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EMPAFISH
European Marine Protected Areas as tools for Fisheries management and conservation

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Suitable methodologies to collect and analyze indicators, and suitable experimental designs to test different situations on MPAs

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Suitable methodologies to collect and analyze indicators to assess the effects of MPAs

Introduction: Overview of the Protocols

Numerous and varied methods of inventorying and monitoring MPAs have been applied by scientific researchers and consultants. Many of these entities already inventory or monitor biodiversity components relevant to conservation and/or management of MPAs over the EU countries, but the efforts are largely uncoordinated or unlinked, have different objectives, use different indicators, and lack support for sharing and statistically analyzing the data. A diversity of methods is desirable in the initial stages of any rapidly developing field, but enough time has passed to now assess the state-of the-science related with MPAs and recommend selected data collection methods that robustly capture data on the effects of protection due to MPAs related to all their proposed objectives. While the geographic scope of data collection methodologies is often initially designed for use at the local level, the use of consistent methodologies across larger regions, in our case the Mediterranean Sea and Central East Atlantic, is now appropriate. The central theme of this document is on protocols for collecting Effects of protection data in MPAs. The protocols in this document outline the steps for obtaining field-, laboratory-, and office-based data about environmental (including physical, biological conditions and ecological), fisheries and socioeconomic records relevant to management objectives and the health of the considered marine systems. While we have not addressed protocols for those tasks more related with the administration and logistics in the management of MPAs, we have included protocols on sampling or handling of fish, macroinvertebrates, plankton, and biomonitoring of selected species and/or communities, as well as fishing and socioeconomic activities.

In this document, we assembled several documents containing a proposal of protocols for data acquisition methods addressing some focus areas relevant to the assessment of MPAs effects. The majorities of these proposals are robust syntheses of science and contain an array of sampling methods suitable to collect data in MPAs. Most of the methods referenced in this document have been previously published.

Recommended Protocols

We recommend a specific subset of protocols for consistent use across the region considered in EMPAFISH project. To capture information on the focus areas, we recommend protocols for use by volunteers and for use by management/research personnel across the Mediterranean and Central East Atlantic. While the protocols recommended in this document are reasonably comprehensive, specialized or research needs may require the development of new or different methodologies. For these needs, we urge users to first review the recommended methods. Thereafter, we direct users to the other documents summarized in this publication, as they are likely to find many of the key building blocks to support their specialized needs.

Linking Restoration and Mitigation Projects with the Protocols

A wide array of agencies, consultants and volunteer groups can undertake MPAs monitoring project data collection, so having consistency in methods is fundamental. Our objective was to provide linkages between the projects and the protocols such that if monitoring of effects of protection data at (or inventory of conditions prior to) projects is desired, there are consistent methodologies to do so. In this document, we identified several types of studies related with the assessment of the effects of protection in MPAs. Also, it is important that the terminology surrounding project types be clear.

The Role of Protocols in Monitoring Strategies

Establishing a baseline and monitoring changes in conditions is fundamental to the recovery and conservation of marine ecosystems. To efficiently undertake these efforts requires a thoughtful approach to monitoring and evaluation. A well structured monitoring and evaluation plan results in the collection of extremely valuable data. In a broad sense, monitoring can be defined as the collection of information necessary to understand the condition and trends of components and processes in a system of interest. More specifically, monitoring efforts provide a context for:

1) Confirming that management decisions were implemented;
2) Making accurate status assessments of the resource to determine whether management objectives are being achieved, and
3) Improved understanding of MPAs functioning and their environments to determine the extent to which changes in status were the result of management actions.

A set of common Objectives for monitoring and evaluation efforts includes the following:

• Measure attributes of environmental conditions and biological resources in the system of interest within relevant temporal and spatial scales.
• Conduct ecological research to better understand the distribution and abundance of ecological variables at the watershed and landscape scales.
• Improve the integration, coordination, and sharing of monitoring efforts across organizations, geographic scales, and relevant elements of the ecosystem.
• Ensure that management decisions are based on the best and most current information.
• Predict future conditions and suggest hypotheses for subsequent scientific testing.

Typically, monitoring and evaluation plans include the driven questions to be addressed and the identification of management questions form the basis of the monitoring effort. Imperative to inventory and monitoring efforts is the prior articulation of specific questions to be addressed (to guide data collection), and the accuracy/quality level of the data developed (to guide uses of the data). More specifically, the questions to be asked should be taking to:

"What questions are we trying to address through this MPA inventory/monitoring effort?
"Are the most appropriate methods being applied?" and
"Where will the data developed from this effort reside?"

Because considerable time and resources are spent on monitoring activities, the clear articulation of the questions to be addressed is fundamental. While questions regarding effects of protection in MPAs are similar across the Mediterranean Sea and the Central East Atlantic, they are not necessarily consistent across the region. Thus, we strongly urge users to think through, and write down, the specific inventory/monitoring objectives and questions they are trying to address.

Monitoring involves a series of observations, measurements, or samples of these attributes collected and analyzed over time. The selection of the appropriate protocol(s), clear definition of the data attributes, and adherence to careful sampling design is essential to fulfil the identified needs. The focus of monitoring efforts should be on the acquisition of data that specifically quantify the effects of MPAs.

Statistically valid approach: Monitoring efforts will need to meet assumptions for standard statistical analysis and results in estimates with known boundaries of error.

Repeatable: The protocols used should provide a statistically defensible method for evaluating and minimizing observer bias and sampling error. This consideration is intended to reduce the inherent variability surrounding many of the data attributes so that replication of sampled attributes will be meaningful across time and space.

Coordinated with other resource entities: It is imperative that the protocols used and the data collected are compatible across the geographic area included in EMPAFISH project. The development of a regional data system for the assessment of the effects of protection in MPAs (centralized or distributed data sets) is clearly warranted at this time. In this context, management actions can be evaluated, trends in ecological, fisheries and socioeconomic responses identified, and changes in recovery and conservation strategies supported.

Cost efficient: Funding resources will always be limited; utilizing focused data collection and analysis procedures by volunteers and management/research personnel will prioritize specific data needs and yield the greatest long-term benefits.

Listed below are components of a standard monitoring plan.

Problem definition
Goal
Objectives
Hypotheses
Site description
Data gathering strategy
Methods
Data Quality
Data Storage and Analysis Methods
Timetable and Staff Requirements
Landowner Permission / Relationships with institutions / Logistic needs

Protocols

Sampling Protocols on Reserve Effects Monitoring

Fish assemblages

1. Background

Fish are ecological dominants in all marine habitats, evolving obvious and striking anatomic, physiological, behavioural and ecological adaptations. Numerically, valid scientific descriptions exist for approximately 24600 species of fishes in 482 families, 58% inhabiting in seawater. Fish are involved in most of the ecological process occurring in the sea. They interact with fish of other species, with invertebrate prey, with bird and mammal predators, and with the plants and sessile animals that provide much of the physical structure of their environment.

As a resource, fish are of considerable importance to the survival of human populations in the form of food of numerous kinds, playing a key role in providing income and employment in many parts of the world. Marine fisheries yield now around 100 million tonnes per year, increasing because a growing human population demands. By other hand, and more recently, fishes represent a charismatic subject for many people visiting tourist places being an important alternative source of incomes for the coastal human communities.

Fish assemblage can be used to evaluate the environmental health degree and estimate societal costs of degradation more directly than other taxa because their economic and aesthetic values are widely recognized. Moreover, fish are highly visible components in marine landscapes but they are too one of the most easily sampled component by professional biologists and managers. Their use as study subject can provide ease interpretations about changes occurring in the marine environment.

2. Measurable objectives

Species identification is necessary in order to establish differences among locations with different protection level (no-take, partially protected, restricted use, unprotected) and changes over time due to the effect of protection in the marine protected area. It is important too in order to analyze the data at the specific level differentiating among target and non target species.

Abundance is the amount of individuals recorded for each observed species. Each observation –noted down by means of the size estimated for the individuals- is assigned to one of a number of predetermined abundance classes (e.g., 1; 2; 3-5; 6-10; 11-30; 31-50; 51-100; 101-200; 201-500; 501-1000), the limits of which coincide approximately with the terms of a base 2 geometric series. However, the real observed abundance can be recorded directly when species appear in low number of individuals. Geometric means of each fish abundance class were used for further calculations.

Size of each individual or group of individuals is estimated visually by means of a rule attached at the end of a stick. Total length is estimated to the nearest 2-cm multiple.

Biomass is estimated using weight-length relationships calculated from data obtained from the same geographical area for each species. If no information is available for a particular species, weight-length relationships from other similar species can be used. Total biomass for the different observations is calculated subsequently multiplying abundance of individuals by the estimated weight for each size.

Habitat structure can be described using substrate characteristics. To estimate it within each transect, we measured (i) the percentage of each kind of substrate present (e.g., rock, sand, *P. oceanica* meadow, etc) (ii) the number of boulders (major length: 50-100 cm), blocks (major length: 100-200 cm) and big blocks (major length: >200 cm), (iii) the maximum and minimum depth and (iv) the maximum verticality of the substrate along the transect.

