INTRODUCTORY GUIDE TO METHODS FOR SELECTED ECOLOGICAL STUDIES IN MARINE RESERVES

MAST - ECOMARE

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FOREWORD

The present Guide was prepared in the framework of the ECOMARE project, a concerted action of the European Community Marine Science and Technology Programme (MAST III) concerned with the ecological effects of Marine Protected Areas (MPAs) in the Mediterranean.

The overall aim of ECOMARE was to unite and coordinate the efforts of a broad group of research teams involved in assessing the effects of protection (cessation of fishing) in Mediterranean littoral ecosystems. The specific objectives of this project were:

- To establish the state of knowledge of the responses of marine communities to protective measures in Mediterranean littoral ecosystems;
- To identify the main research needs and steps forward to progress from the assessment of effects on exploited populations to the assessment of effects at the ecosystem level;
- To review and recommend working methodologies so that future investigations could be carried out in a coordinated and comparable manner.

The second objective lead to the identification of three priority areas for future research in Mediterranean marine reserves: assessing biomass export from MPAs, assessing impacts of human activities in MPAs, and studying indirect effects of protection (e.g., fish-urchin-algae interactions). The third objective lead to the preparation of the present guide on recommended methods for future research, with chapters addressing each of these priorities.

MPAs are gaining increased acceptance as practical means to preserve marine biodiversity and to manage coastal fisheries. Thus, it seemed useful for managers as well as for scientists to gather in one volume the main methods available to study the effects of protection on a variety of ecological processes. The aim of this guide is not to describe each method in depth, but rather to present them briefly, with their advantages and limitations, and to provide references for further reading.

We hope this guide will be a useful aid for future studies in Mediterranean Marine Protected Areas.

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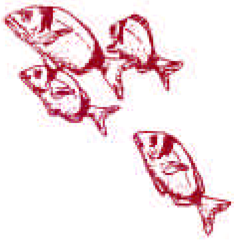
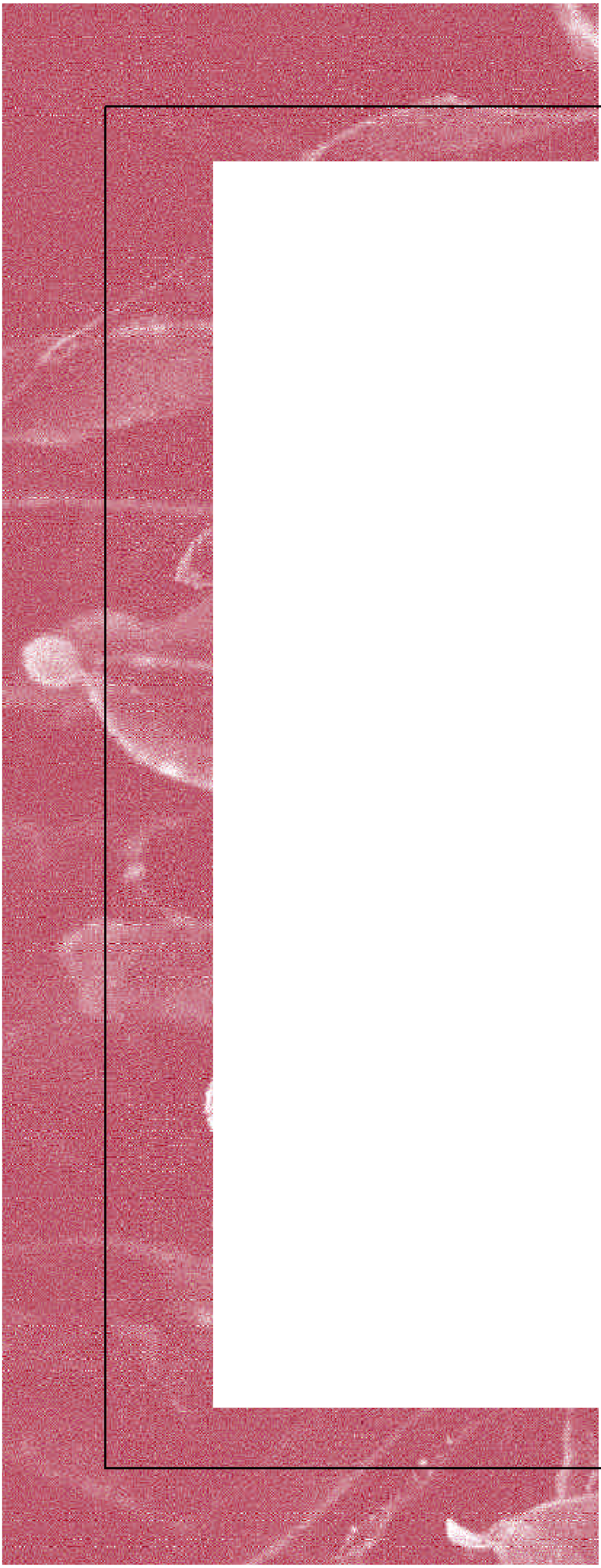


