by AIC are not shown in the table.

	TRT20			TRT10			TRT6			CST		
Random effects												
Parameter	Variance	SD		Variance	SD		Variance	SD		Variance	SD	
ST	0.28	0.53		0.44	0.66		0.48	0.70		0.47	0.69	
Conditional model												
Parameter	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P
Intercept	0.15	0.45	0.74	0.59	0.44	0.18	1.05	0.44	**0.02**	-0.05	0.53	0.93
LOC CP	1.04	0.52	**0.05**	0.86	0.53	0.10	0.72	0.54	0.18	-0.44	0.86	0.61
LOC EF	-0.02	0.56	0.98	-0.65	0.59	0.27	-0.96	0.59	0.10	-0.50	0.70	0.47
LOC MN	-1.33	0.60	**0.03**	-1.15	0.62	0.06	-1.10	0.63	0.08	-1.79	0.81	0.03
LOC TA	-0.18	0.63	0.77	-0.62	0.65	0.34	-0.59	0.66	0.37	-0.29	0.81	0.73
PL BZ	-0.23	0.63	0.71	-0.60	0.70	0.39	-0.54	0.72	0.45	-0.19	0.75	0.80
PL NT	1.49	0.62	**0.02**	1.69	0.64	**0.01**	1.73	0.65	**0.01**	0.29	0.81	0.72
LOCCP: PLBZ	2.73	0.78	**<0.001**	3.34	0.86	**<0.001**	3.17	0.89	**<0.001**	4.22	1.12	**<0.001**

LOCEF: PLBZ	1.72	0.81	**0.03**	2.83	0.89	**0.00**	2.83	0.92	**0.00**	1.82	1.01	0.07
LOCMN: PLBZ	2.29	0.84	**0.01**	2.47	0.92	**0.01**	2.17	0.96	**0.02**	1.44	1.10	0.19
LOCTA: PLBZ	1.00	0.86	0.25	2.00	0.93	**0.03**	1.98	0.98	**0.04**	1.65	1.09	0.13
LOCCP: PLNT	1.46	0.79	0.06	1.39	0.84	0.10	1.25	0.86	0.14	4.46	1.18	**<0.001**
LOCEF: PLNT	0.78	0.85	0.35	0.19	0.91	0.84	0.34	0.92	0.72	2.25	1.13	**0.05**
LOCMN: PLNT	0.38	0.86	0.66	0.07	0.92	0.94	-0.07	0.96	0.94	2.47	1.21	**0.04**
LOCTA: PLNT	1.08	0.87	0.22	1.16	0.92	0.20	1.01	0.95	0.29	1.61	1.15	0.16
Zero-inflation model												
Parameter	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P
Intercept	0.44	0.33	0.18	0.49	0.32	0.13	0.42	0.32	0.19	0.67	0.35	0.05
LOC CP	-3.26	0.79	**<0.001**	-2.90	0.67	**<0.001**	-2.30	0.56	**<0.001**	-0.09	0.46	0.84
LOC EF	-1.95	0.57	**<0.001**	-1.45	0.49	**0.00**	-1.43	0.49	**0.00**	-0.46	0.51	0.38
LOC MN	-0.34	0.41	0.41	-0.08	0.41	0.84	0.16	0.40	0.69	0.93	0.48	0.05
LOC TA	-0.80	0.46	0.08	-0.76	0.45	0.09	-0.21	0.43	0.62	-0.01	0.50	0.98
PL BZ	-0.92	0.36	**0.01**	-0.84	0.34	**0.01**	-0.80	0.33	**0.01**	-1.90	0.36	**<0.001**
PL NT	-1.95	0.58	**<0.001**	-1.87	0.53	**<0.001**	-1.45	0.46	**0.00**	-2.73	0.51	**<0.001**
Dispersion model												
Parameter	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P
Intercept	-0.16	0.18	0.36	0.03	0.19	0.90	0.07	0.20	0.71	-0.10	0.32	0.77
PL BZ	0.10	0.24	0.67	0.00	0.26	0.99	-0.16	0.27	0.54	-0.50	0.38	0.20
PL NT	0.52	0.28	0.07	0.60	0.31	**0.05**	0.62	0.33	0.06	-0.15	0.39	0.71
AIC	3918.71			3672.23			3445.32			2442.64		
Marginal R^2	0.64			0.64			0.61			0.65		

LOC: location. CP: Cabo de Palos. EF: Es Freus. MN: Menorca. TA: Tabarca. PL: protection level. BZ: buffer zone. NT: no-take zone. The final models were: biomass Estimator ~ ZN * PL + ZN + PL + offset(log(area transect) + (1 | ST). The estimate (including the sign), standard error and p-value of the parameters are shown for the conditional, zero-inflated and dispersion models, as well as the variance and standard deviation of the random effects model.

8. Figure legends

Figure 1. Location of the MPAs sampled in the coast of Spain (A): Cabo de Gata MPA (B), Cabo de Palos-Islas Hormigas MPA (C), Tabarca MPA (D), Es Freus MPA (E) and Norte de Menorca MPA (F). No-take zones (light gray) and buffer zones (dark gray) are highlighted in the maps.

Figure 2. Mean richness (A), density (B) and biomass (C) according to the protection level and the location for the estimators included in the study. NT: no-take zone. BZ: buffer zone. UP: unprotected area. CG: Cabo de Gata. CP: Cabo de Palos. TA: Tabarca. EF: Es Freus. MN: Menorca. DS refers to the density value calculated through the distance sampling theory.

Figure 3. Mean sighting distances of the 7 most abundant species included in the analyses by protection level. Data were polled across locations. NT: no-take zone. BZ: buffer zone. UP: unprotected area.

Figure 4. Mean abundance according to the protection level and the location for the ER estimator. NT: no-take zone. BZ: buffer zone. UP: unprotected area. CG: Cabo de Gata. CP: Cabo de Palos. TA: Tabarca. EF: Es Freus. MN: Menorca.

9. Appendices legends

Appendix A. Features characterizing the MPAs included in the study.

Appendix B. Complete set of species recorded in the study, and the percentage occurrence in the transects performed, including the two methodologies TRT+DS and CST. Species selected for the study for being in at least 10 % of the transects are highlighted in bold.