2. Sampling design

Sampling frequency and replication

To assess the effects of protection we need a set of data comparable over time and being representative of what is occurring in the marine protected area. Fish are subject at several sources of variation, comprising almost all the temporal and spatial scales. For this reason, fish data can reflect different pattern of variation depending on the scale selected. A suitable approach might be to carry out the fish survey once a year during summertime because fish diversity is greater and environmental conditions are better for the observers. The suitable sampling frequency will be annual, in such case to detect changes between different areas (fully-protected, partial protected, unprotected, etc) and to detect long-term trends.

Fish monitoring to detect the effects of protection will be based in BACI design. Because spatial variation at small scales (10s meters) is very large, it is recommended to increase the replication at this level including several sites. A hierarchical (nested) sampling design is suggested to estimate this variation and to get a suitable test for variation among areas avoiding pseudoreplication. Recommended minimum number and location of sampling sites are: at least 3 randomly selected sites in each kind of area (fully-protected, partial protected, unprotected, etc). In each site at least 3 random counts will be set. For a properly test of the effects of protection in a single marine protected area, two different and independent control unprotected areas should be considered to achieve finally the assessment by means of an asymmetrical design.

This planning over space should be repeated at least twice (but preferably, at least, three random times) during the sampling period each year in order to check shorter temporal variations and get a suitable source of variation to test differences over years.

Another scale of variation of fish assemblage is depth. It is recommendable to sample in a specific range of depth where fish composition is similar.

Site selection

Whenever possible sites should be of homogeneous substrata (rocky, seagrass, sand, etc) randomly selected. In the contrary, sites should be placed including as much as possible of the substrata of reference recording the habitat structure characteristics as referred above. The sites sampling should not present symptoms of not controlled anthropic impacts. It is recommended to select sites with the most suitable conditions respect to hydrographical and hydrodynamics conditions which facilitate the sampling work.

Level of change that can be detected

This sampling design permits to detect changes among areas with different protection levels over years.

3. Field methods

Details of taking measurements

Abundance of individuals can be recorded along linear strip transects randomly located at each sampling point. Width and length of the strip transects can be adapted to different circumstances and/or limitations. Usually the length of strip transects to count fishes are between 25-50 m long and 5 m wide. In any case, the measures of the strip transect should allow standardization to a certain sampling unit (e.g. individuals×10 m^{-2}) in order to provide comparison among studies from different areas.

Field preparations and equipment setup	***Post-collection processing of samples***
Visual censuses of fish assemblage are carried out by SCUBA diving. Observers have to be competent in diving procedures and technology. Diving equipments have to be in well service. Sampling areas should be well known by observers previously in order to save time looking for suitable places to count fishes.	There is not post-collection processing of samples

4. Data handling, analysis, and reporting

Database procedures	***Recommendation on statistical analyses***
Data should be entered including as much details as possible for each sample. In this sense, it is recommended to include data on size for each individual of each species. Database should include all the needed fields in order to identify properly each sample in the subsequent analysis.	ANOVA including Protected vs. Unprotected and Sampling periods (each year) as main sources of variation should be arranged, including the other complementary sources of variations (spatial and short temporal replication factors and residual). This will allow testing differences in the protected area over the time using the appropriate sources of variation to avoid. Both main factors (Protected vs. Unprotected and Sampling periods) should be included in the multivariate analysis to check changes in the assemblage structure between protected and unprotected over time. It is recommended to pool the data at the short temporal and spatial replication in order to avoid the interference of their variability. For the multivariate analysis will be recommended to use non parametric approaches as those included in the PRIMER package.
Recommended reporting schedule	***Recommended report format***
Reporting should include an introductory part explaining the purposes of the sampling, the objectives and procedures implemented. It have to include the specific description of the methodological procedure performed to gather the data (e.g. sampling unit) in order to allow a posteriori metacomparisons of results with other studies. The report should include the raw data of the recorded species and the assemblage parameters for each sample, and the mean values for each source of variation considered.	The report can be organized in introduction, material and methods, results, discussion of the results and complementary annex reporting the raw data.

5. Personnel requirements and training

Roles, responsibilities and	***Training procedures***

qualifications The team should consist, at least, by three persons in order to fulfil the safety rules.	The personnel should calibrate their skills to count fish in order to avoid skewnees among them in terms of estimations of abundance and size of the individuals recorded.

6. Operational requirements

Schedule	**Equipment**
Each sampling time can take about 8-10 days. Sampling should be carried out between 10-16 a.m.	A suitable 4-5 m long boat, SCUBA diving equipment for each person plus one tank for safety.

Cost considerations

Fuel, air charges, expenses, boat renting.

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Sampling Protocols on Reserve Effects Monitoring

FISHERIES

1. Background

Marine protected areas (MPAs) are a potential tool to enhance the long-term sustainability of fisheries. A substantial amount of evidence indicates that the abundance and average size of organisms targeted by fisheries are increased inside marine protected areas. However, to be useful as fisheries management tools, MPAs need to affect fished areas outside them in a positive manner.

MPAs are predicted to benefit adjacent fisheries through two mechanisms: net emigration of adults and juveniles across borders, termed "spillover", and with the increased production and exportation of pelagic eggs and larvae. The emigration of juveniles and adult fish to surrounding non-protected areas would produce a gradient of abundance across MPAs borders, and should have an influence on the yields and quality of the catches in the surrounding fishing grounds.

When biomass increase brings fishery benefits it is expected to translate in aggregation of effort around MPAs and increases in yields. Thereby, to assess the MPA benefits, as biomass export, it is essential study the spatial and temporal distribution of the fishing effort and the captures of the adjacent local fisheries.

Usually, the fisheries around MPAs are highly diverse, with a variety of gears, target species and fishing grounds, and followed a seasonal rotation. Identify the *métiers* (association between type of gear, target species and season) used in the area is crucial to assess the MPAs benefits.

Measurable objectives

Fishing effort distribution could be a good indicator of the MPA benefits to the fisheries. Fishing effort aggregation along MPAs boundaries suggests biomass export from MPAs to unprotected areas. Fishing effort should be studied for total effort and for each *métier* separately to avoid confounding and interpret correctly the spatial patterns related to the MPA. Fishing effort distribution should be studied by means of effort density, which is calculated as the sum of the fishing effort (measured as number of boats or fishing sets for total effort, and as meters of net, number of hooks, ... for effort of each *métier*) in a determinate area.

Catch per unit effort (CPUE) is used as an index to assess spatial patterns of fish density and catch rates. However, such data are potentially confounded by differences in catchability owing to variation in vessel characteristics, and differences in fishing gears and fishing seasons, etc. Therefore, CPUE should be analyzed for each *métier* separately. CPUE could be calculated for total catch or for some specific species. It is estimated dividing the catch of the sample (expressed in numbers and/or weight) by the fishing effort used. The units of the fishing effort would vary depending on the fishing gear used (meters of net for trammel nets and gill nets, number of hooks for long line and hook and line, surface fished for trawling, …), but have to be took into account the time used during the fishing.

Fishery production is sometimes a better indicator of biomass export from MPAs to unprotected areas than CPUE. This variable considers cumulative spatial effects, because aggregation of effort around MPAs may cause local depletion on CPUE patterns and hampers evaluating MPA benefits to fisheries. Catch per unit area (CPUA), which is a measure of production, is calculated as the sum of the catch of all the samples in a determinate area.

Fish mean size and fish maximum size are good indicators of MPA benefits because closing areas allows animals to live longer and grow to maturity, which is important for supporting fisheries due to the exponential relationship between fecundity and body size. They are estimated measuring the total length of all the individuals caught, and after calculating the mean and maximum size for each species in any sample.

2. Sampling design

Sampling frequency and replication

If there is not prior information on local fisheries, it is necessary to characterize the fisheries identifying all the *métiers* that take place around the MPA. To do so, it is recommended conduct fisher interviews in nearby fishing harbors and random sampling onboard of fishing boats in and around each MPA. This sampling should be done during at least one year to incorporate all the seasonality, and have to be sampled as much boats as possible to obtain a general view of the fisheries.

Subsequently, sampling should target those *métiers* likely to reveal potential biomass export processes. The selection of the *métiers* to be sampled should be done by considering the importance and the intensity of the use in the area and its target species, focusing mainly in target species protected by the MPA. As the final position of the fishing set is not determined by the sampler, it is necessary get as much samples as possible to obtain a good spatial representation.

Level of change that can be detected

This sampling design allows characterize the fisheries adjacent to the MPA and identify the spatial and temporal distribution of their activity. Whit that it is possible detects changes in fishing effort and captures related with distance from the MPA boundaries.

3. Field methods

Field season preparations and equipment setup

When the objective is to calculate the CPUE, the first and indispensable step is to establish collaboration with professional boats to go aboard to carry out the sampling. With a higher number of implied boats, the sampling will be more complete.

When the objective of the sampling is to assess the fishing effort around the MPA, several methodologies can be chosen: i) Onboard sampling, taking the geographical position of the fishing gears which allows know the fishing effort of each *métier* and ii) Sighting, by mean of routes in the influence area. These sighting can be carried out from boat or from plane, and allows sample all the fishing effort of the area.