Chapter 1.

Methods for Assessing Biomass Export from Marine Protected Areas



INTRODUCTION

One of the main expected benefits of protecting species in marine reserves is the emigration of individuals to surrounding non-protected areas. This process could be the net result of random movements of individuals from marine reserves where densities of protected species are higher. Alternatively, emigration from marine reserves could occur by density-dependent movements of competitively subordinate individuals from preferred habitats in protected areas to suboptimal habitats outside. Protected areas are also expected to act as sources of eggs and larvae for downstream habitats.

Despite the relevance of both biomass export and recruitment enhancement in the context of marine protected areas, little work has been done to assess these processes in the Mediterranean Sea or in other marine regions. Due to the lack of suitable methods to empirically test biomass or recruitment export hypotheses, most research efforts in this direction have been on model protected areas. In this chapter a collection of methods applicable to studies of larval, juvenile and adult export from closed areas are presented and evaluated in summary form. The chapter is intended to serve as an introductory guide for researchers interested in studying the effectiveness of marine protected areas in their regions.

1.1. ACOUSTIC TRACKING METHODS

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1.1.1. Introduction and objectives

In recent years, the most significant advances in tagging have come from the development of electronic tags. This technology and its possible evolution (especially with miniaturisation) are a constant source of new applications. The full potential of this method has still to be explored. Even at this early stage of development, compared with conventional tags, the results obtained so far are crucial for fisheries and ecology. This method can provide details of fishes' immediate environment in real time and over a long period, thus allowing study of the factors that most influence their subsequent behaviour.

Electronic tags have been essentially developed with fishes that have a high capacity for dispersal. They include salmon, tuna, cod, plaice, and turbot. Others studies concern crustaceans (lobsters, crabs) and molluscs (cephalopods) (Maynard and Webber, 1987; O'Dor *et al.*, 1995; Freire and Gonzáles-Gurriran, 1998). Three types of electronic tags are used:

Transponder Tags: Transponding tags transmit an acoustic signal when they receive an interrogation pulse from sonar. Sonar Transponding Tags allows the position of a free-ranging fish to be fixed accurately in its relation to a research vessel. The range of reception is between 400 and 1 000m. These tags are currently used to follow large pelagic fish.

Transmitter Tags: These tags send a signal (pulsed or coded) to a receiver which identifies the individual fish. These tags are larger than transponder tags because they require an internal battery to power the transmitter. The lifetime of the tag is a critical consideration in telemetry studies and depends on the trade-off between transmitter size, power supply, range and rate of the signal. Telemetry studies on free swimming fish are generally short-term studies ranging over periods of hours to a month. New sensors can record depth, swimming direction and speed or even heart rate. The detection range of some tags can be up to a kilometre in some instances but is generally less than 100m. Attachment of the tag is internal or external. *Acoustic Pulsed Tags* transmit a simple pulsed signal at a selected pulse rate. Large numbers of fish can be monitored simultaneously using different pulse rates or multiple frequency receivers. In practice, five individual fishes can be surveyed on only one frequency. *Acoustic Coded Tags* operate by emitting a digitally encoded signal on specific acoustic frequencies. Each signal is unique which offers the advantage that many individual fish can be tracked separately on a single frequency. These tags have great potential for increasing data acquisition rates and increasing sample size (Voegeli *et al*., 1998).

Data storage Tags (DST): Also known as archival tags, these tags have ranged from simple data loggers, capable merely of recording depth, temperature, salinity, light, *etc.* of an individual fish to sophisticated programmable devices capable of providing a direct estimate of geographical position at regular intervals over periods of many months. These are the tags specialised for free-ranging fish in the open sea at large spatial scale (100km). Some tags can record data for up to five years and store this information for up to twenty years. However, in order to retrieve the information the tags must be recovered from the fish. An intensive recapture operation is necessary.

In this survey we review the application of electronic tags in relation to studies of biomass export from Marine Protected Areas (MPAs), on the basis of the results of the project (FAIR. CT.96.1394, CATAG) "Improvements of Tagging Methods for Stock Assessment and Research in Fisheries".

Electronic tags are particularly well-suited to studies on the problem of biomass export from MPAs' for several reasons:
- the survey is in real time and particularly precise in time (minutes to days or months) and space (10m to 1km)
- the surface of MPAs' can be totally covered with a few buoys because MPAs' are usually small
- the technological advances make it possible to monitor numerous fish (same species or various species)

Thanks to these technological advances, this method has become a powerful tool to study biomass export in relation to the behaviour and migrations of fishes in MPAs'.

Behaviour: The propensity of fish for erratic movement is a potential source of exportation from MPAs. The study of the home range for resident fish allows the occurrence of to be determined. This information can be obtained by the presence/absence of signal tags in the zone. The percentage of absence informs on the potential exportation outside the MPAs. The diurnal and nocturnal monitoring of movements can inform on the risk of capture outside the MPAs on the basis of fishing pressure over 24 hours. The total disappearance of signals can be interpreted as actual exportation, either by migration to the fishing zone, or by capture by fishermen.
Migration (seasonal, reproduction): Some species have the ability to move long distances. The use of directional hydrophones around the zone at certain periods of the year can inform on the presence of signal tags in adjacent zones. Moreover, the information of presence/absence at certain periods (*e.g.* reproduction period) can inform on the intensity of simultaneous individual migration.

1.1.2. Recommended methods

The advantage of electronic tags in comparison to conventional tags is that during the period of immersion, electronic tags actively give the position of the fish and to store information concerning the environment (depth, temperature, etc). In marine water, we have to use acoustic signals (as opposed to radio signals) because of problems of signal diffusion. The data available for each tag have the same architecture: for each received signal, the receiver registers the date and the location. The new approach uses integrating GIS in spatial analysis (Anderson and Dixon, 1997; Weigle and Flamm, 1997).

There are several approaches: three methods for spatial location and one method for environmental survey.

Spatial location by triangulation

This method is based on an underwater acoustic positioning system. A system of a minimum of three buoys is used to calculate by triangulation the position of a subsurface electronic tag. Each buoy contains a hydrophone, an ultrasonic receiver and a radio link. They send in real time a signal to a base station made of a computer which plots the tag in a 2D graphic and using special software (O'Dor *et al.*, 1998). Succession of positions through time gives the movement of the fish. The distance between buoys is about 100m limiting the covered surface expected unless the number of buoys is increased. If the survey concerns several individuals, it becomes difficult to visualise the results of all tags on the same map. It would be interesting to work in spatial density. The map is divided into regular grid cells (size depending of the scale the study) where the number of signals detected is calculated (Cote *et al.*, 1998).

Spatial location by presence/absence signal

For a large spatial scale survey, submerged hydrophones including receiver and data logging memory are available. Data are collected and stored in a computer in the field in a buoy in the water. In this case, hydrophones are omnidirectional giving information on presence/absence of the tag in the circle of reception (variable according to the power of the tag). The position is less accurate and depends on the relative position of each buoy. At large spatial scale, a large zone (*e.g.* 1-km) can be covered at a low cost by using a large number of hydrophones.

Spatial location by mobile hydrophone

It is also possible to fix a directional hydrophone on a boat equipped with a GPS (Holland *et al.*, 1992, 1993, and 1996). This use of a directional hydrophone means that a tag can be monitored outside of the study zone and fishes can be recaptured .

Environmental survey

The environmental survey method is based on the storage of environmental information on the area where the fish lives (temperature, luminosity, depth, *etc.*). The geographical position at the different times when information is stored can be indicated. This technique is particularly well-suited to offshore surveys specific to certain fishes (*e.g.* tuna, cod). In this case, there is no a real time survey and a recapture operation is necessary for the interpretation of data. The results are represented as graphs showing the patterns of change of the studied parameters (Sturlaugsson, 1995; Thorsteinsson, 1995).