Sequence of events during field season

To georeference as accurately as possible the sampling area. At least, the initial and final points, although a better detail will help later in the interpretation of results. When it is possible, to obtain the plotter representation of the sampling area.

When the catches are taken on board, the sampler must count the **abundance** of each species, and for each individual caught, the **length** (to the nearest cm) and the **weight** (to the nearest 0.01 g) must be measured. These data will be filled in the polyester paper.

Post-collection processing of samples

When the objective of the sampling is to estimate the spillover from the MPA and to value its influence on the surrounding fisheries, it is not necessary to retain the obtained samples. However, if later these samples are going to be used for another type of works (otholits, stomach contents ...), the samples can be preserved in ice or in seawater formaline (10%). But it is important to remain that the owners of these catches are the fishermen.

4. Data handling, analysis, and reporting

Recommendation on statistical analyses

To detect changes between different assemblages associated to distances from MPA are recommended multivariant and univariant analysis. Multivariant analysis to test the catch structure and univariant analysis to compare assemblage parameters or species.

To test the relation between a parameter (CPUE, mean size, effort density …) with distance, the use of linear regressions is recommenced.

Metadata procedures in connection with GIS information on the MPA are counselled to represent the fishing effort and capture data.

Recommended report format

The report would contain all the possible information on the objectives, the employed methodology, the main results and conclusions with special emphasis in the spillover and the effect on the fisheries and recommendations.

5. Personnel requirements and training

Roles, responsibilities and qualifications

The requirements for develop this monitoring are sailor certificate, experience in the *in situ* determination of species, technical with experience in statistical analysis, geographic information system and interpretation of biological information.

6. Operational requirements

Facility and equipment needs

Equipment needs: professional sailors and boats; plane and pilot; selected gear, ichthyometer; precision balance; GPS; polyester paper; waterproof clothes.

Cost considerations

Fuel, expenses, travel costs, boat renting, fishing gears.

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<table>
<tr><td colspan="2">Locality:</td><td colspan="2">Sample code:</td><td colspan="2">Date:</td></tr>
<tr><td colspan="3">Sampler:</td><td colspan="3">Boat name:</td></tr>
<tr><td rowspan="2">Geographic position</td><td colspan="2">Beginning:</td><td rowspan="2">Gear</td><td colspan="2">Fishing effort:</td></tr>
<tr><td colspan="2">End:</td><td colspan="2">Technical Characteristics:</td></tr>
<tr><td colspan="2">Direction:</td><td colspan="2">Set time.</td><td>Beginning:</td><td>End:</td></tr>
<tr><td colspan="2">Habitat type:</td><td colspan="2">Recover time.</td><td>Beginning:</td><td>End:</td></tr>
<tr><td rowspan="3">Depth</td><td>Beginning:</td><td colspan="4" rowspan="3">Observations</td></tr>
<tr><td>End:</td></tr>
<tr><td>Mean:</td></tr>
</table>

Species	**Abundance (size)**	**Total biomass**
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
1		
2		
3		
4		
5		
6		
7		
8		
9		
20		
1		
2		
3		

Sampling Protocols on Reserve Effects Monitoring

Socioeconomic issues

1. Background

During the last few years, new conservation policies for coastal marine systems started around the world to establish new marine protected areas. Mass-tourism has occurred at around these areas, causing high tourism pressure with consequent environmental and socio-economics transformations.

Mass tourism involved the development of recreational activities as snorkel, SCUBA diving, recreational fishing, boating, glass bottom boats, etc. These leisure activities provide significant business in the local economy and may supply AMP management cost. But, this mass-tourism can too produce fatal ecological consequences and reduce the aesthetic valour of these areas, reducing the degree satisfaction's user and his "willingness to pay". This satisfaction level is related with the desire of achieve his expectations and marine protected area perception. People agglomeration in the same time and site may contribute to reduce this satisfaction level and create user's conflicts.

In order to make a correct marine protected area management and avoid these undesirable effects, socio-economic and ecologic aspects may be to take into account.

Measurable objectives

Level frequentation. Number of user for each leisure activity: recreational fishers (angling, spear fishing), divers, number of participants in glass bottom trips, boaters, etc. Spatial and temporal distribution of each activity may be study.

Economic Setting. Assess business activity of each recreational activity. For this, is necessary know cost and benefits of recreational activities operators and the expenditure's users in each activity (accommodation cost, trip, equipment, etc).

User Satisfaction level's. Identify user's motivations and the most valuables characteristics for users (size of fishes, per example for the recreational fishers or divers). This is relates to their expectative and satisfaction level before and after of visit the AMP and have to evaluate too.

User conflicts. Detect some possible user conflict between different activities. Usually this conflicts, if these exists, is for the space; scuba divers and anglers per example.

2. Sampling design

Sampling frequency and replication

The sampling must cover all the activities within the influence area of the marine protected area. The independent sampling units must be identified based on the spatial and temporal distributions of the activities. If an activity is developed mainly in the summer season or at a determinate time e.g.: in the afternoon or in a determinate zone, this will be the sampling unit. But if the activity is not distributed uniformly in time and/or space the sampling must be repeated in each different periods and areas along time. In the same way, if the aims of the sampling are stakeholders social perceptions (e.g.: satisfaction, money willing to pay to enter to the MPA) the sampling methodology must be done randomly incorporating different stakeholders characteristics (e.g.: ages, economical status, origin place).

We recommend identifying the different activities performed in the marine protected area and develop a long temporal sampling methodology. This will help to evaluate possible fluctuations in time and space of the stakeholders' preferences.

3. Field methods

A questionnaire is developed for the visitors and operators and is administered using face to face interviews, if is possible. The answer can be open answers or categorical answers (by closed sentences or punctuations). It is necessary to ensure that the sample adequately represented the visitor population and operators of most popular leisure activities.

The main recreational uses will be: recreational fishing (spear fishing, anglers and boat fishing), scuba diving, shipping and glass bottom boat trips. For each activity is recommended elaborate one questionnaire for users and one for users. People interviewed once must not been interviewed any more.

- **Users,** more specifically, several questions that are aimed at finding out about socio-economic background of those visiting are included (age, usual residence, occupation and household income). Also, must be included questions about the activities that different visitors engaged as number of times that they visit the AMP and economic aspects of each activity (cost of activity, equipment, accommodation and travel). The survey also should elicited comment from visitors regarding the importance of different factors that attracted them to the AMP and their expectations and level satisfaction. Other questions about their attitudes to any proposal or their willingness to financial contribution must been recorded too.
- **Operators.** Questions about cost and benefits of their business are very important in order to evaluate the economic activity in the marine protected area. Aspects as number of jobs, fixed cost (harbour dues, licence, buildings, insurance, management costs, etc), variable cost (maintenance, equipments, etc), benefits (per customer, per season, etc) must be into account. Other aspects related with their perception of AMP, conflict with others AMP user, their willingness to make a financial contribution for maintenance and management AMP aspects should be recorded too.

Post-collection processing of samples

The survey dates have to reported in database file, access programme should be use for this propose because it allow introduce a lot of variables.

Statistical analysis of visitors' profile can be made to explore the dependence of any variables as gender or education level with others as awareness or satisfaction level with each recreational activity.

4. Data handling, analysis, and reporting

Recommendation on statistical analyses

Data should be entered including as much details as possible for each interview. Its recommended to maintain the raw data at the individual interviewed in order to manage the information at minimum detail. Database should include all the needed fields in order to identify properly each sample in the subsequent analysis. It is high recommended to include an specific identification for each sample.

Statistical analysis including Protected vs. Unprotected, sampling periods and localities as main sources of variation should be arranged, including the other complementary sources of variations (spatial and short temporal replication factors and residual) to test differences in the protected area over the time. It is recommended to pool the data at annual temporal scale to avoid the interference of the variability at shorter temporal scales. For the multivariate analysis will be recommended to use non parametric approaches as those included in the PRIMER package.

Recommended report format

Reporting should include an introductory part explaining the purposes of the sampling, the objectives and procedures implemented. It have to include the specific description of the methodological procedure performed to gather the data in order to allow a posteriori metacomparisons of results with other studies. The report should include the raw data of the interviews, and the mean values for each source of variation considered.

The report can be organized in introduction, material and methods, results, discussion of the results and complementary annex reporting the raw data.

5. Personnel requirements and training

Roles, responsibilities and qualifications

The main requirement for the development this study is personal with knowledge and experience in: elaborate questionnaires, interpretation of socio-economic results and statistical analysis.

6. Operational requirements

Facility and equipment needs

Specific questionnaires, tape recorders.

Cost considerations

Expenses for interviewers and travel costs.

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Sampling Protocols on Reserve Effects Monitoring

SCUBA diving

1. Background

Until recent, diving was generally considered a non-destructive activity, but scuba diving may result in the deterioration of benthic communities because divers can easily damage marine organisms through physical contact with their hands, body, equipment and fins. Although the damage produced by individuals is usually quite minor, there is some evidence that the cumulative effects of these disturbances can cause significant localised destruction of sensitive organisms.