1.1.3. Problems and limitations

Financial aspect

The most important limiting factor is the cost of these technologies. The miniaturisation of electronic tags makes it possible to monitor smaller fish but the consequence is an increase in price. The equipment necessary to monitor fish in real time is also expensive. If the exact position of fish is not indispensable, underwater hydrophones are preferable because they are cheaper.

Time consuming

Spatial location by mobile hydrophone is the most time consuming method. As it is possible to monitor only one individual during a survey, monitoring several individuals requires a lot of ship-time.

The use of submerged hydrophones with data logging memory (spatial location presence /absence) requires human intervention to collect data directly from the field. If the number of hydrophones is important or if sea conditions are not good, the work time may be increased.

Loss of signal

The position of buoys is critical for the interpretation of the signal sent by the fish. They have to cover the zone in its entirety. The signals can be lost when fishes shelter in holes. If the signal is lost inside the area, this absence of signal will be interpreted as a migration outside, thus as export. Even with good positioning, areas may include a non-reception zone. The mobile hydrophone is generally attached to a boat to track in real time the position of fish located with a GPS. The distance of signal reception, which depends on the characteristics of each type of tag, limits their utilisation.

Fish mortality and loss of tag

Individuals must be caught with care to limit mortality. Whatever the capture techniques used (trawls, seines, *etc.*), there is always the risk of skin damage and scale loss. Tags can be fixed externally (in a variety of locations) or internally by insertion in the stomach or by surgical implantation (muscle or peritoneum). There are advantages and disadvantages in each of these methods and the choice depends on the type of tag, the type of fish and its lifestyle and the purpose of the research. If the use of internal tags limits tag loss, it may produce a signal attenuation. External tags have the advantage of being simpler and quicker to apply than internal tags. Each manufacturer offers recommendations about fixation in the two cases.

Tag recapture

In the case of Data Storage Tags, as well as for other electronic tags, recapture must be maximised because of the cost of the tag. A recapture campaign must be accompanied by good advertising of the tagging programme in the appropriate zone by means of local newspapers, posters, public presentations, interviews, *etc.*

For the transmitter tags, even if it is possible to know the exact position of a fish, rapid recapture is difficult. One way is to use diving with a special hydrophone to locate the fish precisely. Other methods of recapture are time-consuming (nets, fishermen).

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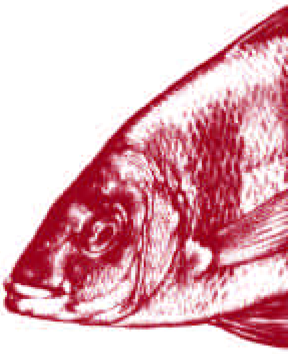

1.2. LARVAL OTOLITH METHODS

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1.2.1. Introduction and objectives

The discovery of daily increments on the otoliths of fishes by Panella (1971) introduced a powerful new method for studying the age and growth of fishes. Daily increments are often particularly clear on the otoliths of larval and juvenile fishes, making this technique a valuable tool for early life history studies. While the general methodology has become well-established (Campana and Neilson, 1985), many promising applications have yet to be explored.

Otoliths are accretions of calcium carbonate within the semicircular canals of the bony fishes. The production of visible daily increments depends on a daily cycle of differing rates of accretion of a protein matrix (darker under transmitted light microscopy) and crystalline inclusions (lighter). Among the three pairs of otoliths, the largest pair, the sagittae, and the next largest, the lapilli, are used for ageing.

The most basic information available from the otolith is the total number of daily increments for a fish of a given size. A more commonly used application of this technique is the estimation of the duration of the planktonic larval phase. The presence of a settlement mark allows one to easily calculate the date of settlement, simply by subtracting the number of daily increments between the mark and the edge of the otolith from the date of capture. This technique has been used to reconstruct the daily pattern of settlement. Comparisons of reconstructed settlement patterns have been used to measure the persistence of age cohorts over a season, to assess the effect of recruitment on subsequent population sizes, and to infer the patch size of planktonic fish larvae by estimating the spatial scale of settlement events. Similarly, the daily pattern of spawning can be back calculated by subtracting total age from the collection date. Of course, this would only reflect the ultimately successful spawning patterns, which may well have little correspondence to true spawning patterns.

In this section we explore the application of larvae otolith methods in relation to the study of larval export from MPAs.

1.2.2. Recommended methods

Otolith tagging

The only way to estimate larval dispersal unequivocally is to mark larvae so that they can be traced from their origin to their ultimate destination. Jones *et al.* (1999) developed a technique of marking the otoliths of developing embryos of some small coral reef fish. It allows them to be marked en masse in their natural habitat, and to hatch and disperse with minimal interference. The tagging method adapted the use of the fluorescent compound tetracycline, which had been widely used to mark otoliths in fishes and validate age rings, and more recently to mark the hatchery-reared larvae of freshwater and estuarine fishes (Secor *et al.*, 1991; Tsukamoto, 1998; Reinert *et al.*, 1998). Experimental works have shown that immersion of embryos in tetracycline resulted in clear fluorescent marks at all stages of development. Tetracycline produced a signature core of yellow fluorescence that persists through larval development and metamorphosis. Moreover, such treatment does not induce significant reduction in survivorship.

With this approach, it is technically possible to tag embryos and to recover the tag in the new recruits. In this way Jones *et al.* (1999) were able to demonstrate that coral reef fish larvae are capable of returning to their natal reef. They also showed that some tagged individuals were recovered in adjacent reefs demonstrating effective dispersal from reef to reef.

In addition to this technique, recent unpublished experimental works have showed that injection of tetracycline into gravid females leads to tagged embryos. This approach could be promising for species releasing pelagic eggs (most commercial species). Given the recent development of tagging techniques on otoliths, this method could be useful to demonstrate export of eggs and larvae from marine protected area by tagging populations inside MPAs.

Otolith microchemistry

Since daily increments are preserved unmodified within the otolith, it is possible to derive information about past environmental conditions. To date, it has proven feasible to infer past temperature exposure by analysis of stable isotope compositions, such as oxygen and carbon isotopes, or by strontium-to-calcium ratios (Radtke *et al.*, 1987). These techniques may make it possible to discover where in the plankton the larvae are travelling at various ages or from what oceanic region larvae are derived. Analyses of the elemental composition of otolith sections may be particularly valuable if they could identify the source population of larval recruits (Campana, 1999).

Otholiths may thus be used as recorders of growth history and trace-element environmental signatures. Because they are formed at birth, new layers of calcium carbonate are deposited daily as discernible growth bands and elements from the surrounding sea water can become permanently incorporated into otoliths (Mugiya *et al.*, 1991). In addition otolith trace-element signatures have recently been used to identify stocks (Campana *et al.*, 1995) and nursery habitats (Gillanders and Kingsford, 1996; Thorrold *et al.*, 1997) for several temperate and tropical species (Swearer *et al.*, 1999).

1.2.3. Problems and limitations

As pointed out recently by Thresher (1999) in a review, analysis of otolith microchemistry requires validation to determine environmental vs. genetic component of the variation in the trace-element composition of otoliths. In fact recent validation experiments have shown that variation in otolith microchemistry could appear between individuals issued from different areas but reared in the same conditions. In that case, the elements involved must be excluded from any analysis because they are partly inherited.

One basic problem of otolith studies is whether the observed increments on the otolith are in fact formed daily. Despite widespread use of the adjective "daily," there are few direct demonstrations of daily production of increments for reef fishes. Since Geffen (1982) first documented that increments were not always produced daily on the otoliths of larval herring, there have been a number of reports of increments being formed at a rate of less than one per day (Campana *et al.*, 1987). There often appear to be additional arrays of "subdaily" increments within the larval period. The higher-order array (with fewer increments) is usually visible over more quadrants of the otolith and at wider planes of focus than the finer array of "subincrements," which tend to occur over a smaller section of the otolith and at a single plane of focus (Victor, 1986). Clearly, the presence of narrow increments requires thorough validation of their periodicity. This is a difficult experiment to perform in the field, especially given the necessity that the larvae be permitted normal planktonic behaviour and migration. Marking individuals within a very large enclosure drifting with the plankton is probably the only practical method possible at present.