There is a bigger problem when the diving activity focuses in marine protected areas. In some cases, the effects of a massive number of divers in few places of a marine reserve can be opposite to the main objectives of the creation of the MPA. However, some authors state that the impact that divers produce in a site may be influenced more by their experience and behaviour than by the number of people who frequent the site.

Effects of scuba diving on marine protected area should be assess in order to ensure the sustainability of this activity and the protection and conservation MPAs objectives. For this, it is necessary evaluate and quantify the scuba diving damage and his effects on the fragile species and habitat.

Selection of habitat or indicator species

The first step that may be question is the indicator selected, this is, what is the habitat or groups of susceptible species at diving's damage. This habitats or species may meet some characteristics for to be selected:

- Sensibility at diving effect because of your fragile structure's form as your physiology (filter feedings).
- Spatial and temporal consistency. It should be present in large spatial and temporal scale. Seasonality specie don't is a good indicator of scuba diving impact e.g.
- Measurability and effectiveness in terms of precision, accuracy and risk of error.
- Relevance in relation at objective to assess.

Measurable objectives

Density or coverage. Density is defined as numbers of individuals in determine area and coverage is the percentage of area occupied by concrete specie. Abundance is most employed for singles individuals (ascidians, nacres) and coverage for animal's colonies (corals, bryozoans), algae, plants or undefined individuals (e.g. sponges).

Size. Maximum height or diameter may be useful for study changes in the structure of the community.

Damage. Percentage of individuals cankered or affected for necrosis are parameters that indicate the injuries magnitude. The proportion of each individual or colony damage may be recorded too.

Degree of exposure. To evaluate the degree of exposure of individuals or colonies to abrasion, this are classified into one of five categories, according to decreasing degree of exposition (1: as epibionts; 2: on convex surface; 3: on flat surface; 4: in concavity; 5: under overhang).

2. Sampling design

Sampling frequency and replication

A before-after/ control-impact (BACI) assessment provides an appropriate framework to detect ecological degradation in natural habitats. The simplest BACI design would consist of one sampling time before and one afters the impact and more than one (sampling) location per each of two groups. Others authors considered more than one sampling time before and after the impact and a greater number of locations (control-impact) to be more efficient, called "beyond BACI" design.

From the practical point view, if the density of the individuals is low or if the depth is too great in order to record enough replicates, the "split-plot" or repeated measures design would be useful in this cases. This design may consider impact/control factor spatial replicated and before/after time replicated too.

Usually, there aren't dates of before dive activity on impact localities and for supply this gap in the design, before/after factor is refereed at peak dive activity and don't when the diving didn't has started.

Finally, the choose design depends of our particular case and our available resources (time, personal, etc) in order to develop this study.

The main coralligenous or coral reefs species not exhibit great seasonal fluctuations and consequently, one survey per year, before and after of the peak diving season is generally sufficient to detect and quantify changes in the populations.

Site selection

It is recommended select sites with the same depth, morphological features and habitat because these factors determine the presence and dynamic of some community. Also, with the aim of incorporating the influence of these factors (e.g. slope) in assess of diving impact, they could be included in the experimental design. It is important know estimate data of dive frequentation in each sampling site.

Level of change that can be detected

This sampling design allows detect changes among sites with different intensities of diving activity and the response of communities at this type of impact. Also, assess the recovery ability of this species and identify the most sensitive specie at diving impact. Identify the morphological habitat features that more influenced the potential diving damage may be possible too if this factor is include in the design.

3. Field methods

Field season preparations and equipment setup

With the objective of recorded samplings adequately, it is needed several previous considerations in order to select the method sampling in our particular case.

The density benthic organism and others biometrics parameters (diameter, height…) is usually estimated on a 1m^2 frame (or sometimes 0.5m^2) or circular plots too. From the practical point of view, to avoid damage to individuals (cankered or necrosis), the $1m^2$ surface can be delimited with a rope or two 1m solid rods linked by two 1m ropes. The proportion of damage individuals can be measured on the same plot. Other sampling method is to establish a big fixed transect or plot and sampling randomly inside with minor units, but it is not useful if the organism density is very low.

If the density of sampled organism is low or if the depth is too great, the density can be measured along 5 x $2m^2$ transects. They are delimited by a rope laid on the bottom with a 2m rod at one end and weight on the other end. The proportion of cankered or necroses organisms is visually estimated by counting the number of healthy individuals and the number of damaged organisms.

Some authors analysed biological or biometrics parameters and abundance on underwater photographs taken in plots in order to obtain great number of replicates.

Sequence of events during field season

The *in situ* sampler must count the abundance of each species in the plot, and for each individual the height or maximum diameter, the degree exposition and damage proportion must be measured. These data will be filled in the polyester paper.

The photographer sampler must to take the samples and recorded on in situ the degree exposition. It's very important to employ some reference when take the photo in order to posterior adjust the spatial scale.

Post-collection processing of samples

If underwater photographs is chosen for survey method, the samples must be post-collection analyzed with a treatment image software.

4. Data handling, analysis, and reporting

Recommendation on statistical analyses

To detect changes in the assemblage between different times and sites are recommended multivariant and univariant analysis. Multivariant analysis to test change in composition community and univariant to identify changes in abundance or population structure for each species.

To test the relation between a parameter (abundance, necrosis …) with diving intensity, the use of linear regressions is recommenced.

Recommended report format

The report would contain all the possible information on the objectives, the employed methodology, the main results and conclusions with special emphasis in management strategies and recommendations for the SCUBA-diving activity.

5. Personnel requirements and training

Roles, responsibilities and qualifications

The requirements for develop this monitoring are professional divers with experience in the *in situ* determination of benthic species, technical with experience in statistical analysis and interpretation of biological information.

6. Operational requirements

Facility and equipment needs

Equipment needs: professional divers with complete dive equipment and vessel; tape measure, rods and/or frame (1m^2 or 0.5m^2 surface); photos camera; polyester paper; ruler or square calliper.

Cost considerations

Air charges, travel costs, expenses, boat renting, dive center fees.

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Sampling Protocols on Reserve Effects Monitoring

Recreational Fisheries

1. Background

The establishment of marine protected area attracts at visitants, that may be incompatible with conservation objectives if a correct management don't is applied in order to avoid increasing of tourism frequentation. Massive tourism involved the growth of group activities: recreational fishing, diving and anchoring. The MPA establishment without correct planning and regulation that don't take into account all implied sectors could be victim of your own success because of "overfrequentation" effect.

Diving or anchoring are damaging to habitat and have indirect effects on marine ecosystems, but the recreational fishing have direct effects on fish assemblages by means of catch target species, bait or "by-catch". The recreational fishing may be a risk for some rare or threatened species.

In multiple-use marine protected areas, the allow activities should be suitable management in order to reduce conflict, carry out the protection and conservation objectives and allow the development of tourism activities through sustainable use of his resource. For these objectives, to know the typology, frequency and intensity of these activities is necessary, including the recreational fishing.

Measurable objectives

Recreational fishing effort is a good indicator of the fishing intensity in area or time. Spatial and seasonal distribution of fishing effort should be studied by means total effort per type of fishing (spear fishing, boat angling or on shore angling) in each area and each stratum of time. Total effort is calculated by instantaneous counts in a portion of time and space (measured as number of boats or anglers/hour or day or morning, afternoon,…), in order to determine the effort in this portion, and then expand the observations to the whole fishery

Catch per unit effort (CPUE) is estimated dividing the catch of the sample (expressed in numbers and/or weight) by the fishing effort employed. In practice, catch is more easy recorded as size for specie and after calculate weight mean size-weight relations. The methodology is the same of the estimating the effort, determine the catch in an observed portion of the fishery (space and/or time) and expand the observations to the whole fishery. The ***total catch*** may be estimated multiplying the total effort in a stratum (space or time) by rate catch in this stratum.

2. Sampling design

Sampling frequency and replication

Sampling design by each fishing types (on shore, boat or spear fishing) should be stratified

into: weekend/ weekdays, daily period (morning, afternoon or evening) and season. These three factors is going to determine the effort temporal pattern and no true bias could be produced by ignored these. If study area is sufficiently large size this one should be spatial fragmented in minor units (in different study cells, different point's access or different routes).
Ideally, adequate survey design may requires that 10% of the fishing activity (in space and time) is been sampling but number of replicates and sampling frequency depends of economic and personal available supports, in fact. One year might be enough time for obtain information in order to assess the recreational fishing effects on MPA. Long-time monitoring is suggested.

Level of change that can be detected

This sampling design allows characterize the recreational fishing on MPA and identify the spatial and temporal distribution of their activity.

3. Field methods

Prior considerations

There is a controversy over the best method for collect catch, effort and rate catch data. Practical survey methods may require that a different method be used for each parameter, effort and catch (or catch rate). Such approaches are termed complemented or combined survey according to spatial, social and economics AMP characteristic. When initiating an angler survey the "best" methods are not always obvious and what is appropriate at one location may not be for another. Making preliminary counts and interviews during a prior representative time period aides in this decision process.