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1.3. GENETIC STUDIES

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1.3.1. Introduction and objectives

Population genetics can contribute to the study of dispersal of larvae and migration capabilities of adults, because the use of intra-specific markers, such as allozymes or DNA haplotypes, offers a new opportunity to distinguish populations of different spatial scale. Detectable allele frequencies can be used to estimate levels of migration if it can be assumed that these frequencies reflect a balance between the opposing forces of migration (gene flow) and random divergence of allele frequencies (genetic drift). While this theory is silent about allopatric populations with identical genetic profiles (Shaklee *et al.*, 1982), differentiation provides unequivocal evidence of reproductive isolation. The main difficulty with this approach is that other forces, notably natural selection and historical contact, can influence allele frequencies, and the relative importance of these forces in natural populations has proved extremely difficult to evaluate directly.

One effective migrant exchange between subpopulations per generation is sufficient to stop fixation of alleles (Allendorf and Phelps, 1981). Such a model indicates that gene flow is a powerful means of homogenising subpopulations even with a small number of migrants. Any significant divergence between allelic and/or genotypic frequencies of different samples indicates at least some restriction of gene flow, provided that genes are not subject to local selection.

1.3.2. Recommended methods

The recent development of molecular techniques offers a palette of technical approaches for population biologists interested in a wide range of questions. For example, these tools can be used to determine individual reproductive success or to measure rates of genetic divergence among populations. Which technique is the most appropriate for a particular question depends upon the extent of genetic polymorphism required to best answer the question, the analytical or statistical approaches available for the technique, and the pragmatics of time and cost of materials. Empirical studies that employ allozyme polymorphism as genetic marker have contributed a great deal to our understanding of population processes (Smith *et al.*, 1990). The hope of obtaining highly informative genetic markers for tracking individuals and/or their genes under field conditions had led many population biologists to consider switching to DNA-based techniques. The actual rate at which new molecular techniques are being developed far outstrips their efficient and reasoned incorporation into studies of population ecology (Parker *et al.*, 1998). This had led to confusion among population biologists about various new techniques, often causing people to prematurely abandon standard techniques (such as allozymes electrophoresis) that provide readily interpretable data. The expectation of exploring variation in DNA sequences needs to be tempered while considering the drawbacks associated with different techniques and the types of data obtained from them.

We should recall here that the choice of marker also has implications on evolutionary processes that will be investigated. Choosing markers under genetic selection such as allozymes or cytochrome b gene of mtDNA may provide different results than choosing neutral markers such as microsatellites. Differences in results may not only be a consequence of the higher variability investigated but more likely related to the fact we are looking at different evolutionary processes (selection *vs*. genetic drift). This fact has often been omitted in previous studies looking at allozymes and assuming that they are neutral. It is necessary to demonstrate the neutrality of markers.

When it comes to choosing a technique, there are many currently available: protein electrophoresis, nuclear and mitocondrial RFLPs (restriction fragment length polymorphism), minisatellite and microsatellite VNTRs (variable number tandem repeats), RAPDs (random amplified polymorph DNA), and DNA sequencing (techniques are described in Parker *et al.*, 1998). This section presents details and advantages of techniques already applied to describe larval dispersal.

Gel electrophoresis is a powerful yet relatively simple technique that separates proteins in an electrical field according to their net charge and size. The key to the electrophoretic method is that a single gene encodes each specific protein, and differences in proteins give an indirect estimate of differences in the sequence of the DNA. Because some changes in the DNA sequence do not result in change of charge and/or size of protein, it has been estimated that electrophoretic techniques detect only about one-third of base substitutions in DNA (Lewontin, 1972). Moreover, only about 10% of the total genome will encode proteins. Consequently, the variations detected at protein gene loci may not be representative of the genome as a whole and some species may lack allozyme polymorphism. A further limitation of using allozymes rather than non-coding DNA as genetic markers to separate populations is that allozymes may differ in metabolic function. Many statistical models in population genetics assume that phenotypic differences among allozymes are minimal and selectively neutral, but exceptions are known even in marine fish where selection can maintain genetic variation itself (DiMichele and Power, 1982). Selection must be considered a possibility when differentiation originates from a single locus. However, most multilocus differentiation patterns are probably robust with regard to concerns about on-going selection. There is substantial evidence that the majority of observed variation in enzymes is neutral (Kimura, 1983). In spite of these limitations, protein electrophoresis remains a powerful technique to exhibit genetic variation (Smith *et al.*, 1990).

RFLSP techniques use restriction enzymes to cut the DNA at a specific nucleotide sequence recognition site, and generate sets of DNA fragments that differ in size when mutations have created or destroyed restriction sites. The most extensive use of restriction enzymes in population studies has been for the survey of allelic diversity and population differentiation in mitocondrial DNA. Enzymatic digestion of a small mtDNA molecule results in a number of DNA fragments that can be resolved and identified on agarose gel. Allele frequencies are estimated from absence/presence of the restriction site among individuals and several

(>100) restriction enzymes are commercially available. Larger molecules such as nuclear DNA cannot be analysed following this protocol because they produce too many segments to be separated on gels. The small size of the mitocondrial genome, its predominately maternal inheritance, and its relatively high rate of base-pair substitutions (especially in the control region) make it a valuable tool for studying relationships among populations (Avise *et al.*, 1987).

The most detailed analysis of DNA differentiation can be obtained by sequencing the region of interest from different individuals. The most difficult problem for analysing genetic markers lies in the selection of the region of the genome that both reveals allelic variation and can be sequenced efficiently. Some genes, such as cytochrome b in the mtDNA, have been time-calibrated and allow accurate estimates of time divergence between populations. Analysis of sequence differences is based on mutation of nucleotides within a position. Thus it only looks at mutation rate and does not take into account genetic drift, mostly affected by effective population size. Consequently sequence analysis often shows lower levels of differentiation between populations than other methods.

Among other available techniques, the microsatelite VNTRs seem certainly the most promising approach (O'Connel and Wright, 1997) because: (1) they provide information for individual loci and therefore are amenable to calculations of allele frequencies necessary for population studies; (2) they typically exhibit high levels of gene diversity and show high level of polymorphism; and (3) once primers have been developed, the protocol consists only of PCR (Polymerase Chain Reaction) and electrophoresis. Some new technical advances are even starting to eliminate the electrophoresis step by looking at fluorescence or weight analysis of microsatelites. Such approaches may get around slippage during PCR process that can make individual alleles difficult to identify among false bands. In terms of analysis, interpretation of variation of allelic frequencies remains problematic. Measures of population subdivision based on micosatellite allele frequencies have been highly conflicting because Fst estimates are based on a model with low mutation rates; a postulate that is reasonable for enzymes but not for microsatellites. New models have now been proposed to estimate differences (Slatkin, 1995) and distances (Goldstein *et al.*, 1995) using microsatellites. However, the fundamental question remains open because it still is not clear whether all mutations at microsatellite loci involve changes of only one or two repeats as assumed in many models (Valdes *et al.*, 1993) or whether mutations of larger effect occur occasionally (Di Rienzo *et al.*, 1994). Despite some estimates using genetic divergence from microsatellites to estimate coalescence times (Slatkin, 1995), this statistic must be interpreted with caution. Technically, microsatelite analysis requires primers to be set up for each locus. This is a time-consuming but necessary step. Because of this technical step that is unique for each species, microsatelite VNTPs can only be viewed as markers for study projects involving few species (1 to 3) and numerous samples. At present, microsatellites are only used for commercial species that require large sample size analyses and long-term projects focused on one species. This is the case for salmon (Tessier *et al.*, 1995), cod (Bentzen *et al.*, 1996), and herring (O'Connel *et al.*, 1998). In these cases, it will save time in processing all individuals compared to other DNA approaches, and it is as easy to use as allozymes, despite the time needed to set-up primers for each locus. Among other possible developments, the microsatellite approach allows surveys of oceanic larvae (Ruzzante *et al.*, 1996), which may identify the origin of larvae caught directly in the ocean.

1.3.3. Problems and limitations

When used in marine reserve biomass export studies, population genetics can provide some useful information but it requires some prior explanation. Export from a marine reserve may concern eggs and larvae drifting in the water column or juveniles and adults migrating outside protected area habitats. As we mentioned earlier, the fact that a small amount of gene flow is sufficient to homogenise populations limits the power of genetic markers. This makes it difficult to differentiate populations that have essentially separate dynamics but are linked by trivial amounts of gene flow, from populations that exchange numerous migrants and are effectively a single stock.