Off site survey

An advantage of the use of this type surveys is that one can obtain more information on effort and catch rates for fishers not easily reachable in an onsite survey (night fishers or anglers fishing from private jetties). However, a key concern is that effort and catch-rate date that are self-reported may contain large measurement errors. These errors may be due to wilful deception, recall bias, prestige bias or lack of knowledge (e.g. species identifications or precise spatial location). There are some possibilities:

i) ***Telephone, personal, mail or internet survey***. Questionnaire is elaborated in order to record information of effort, catch species, rate catch, spatial and temporal effort distribution, and satisfaction level, among others. The goal of these types of surveys depends on the availability of a list or database of the population of all marine recreational fishers that use marine protected area. Such licenses file list, with information by harbours, are available in some states but not others. Sometimes, fisheries agencies use off-site interviews for estimating fishing effort whereas not use this method for rate catch.

ii) ***Recreational fishing club records***. Data facilitate for the amateurs recreational fishing clubs may be a good source of more information. Usually, these celebrate fishing competitions and recorder date of captures and effort. This methodology might be a good estimator of the rate catch but not is more recommended for effort estimations.

In situ survey

These type of methods allow obtain an estimation more accurate of effort and rate catch because the observer is unbiased and training subject t. The observer may identify and measure the catch and estimate and reference spatially the effort more correctly. There are different on site methods according to our particular situation.

- ***i)*** ***Access point survey.*** This method is based in to observe a portion (e.g. 10% of the days in the season) of the fishery, determine the catch and effort in this portion, and then expand the observations to the whole fishery by dividing by the fraction of the fishery observed. Anglers are intercepted as they leave the fishery, complete trip interviews to estimate catch in this effort portion. The seasonal totals would then be obtained by multiplying the observed catch and effort by the observed portion (e.g. by 10). This method may be preferred when fishery has one or two access point and this are well identified.
- ***ii)*** ***Roving creel survey.*** The strategy is to estimate the total fishing effort and the catch per unit of fishing effort (catch rate). The total catch is then estimated as total effort x catch rate. For example, in a given day, the fishing effort might be estimated by making one (or more) instantaneous count for the number of anglers fishing at one (or more) instants. The instantaneous counts provide an estimate of the average number of anglers fishing during the day. The product of the average number of anglers fishing multiplied by the length of the day is an estimate of the fishing effort in angler-hours and can be multiplied by an estimate of the catch per angler hour to estimate the total catch in the day. In this case, anglers are interviewed using incomplete trip interviews because they doesn't finally their fishing day when they are intercepted. This method may be preferred at larger size fisheries with much access points or this are not well identified.
- ***iii)*** ***Aerial survey***. This method is considered as roving instantaneous count. Aerial survey has frequently been used to assess recreational fishing effort. These are an efficient method of estimating angler or boat fishing over large areas. The basic strategy is to observe a time portion of the fishery, determine the effort in that portion and then expand the observations to the whole fishery.

4. Data handling, analysis, and reporting

Metadata procedures

Effort and catch dates are recommended to entry in a GIS database in order to elaborate maps and analyze spatial and temporal trends in recreational fishing effort and catch in the AMP. This database and analyse would be making for an expert.

Recommendation on statistical analyses

To detect change in catch and/or effort between different locations, seasons, type of fishing, type of days (weekend or weekday) or period of day (morning, afternoon or evening) is recommended univariant and multivariant treatments of data.

Recommended report format

The report would contain all the possible information on the objectives, the employed methodology, the main results, conclusions and possible recommendations for the recreational fishing management.

5. Personnel requirements and training

Roles, responsibilities and qualifications

The main requirement for the development this study is personal with knowledge of procedures in recreational fishery and experience in: elaborate questionnaires, identify in situ species and interpretation of biological results and statistical analysis.

6. Operational requirements

Facility and equipment needs

Airplane, ichthyometer or precision balance; GPS; in press questionnaires, polyester paper; waterproof clothes.

Cost considerations

It is widely know that there are tradeoffs between survey costs and the precision of the estimates, but it is also true that methods that reduce bias in the estimates may be much more expensive. On site catch-rate estimates are much more expensive than offsite self-reported catch-rate estimates. In other hand, the spatial scale of marine protected area will be a crucial component for the decisions of method survey use.

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Sampling Protocols on Reserve Effects Monitoring

EXPERIMENTAL FISHING

1. Background

Marine protected areas (MPAs) are a potential tool to enhance the long-term sustainability of fisheries. A substantial amount of evidence indicates that the abundance and average size of organisms targeted by fisheries are increased inside marine protected areas. However, to be useful as fisheries management tools, MPAs need to affect fished areas outside them in a positive manner.

MPAs are predicted to benefit adjacent fisheries through two mechanisms: net emigration of adults and juveniles across borders, termed "spillover", and with the increased production and exportation of pelagic eggs and larvae. The emigration of juveniles and adult fish to surrounding non-protected areas would produce a gradient of abundance across MPAs borders, and should have an influence on the yields and quality of the catches in the surrounding fishing grounds.

An approach based on experimental fishing allows for testing the hypothesis of increased catches along the border of MPAs in comparison with other fishing grounds, whereas controlling some factors (e.g. habitat, depth …) that could influence in the yields and confound interpretation.

Measurable objectives

Catch per unit effort (CPUE) is used as an index to assess spatial patterns of fish density and catch rates. It could be calculated for total catch or for some specific species. It is estimated dividing the catch of the sample (expressed in numbers and/or weight) by the fishing effort used. The units of the fishing effort would vary depending on the fishing gear used (meters of net for trammel nets and gill nets, number of hooks for long line and hook and line, surface fished for trawling, …), but have to be took into account the time used during the fishing. The aggregation of fishing effort around MPAs may cause local depletion on CPUE patterns and hampers evaluating MPA benefits to fisheries, therefore could be necessary to correct the data of CPUE obtained in the experimental fishing with the fishing effort existing in the area.

Fish mean and maximum size are good indicators of MPA benefits because closing areas allows animals to live longer and grow to maturity, which is important for supporting fisheries due to the exponential relationship between fecundity and body size. They are estimated measuring the total length of all the individuals caught, and after calculating the mean and maximum size for each species in any sample.

2. Sampling design

Sampling frequency and replication

The fishing gear selected for the experimental fishing would vary depending on the species to analyze. It should be chosen these gears that have a high catchability over the selected species.

The experimental fishing should be carried out at different distances from the MPA boundaries (at least 3 distances), for comparing captures along the border of MPAs with other fishing grounds located at medium and far distances from the MPA. Due to the variability observed in the abundance or catchability of the species as a result of seasonal changes in temperate systems, it is recommended to sample in at least 3 different times distributed along the year. To assure the temporal consistency of the results, should be repeated the study in at least two years.

It is recommended increase the spatial and temporal replication in a small scale due to the high variability observed in the captures. A hierarchical (nested) sampling design is suggested to estimate this variation, using 2 locations at each distance from the MPA, and 6 days at each time sampled with 6 replicates.

Site selection	***Level of change that can be detected***
It is recommended select sites with the same habitat characteristics and depth because these factors could influence the CPUE and confound interpretation. Also, with the aim of incorporating the influence of these factors in the biomass export effect assessment, they could be included in the experimental design.	This sampling design allows detect changes among different distances from the MPA boundaries, and also between those factors included in the experimental design (habitat, depth, …)

3. Field methods

Field season preparations and equipment setup

With the objective of getting samplings next to the real fishing, it is needed several previous preparations: *i*) To establish collaboration with a professional fish boat to carry out the sampling with a real features; *ii*) To select the gear to sampling; *iii*) To manufacture, to rent or to buy the selected gear.

Sequence of events during field season

To georeference as accurately as possible the sampling area. At least, the initial and final points, although a better detail will help later in the interpretation of results. When it is possible, to obtain the plotter representation of the sampling area.

When the catches are taken on board, the sampler must count the **abundance** of each specie, and for each individual caught, the **length** (to the nearest cm) and the **weight** (to the nearest 0.01 g) must be measured. These data will be filled in the polyester paper.

Post-collection processing of samples

When the objective of the sampling is to estimate the spillover from the MPA and to value its influence on the surrounding fisheries, it is not necessary to retain the obtained samples. However, if later these samples are going to be used for another type of works (otholits, stomach contents,....), the samples can be preserved in ice or in seaformaline (10%).

4. Data handling, analysis, and reporting

Recommendation on statistical analyses

To detect changes between different assemblages associated to distances from MPA are recommended multivariant and univariant analyses. Multivariant to test the cacth structure and univariant to compare assemblage parameters or species.

To test the relation between a parameter (CPUE, mean size …) with distance, the use of linear regressions is recommenced.

Recommended report format

The report would contain all the possible information on the objetives, the employed methodology, the main results and conclusions with special emphasis in the spillover and the effect on the fisheries and recommendations.

5. Personnel requirements and training

Roles, responsibilities and qualifications

The requirements for develop this monitoring are sailor certificate, experience in the *in situ* determination of species, technical with experience in statistical analysis, geographic information system and interpretation of biological information.

6. Operational requirements

Facility and equipment needs

Equipment needs: proffesional sailors and boats; selected gear, ichthyometer; precision balance; GPS; polyester paper; waterproof clothes.

Cost considerations

Boat renting, fuel, travel costs, expenses, fishing rears.

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<table>
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<tr><td rowspan="3">Depth</td><td>Beginning:</td><td colspan="3" rowspan="3">Observations</td></tr>
<tr><td>End:</td></tr>
<tr><td>Mean:</td></tr>
</table>

Species	Abundance (size)	Total biomass
1		
2		
3		
4		
5		
6		
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Sampling Protocols on Reserve Effects Monitoring

SEAGRASS

1. Background

The most important habitats on soft bottoms are these occupied for seagrass meadows: *Posidonia oceanica* (exclusive of Mediterranean coastal areas), *Cymodocea nodosa, Zoostera nolti, Z. marina* and *Halophila* sp. These communities are considering habitats of community interest for the Habitat Directive. The species are listed as protected in the different international and regional laws related with marine biodiversity conservation and are, consequently, key elements for management.

The structural complexity of meadows represents an excellent indicator to determine the degree of conservation state and the quality of environmental. The natural development of seagrass can be affected for a variety of disturbances of different origins (coastal constructions, sedimentation, contamination, anchoring, etc) that can be easily identified, measured and quantified for standardised and well know methods.

The majority of studies on the structure of seagrass distinguishes between macro and microscale approximation, and are related with the spatial distribution of seagrass. From the macroscale point of view the meadows appear as a heterogeneous habitat with a mosaic of patches, sediment, dead roots, rocks or others vegetated habitats. Inside the meadows patch, the roots growth in different directions and with diverse elongation intensity, and determine the spatial microstructure.

2. Measurable objectives

Coverage is the descriptor of the degree of heterogeneity of meadow (macroscale structure) and is calculated it as the percentage of living meadows occupying a determined area (transects or quadrate).

Shoot density is the amount of shoot in a determined area (microscale structure). Giraud (1977) established a first scale of *Posidonia oceanica* meadow classification depending on the shoot density: very dense (>700 shoots/m^2), dense (400-700 shoots/m^2), sparse (300-400 shoots/m^2), very sparse (150-300 shoots/m^2), semi-meadow (50-150 shoots/m^2) and isolated shoots (< 50 shoots/m^2). For *Cymodocea nodosa* meadows the value of density reported from Canary Islands oscillated between 164-2002 shoots/m^2 and those reported from the Mediterranean Sea oscillated between 757–1925 shoots/m^2. Other interesting descriptors from the microscale point of view are ***average length of leaves*** (distance from the apex of leave to the base) and ***baring shoots*** (distance from sediment to the zone of contact between leave base and rhizome).

Fragmentation is a concept related with the discontinuity of habitat and very applied in the case of seagrass beds. For example, *P. oceanica* in shallow areas use to naturally be eroded by hydrodynamism. *Cymodocea nodosa* is commonly found on shallow bottom, growing on unvegetated areas within *P. oceanica* meadows. Thus, both seagrass meadows and interspersed sandy substrate coexist, this providing a highly fragmented ecosystem in comparison with an extensive *P. oceanica* meadow or unvegetated substrate. Under the impact of disturbances (dumping, coastal construction, etc.) the *Posidonia* meadows boundaries are fragmenting into small patches. Also in case of anchoring impact the meadows can shows a high fragmentation in all the extension.

2. Sampling design

Sampling frequency and replication

Seagrass monitoring to detect the effects of reserve is supported in the sampling designs explain previously, based in BACI design.

The suitable sampling frequency is annual, in both case, for detect changes between different areas and to detect long-term trend. The interannual changes in seagrass are not very important in magnitude, because the growth of seagrass is relatively slow, but the changes between seasons are significant. For this reason is necessary to sample in the same period of year.

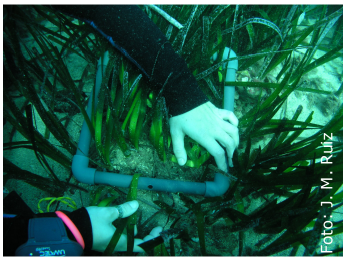
In the case of seagrass is recommended increase the spatial replication, because the descriptors defined before change due to diverse factors at different spatial scales. A hierarchical (nested) sampling design is suggested to estimate this variation. Recommended minimum number and location of sampling sites are: 3 locations (1 location in the reserve area and 2 outside) and 3 randomly selected sites. In each site it is necessary replicate the samples of coverage, as minimum three, randomly located. The shoot density, leaves length and baring rhizome are more variable parameter and precise a replication of each observation least 3 measures at each sampling point of coverage). A second scale of variation of density of seagrass includes the influence of depth and it recommended sample in the meadows boundaries, in shallow and in deep.

Site selection	***Level of change that can be detected***
The sites sampling should not present symptoms of anthropic impacts not controlled. It is recommended select sites with the best quality respect to hydrographical and hydrodynamics conditions, that facilities the sampling work.	This sampling design permit detect changes between locations, with different zoning and uses

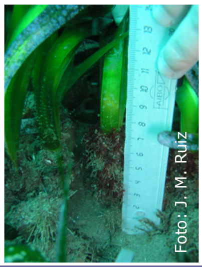
3. Field methods

Details of taking measurements

Coverage can be measured using linear transects randomly located at each sampling point. Surface distance under the transect covered by the seagrass and different algal species is measured to the nearest cm, and percent cover is calculated. A suitable length for these transects would be 10 metres. If the objective of census is also other component of community (fish, invertebrates, etc.) the longitude of transect should be the same, for extract the information of habitat in all the space of census. Another method has been standardised using a transparent PVC sheet (30x30 cm) subdivided into 9 squares (10x10 cm). The observer positioned some metres above the substrate counts the number of squares occupied by seagrass. This method is more appropriated for small seagrass as *Cymodocea nodosa, Zostera* sp. and *Halophila* sp.



Shoot density can be measured by counting the total number of shoots present inside a 400 cm^2 quadrate (20x20 cm). For *Cymodocea nodosa* can use the small squares (10X10 cm) of subdivided quadrate.



Average length of leaves and ***baring shoots*** (distance from sediment to the zone of contact between leave base and rhizome) would to measure with a simple ruler.

Field preparations and equipment setup

The suitable and more precise method of sampling for shoot density is estimate *in situ* by autonomous diving. But another methods, and often combine, are used, involving aerial photographies, video-camera, echosounders and side-scan sonars to map the distribution of seagrasses. These methods are more expensive and precise a more complicate post- processing of samples, but permit processes information on cover in large superficies. To estimated density sometimes utilising stochastic interpolation techniques such as "kriging".

Post-collection processing of samples

Methods based in interpretation of image precise processes post-collection.
Cover and density descriptor *in situ* not required any post-collection processes.

4. Data handling, analysis, and reporting

Database procedures

Metadata procedures in connection with GIS information on the MPA are recommended. The database design would be making for an "expert", for example in *Access* software, which is a tool easily accessibly for data entry, verification, and editing. The database and GIS can have an easy consultation entry programmed for the "expert", that permit produce more accurate data report and maps.

Recommendation on statistical analyses

To detect change changes between different locations or control-impact location is recommended *ANOVA treatments* of data, concretely asymmetric design.

To test the relation between a parameter with other (example: number of divers and number of fish in a diving point), *regressions.*

For long-term trend (e.g., every 5 or 10 years) a simple representation in the time.

Recommended reporting schedule

Recommended report format

The sampling period recommended is in summer season, but not in the tourist season, to facilitate the field works. For example, in Mediterranean areas, in September.	The report would contains all the possible information on methods, atmospheric events, hydrographical and hydrodynamics information and the results of each sampling period, comparing with others years.

5. Personnel requirements and training

Roles, responsibilities and qualifications	***Training procedures***
The requirements for develop this monitoring are professional divers, and technical with experience in interpretation of biological and oceanographical information and statistical processes.	Important: remember the protocol of security in before diving tasks. The responsible of study would remember it, and the procedure of diving permits.

6. Operational requirements

Schedule	***Equipment***
The sampling will be done once per year during summertime. Time effort needed will depend on the level of replication selected, comprising tentatively between 1-2 weeks.	Equipment needs: complete diving equipment; quadrates for density (20x 20 cm or 10 x 10 cm); lineal transect for cover and 2 pick to fix it in the substrate, or a 50x50 square subdivide; something to write, a bag to take all the material. Vessel and skipper, depending of sampling area.

Cost considerations
Travel costs, expenses, air charges, boat renting, samples preservatives.

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Guidelines on suitable experimental designs to test different situations on MPAs

General introduction to detecting ecological impact and monitoring

Needs for manager: field assessment of protection effects.

After the creation of a MPA, managers will require information for understanding the biological and socio-economic process derived from uses restrictions (e.g. stop fishing). The process for obtaining data can be carried out applying very different methods. However, some general assumptions and analytical aspect should be considered for avoiding confused interpretation of results. Generally, studies on the influence of MPA are treated as impact studies. The justification for declaring MPAs are numerous, including arguments that focus on social, economical or biodiversity conservation. The implications for protection vary greatly form criteria such a protected an overexploited assemblage to more complex justifications as conservation of ecosystem heaths and biodiversity. Evidences are required to support the management decision, before or after the creation of a MPA. Therefore, the estimation of temporal and spatial changes on target indicator should be done in the most correct way, for preventing misunderstanding.

Before to start a monitoring program, manager should consider this is the logical structure of the measurement or experimental study. This experimental design will be fully based on the preliminary hypothesis or questions to be answer related with the special conditions of management of certain area, namely considered as MPA, e.g. is the fish abundance of target species increasing related with the various MPA zones and is it consistent among the main habitats? A full description of the objectives, the nature of the experimental units to be employed, the number and kinds of treatments and the response (experimental units or variables) that will be measured should be specify before to start any survey.

The spatial and temporal display of sampling units might be also considered, as well as the use of control location outside the MPA. Some decision should bear in mind about the spatial heterogeneity across the MPA, depth ranges and habitat complexity and heterogeneity. These considerations will be important to attempt the correct sampling design for reducing the sampling error related to spatial variability on population distribution. Additionally, approach from different spatial scales can help to understanding biological processes.

Furthermore temporal variability of variables to be measured requires especial attention from manager. Temporal scales of change (e.g. daily, seasonal, annual or interannual) will affect naturally to populations and will influence to results obtained from a determinate experimental design.

The next sections try to clarify some of the most important aspects to be taken into account by manager during the design of monitoring protocol, evaluation of MPA effect or, even, the design of preliminary studies before to create a MPA.

Hypothesis definition and experimental design.

Before to start any study, the manager should make a prediction deduced from the potential effects of protection on marine systems. This prediction is called research hypothesis. It is important to define a model under which it will be inferred the changes on biological systems. Therefore, hypothesis will be a statement that is tested by investigation, experimentally if possible, in contracts to a model or theory and also in contracts to a postulate, generally the effect of protection at different level or indirect ecological effects derivate from protections. Hypothesis should be able to discompose in several experimental factors. Experimental factor is, therefore, on of the experimental imposition that the manager consider important for its hypothesis. The number and kind of factors to be considered into the experiment should be defined, as well as the number of treatment bellowing to each factor.

After defining a hypothesis, manager should specify a null hypothesis, which includes all possibilities except the prediction in the hypothesis. The final phase in the study will be the experimental test of the hypothesis. If the null hypothesis is rejected, the logical hypothesis, and therefore the model, is supported. If the null hypothesis is not rejected, then it should be retained and the hypothesis and the model from which is derived, are incorrect (Quinn & Keough, 2002).

Comparison of MPA with control(s) location(s) and monitoring programs.

For detecting the changes due to protection, it should be considered necessary the comparison of location within the MPA with other locations with normal uses and no restrictions. Also different management on zones within MPA can be also weigh against external location for this purpose. Therefore in many case the use of external location, considered namely as control are completely necessary.

In the present case, from the experimental design point of view, control will be an untreated treatment (no protected) against which the other treatment (MPA) is to be compared. Controls are required primarily because biological and socio-economic systems either exhibit temporal changes. In many case, inference of protection effects will showed a substantial seasonal change and temporal patterns will mask the real protection effects. The use of several control location will help to obtain correct conclusion. Furthermore, manager will need to use several control locations because the inherent spatial variability of coastal areas. This aspect will be discussed later on.

At other hand, monitoring programs can be considered as a management tool. Monitoring refers to repeated sampling design over time. A more precise definition would be sampling in time with adequate replication to detect variations over a temporal range from short to long time periods, preferably done at more than one location. Seasonal patterns have a great influence for detecting changes in biological variables. For monitoring studies, replication in time is crucial, as well as the use of control locations. Therefore sampling within these time intervals (seasons) is completely necessary.

In some situation, long term monitoring programs, even before the creation of a MPA, can be carried out. In this case, the scale of temporal replication: monthly, seasonal or yearly, should be considered related with the hypothesis and budget.

Field experiments on MPAs.

Research on MPAs about the effects of protection can be carried out by an experimental approach and MPA can offer optimal conditions for developing ecological experiments because the more natural conditions of marine ecosystems protected for human impacts. Hypothesis testing related to protection or other ecological questions can be done by experiments. A definition of experiment could be any logical derived procedure used to test unambiguously a proposed null hypothesis. Normally, the kinds of experiments applied on MPA are mensurative experiments, which test hypotheses about patterns, where the selection of sites is not by random procedures (Hurlbert, 1984). Mensurative experiment involves the making of measurements at one or more points in space or time. In this case, space or time is the experimental factor. For example, the changes on fish abundance populations due to protection may be tested comparing the abundance from several sites, some from protected areas and some from open areas.

Manipulative experiments can be use for identifying causality of protection on ecosystem process. This experiments employ an intrusive approach by manipulating biological systems to test the effects of protection. In this case, experiment involves the imposition by the experimenter of some external factors on experimental units. The impact of human trampling on intertidal habitats has been research by manipulative experiments, where the experimenter imposed several grades of trampling to experimental units, measuring the impact on target variables (e.g. invertebrate abundance or seagrass density).

Cost-Benefit analysis and Power of analysis from pilot studies.

In many cases, economical budget and time use to be limited for MPA managers. Information for a pilot study may help in allocating effort as economically possible. The data obtained from a pilot studies can be used in a cost-benefit analysis where the aim is to maximize precision and minimize the cost (Underwood, 1997). The cost may be expressed in monetary terms or processing time, and decision have to be made on the best way to allocate these cost (Kingsford and Battershill, 1998). Cost-benefit analysis is useful for decisions on precision that are relevant to management hypothesis. This aspect will be strongly link to the power analysis concept.

Statistical power is the probability that a particular experiment will results, after a statistical test, in the rejection of the null hypothesis at a particular level of significance (α) when the null hypothesis is false. From a management point of view, it is very important to calculate a priori the power of an analysis- This aspect involves calculations of a power for a proposed design which is expected to be able to detect a significant results for a given effect size (e.g. differences on fish abundance between different habitats or locations; Kingsford and Battershill, 1998). As a result the use of a pilot study can be crucial for obtain information of effect sizes that are used in the development of a large sampling design. Manager should decide, based on biological and ecological knowledge, the smaller difference that it is wished to detect.

Power will depend on the number of samples (n), the level of statistical significance chosen (α) and the spatial and temporal variability of measured variable. Optimization of replication is appropriate to ensure that the number of replicated per experimental unit and the number of experimental units per treatment are chosen to maximize the power of the test. Therefore the calculation of which is the minimal number of samples for being able to detect the effects of protection may be decisive, but the objectives of sampling program need to be specified for a correct calculation of sample size (Krebs, 1989). For nested designs, it could be also important to consider the replication at different spatial and temporal level for get an optimal power analysis.

Basic knowledge on experimental design

Sampling design and randomization

There are excellent reviews of approaches to sampling design and ecological methodology for carrying out studies on marine ecosystems (Andrew and Mapstone; 1987, Schmitt and Osenberg, 1996; Kingsford and Battershill, 1998). Managers generally use "statistical inference" in decision making (hypothesis contrasting by an experiment) and this is one of the first questions that the managers should decide. There are other approaches (e.g. gradient analysis, spatial statistical analysis such as kriging) but the statistical inference will help to distinguish the natural variability between a group of samples from variability induced by a experimental treatment (effects of protection).

Following Kingsford (1998), several recommendations can be done related to sampling design and replication before to define an experimental design. It is already obvious for managers that any sampling program need replication, but samples should be independent each to other, and therefore, random samples should be taken if you expect to generalize the results (Underwood, 1997). For a determinate budget measured as total number of samples to be taken, it is better if effort is not concentrated within one locality, and generally it is more informative to consider few replicates and more locations rather than opposite but we should be cautious with the minimal number of replicates if small scale variation is high.

The sampling size is also very important to consider. It is often better to have a large number of small sample units than a few large sampling units (Andrew and Mapstone, 1987). It is important to consider the size and patchiness of indicators to be measured and carried out a preliminary sampling to determine relationships between precision and sample unit size and number. Sample size can be, for many cases, achieved from scientific paper related with the study subject or discuss with other colleagues with enough experience.

Other important consideration is to decide the additional factor to be included in the identification of the "protection effect", for example season, depth or habitats. Every level of these factors should be replicated. If it is taken into account several habitats, sampling should be done at several random sites within each habitat and for seasons, several random times within season. Stochastic fluctuations in fish abundance, for example, can be great and happen over a short spatial or temporal scale. Therefore, variation within habitats or seasons is very important. Regular sampling at monthly intervals or repetitive sampling in permanent sites or regular distances can miss real changes in the selected indicator of protection. It could be important, if the data will be analyzed by parametric techniques as analysis of variance, that manager avoid repeated measurements over time on individual organism (e.g. gorgonian growth) or individual plots (e.g. seagrass density). This kind of sampling produce non independent replicates and statistical analysis for generalizing the results is more difficult. However legitimate ecological reasons why repeated sampling of the same sample units should be done can exist (e.g. monitoring of artificial reef modules, recolonization experiments or recovery of damage organisms). In this case, temporal patterns can be distinguish using other techniques as general additive models or use more complex data analysis as repeated measured analysis of variance.

Balance sampling designs are also important. Unequal replication in an otherwise balanced design leads to difficulties in analyses; therefore same number of replicates should be obtained for a determined experimental unit. Also it is important to have a balance sampling at other level of replications, choosing the same number of random times within a season or site within a location.

Types of factors.

A crucial aspect of experimental design is to clarify the kind of factors that it will be included in the study. Factors can be described as fixed or random (Underwood, 1997). Fixed factors indicate that you have randomly allocated sample unit in all of the possible treatments that are available and are relevant to the hypothesis. For example, if season is a worthy factor, the four seasons will be sampled; if habitat is important for manager decisions, the main habitats will be sampled. It is necessary to replicate within a fixed factor because if samples are taken from a single time within season or site within habitat, samples may give not a general interpretation of variability.

Random factor are when the manager choose treatments as representative of a much larger set of possibilities. Random factor are used for enabling to make more general statements about the results derived from an experiment. For example, if manager want to test the spatial variability of fish abundance across seagrass meadow, several random sites will be selected from many potential sites within seagrass; for a determine season, manager samples five random time for determining fish abundance from the total of day that can be possible to sampling.

The factor that the manager will decided to included in the study, fixed and random, will be coupled following the next classification. A deeper explanation of these concepts can be found in Underwood (1997).

Orthogonal sampling designs.

When the manager will like to evaluate the effects of two or more factor simultaneously will be use an orthogonal or factorial experiment. For example for evaluating the effects of protection and habitat on fish abundance, the manager will considered an experiment with these two factors which will be orthogonal between them. For each level of one factor (e.g. habitat A), the experiment will exam the answer of the variable for the total number of treatment of the other factor (e.g. protection and non protection).

The most interesting aspects of orthogonal designs is that the analysis will evaluate the combined effects of the factors by the analysis of the interaction affects. Manager will be able to know if there are different effects of protection on fish abundance depending on habitat type.

Nested sampling designs.

Nested or hierarchical designs is needed for replication of experimental units, which is mandatory to maintain any logical bases for making inferences from experiment (Underwood, 1997). In this case, two factors will be nested when the treatments of the nested factor will have different level or treatment in each of the levels of the main factor. Therefore one of the major uses of nested or hierarchical designs of experiments is to ensure appropriate replication and nested factors will be always a random factor.

For example, if manager will like to assess the effect of protection by comparing fish abundance from a MPA and a control location, several random locations should be randomly selected at each level. Therefore, the factor "location" is nested within "protection". For manipulative experiment it will be also required replication of the experimental plots. In both case, the factor used for replicating the treatments of the main factor to be research is defined as a nested factor.

Nested designs are also useful for analyzing the variation though several spatial or temporal scales. Generally, one of the problems meet at the start of a management program is to know at which scales an indicator of protection (e.g. fish biomass) may be changing. It is easy to understand that different ecological interactions will work at different spatial and temporal scales. Nested sampling designs are one approach to explore. For sample, sea urchin abundance in a MPA will depend on population patchiness distribution and habitat complexity. If this aspects are not well know, a spatial hierarchical design can be applied, sampling at different regions separated 10's of km, different locations separated km's within a region, different sites separated 100's m within a location and taken several samples (quadrats) separated meters within each site.

Partially hierarchical sampling designs.

In most cases, it is common for ecological research to use orthogonal and nested components in an experimental design, which are called partially hierarchical or mixed model. Mangers will be interested on examining the effect of protection, linked to other important factors such as habitat or season. Because it will be necessary to replicate at each level of a orthogonal factor, a nested factor will be need for an adequate replication. For the construction of any experimental design, combining several orthogonal and nested factor, see Underwood (1997).

Different common scenarios, applied experimental design and examples for detecting the effects of protection.

Control-Impact designs: from simple to complex sampling design.

Many studies related with the effects of protection on MPAs are designed as a experiment for detecting environmental impact. Generally impact can be considered as a perturbation causing the alteration of the population of target species or assemblage structure, including physiological variables, size, density, biomass or behavior. In the particular case of a MPA, it is considered that the impact is the perturbation derived from the different ranges of protection, e.g. stop fishing, or a particular management, at a determine location.

The persistence of a measurable impact can divided on two different kinds of perturbations, "pulse" and "press" (Bender et al, 1984). For a MPA, we expect that the impact on target populations will be press impact. A press is a sustained alteration of measured variables, in contrast to pulse perturbation which is a relatively instantaneous alteration, after it the system will return to the previous state. If protection is continuing along time because a correct enforcement, therefore the positive impact should be persistent along time.

At most of the case managers will be considering the evaluation of the effect of a single marine protected area. In most of the case, the hypothesis will be related to the process derived from the protection of a location of restricted dimensions. Therefore it will be impossible to replicate at several impact location, several MPA. In most cases managers should compare the changes on indicators on one MPA with multiple control areas. For the forthcoming data analysis, this type of sampling design is considered asymmetrical because the number of impact sites does not equal of controls sites. It is very important to remark that it is crucial the use of appropriates spatial replication in assessment the environmental impact of MPA. If it is compared the effect derived of protection with a single control area any interpretation of the results is confounded. Differences from MAP and a single control area can not be attributed to the effect of protection and they can be produce for natural differences between these two locations. Therefore it is very important to select several control areas for a correct evaluation of protection effects.

Furthermore, for detecting environmental impact it is necessary to obtain quantitative data before the establishment of a MPA because it is possible to demonstrate that the effects of protection has happen because the changes of management. In other situation, e.g. oil spill accident, it is impossible to anticipate that the perturbation will be take place, but the creation of a MPA use to be planning during a long period of time, allowing to obtain information on indicators before the uses regulation on the are to be declared MPA. Green (1979) coined to term BACI (Before, After, Control, Impact) in reference to sampling design for the detection of environmental impacts where indicator measurement is done both before and after the impact and in both control and impacted area.

Underwood (1991, 1992, 1993) develop the concept to "beyond BACI" designs which make use of multiple control in space and multiple samples in time, both before and after the impact. For a more extensive explanation about the different consideration for an optimal design for detecting human impact on natural ecosystem see these papers. Consequently, to follow the beyond BACI philosophy is the most correct way to interpret the effects of protection. Comparison of a MPA with several controls, before and after the use restrictions, will likely permit to judge the management effect without confusion due to natural temporal and spatial variability of marine systems. If there are several MPA to compare it is possible to use a MBACI (Multiple Before After Control Impact; Keough and Mapstone, 1995).

However, optimal sampling design will depend on budget feasibility of managers or other technical restriction. Therefore, under the real achievability of the beyond BACI or MBACI design, manager should choose the best sampling design for the most correct interpretation of protection effects. The nest section shows several examples of common evaluations of MPA, depending on sampling restrictions.

Asymmetrical sampling design

Manager will compared a MPA with several controls will need to use asymmetrical designs, which can be quite complex to analyse. Some parts of the experiment are unbalanced: one location protected and several no protected. However, any level within a group of treatments will have the same number of replicates per treatment. Analysis of variance of this design is not straight and needs calculations based on the results of more than one analysis of variance (Underwood, 1993).

Different sampling design used for the detection of MPA effects.

- MPA is projected but already without protection
  availability of control
- MPA is already establish
  availability of control
- Interested on spatial process: variability among zonation, habitats and depth

- Interested on temporal process: monitoring programs

- Comparison of protection effects for several MPA.

Summary and general recommendations

Before to start any study, preliminary sampling can be recommended for minimizing logistical and methodological problems that manager can meet in the field and for making best guesses on sample unit size, number, etc (Kingsford and Battershill, 1998). Preliminary sampling can be essential to the success of experiments, estimated the cost-benefit of a particular sampling effort, determine the power analysis and gain understating of the temporal and spatial scales of variability of selected experimental unit.

A correct experimental design must be also define with the aim to obtain correct interpretation about the process related to protection, avoiding pseudoreplication. Pseudoreplication may be defined, in analysis of variance terminology, as the testing for treatment effects with an error term inappropriate to the hypothesis being considered (Hurlbert, 1984). Generally, pseudoreplication is related to a statistical error of using replicates from experiments which violated the principle of interspersion. The basic statistical problem is that in these cases the replicates are not independent, and the first assumption of statistical inferences is violated. Hurlbert (1984; see Krebs, 1996) define several types of pseudoreplication. The most simple and common type occurs when there is only one replicate per treatment or experimental unit. If it is compared the fish abundance from one protected location and one no protected location, the test will answer the specific question of whether this particular locations are different but not to the more general hypothesis of effect of protections. Temporal pseudoreplication is also common in MPA management. In this case, a time series of data is accumulated by successive samples from a single experimental unit, and the samples are not independent samples. A good experimental design will help to reduce the size of the experimental error, avoiding the pseudoreplication, an obtain conclusions about the effects of protection more precisely.

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