In this context, permanent export of individuals (larvae and/or adults) will be impossible to detect because it will result in homogenisation of outside and inside marine protected area populations. However, a recent and pioneer work was able to detect variation among larval pools in the ocean (Ruzzante *et al.*, 1996). Such variations were based on microsatellite analysis and suggest that each spawn results from limited effective population size that induces genetic drift. Such variation is detectable using molecular surveys and may be monitored over time to deduct dispersal patterns. The results of Ruzzante *et al.* (1996) are unique because they were linked with a detailed oceanographic sampling protocol. Genetic markers cannot inform on dispersal alone and a combination of currents and genetic studies can provide such results, when linked with an appropriate sampling protocol. Finally, this approach leads to identification of a larval pool from their gene frequencies and its follow-up in the ocean.

The main constraint in any genetic survey is to be able to identify individuals issued from the marine protected area. In this context, one alternative would be the release of genetically tagged individuals. Artificial genetic tags consisting in the introduction of a small sequence inside the genome of the targeted species is ecologically dangerous. However, one could select individuals showing a rare allele for some microsatellite marker and release eggs, larvae and juveniles in the marine protected area. Analysis of adjacent new recruits, juveniles and adults over the first three years compared to a sample from the marine protected area will inform on the dispersal from the site where they were released and consequently on the export from marine protected area.

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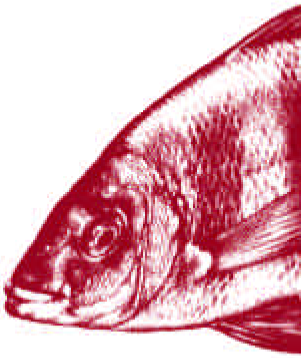

1.4. ICHTHYOPLANKTON SAMPLING

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1.4.1. Introduction

An ichthyoplankton survey may help to assess fish biomass export from MPAs by monitoring the distribution and abundance of planktonic stages (eggs and larvae) inside and outside the protected area. The distribution of abundance of the different stages aims to detect the direction and intensity of the flux and the presence or absence of a density gradient (Gulland, 1973; Ahlström and Moser, 1980).
A spatial and/or a temporal sampling may be conducted inside and outside marine protected areas:
- to evaluate the quantity of fish larvae and to assess spatial variations in larval abundance, inside and outside the protected area or according to different aspects of the shore such as embayments, cliffs, *etc.*, or hydrological particularities;
- to estimate temporal variations of abundance of the different species at different scales (day, month, season, year).

The best evaluation of the abundance of surviving large larvae will not necessarily predict, in a correlative sense, the level of recruitment. The composition of the ichthyoplankton assemblage does not necessarily reflect the composition of the adult fish fauna. As habitats and behaviours of young and adults differ, ichthyoplankton surveys must sample larvae around (offshore areas) and within (inshore habitats) MPAs in order to obtain a complete picture (Leis and Miller, 1976). Exportation is a dynamic phenomenon, and the displacement of larvae may be studied by monitoring the larval transport or by studying larvae passing in a short window (one point) or several windows between the starting and the arrival points (Fortier and Leggett, 1985). The questions we have to answer are : what? how many? when? how?

1.4.2. Methodology

Sampling design

The sampling design ought to provide measures of spatial and temporal variability, within acceptable confidence limits in order to estimate changes in abundance levels of larvae and juveniles. A systematic grid of stations inside and outside the protected area is recommended for multispecies ichthyoplankton sampling. Within this scale, time-series surveys (weekly, bimonthly, monthly) or studies of the recruitment process are nested for target species on a finer horizontal and vertical scale, which aim at discovering the processes controlling annual recruitment success of new year classes (Sherman *et al.*, 1983).

The sampling strategy includes: collection of larvae, sorting, identifying and measuring them, and analysis of the data. The choice of species studied will determine specific sampling requirements. A space-extensive survey might be required to test the null hypothesis about larval transport. It probably will be preferred to a time intensive survey, which would be required to test the null hypothesis about mortality/critical period. In any case, without previous data on recruitment or spawning areas, a first cruise is recommended in order to obtain samples from the different areas (pre-sampling) or at different periods (nycthemeral variations included) as a basis for developing a suitable sampling strategy.

Gear

In order to conduct qualitative and quantitative surveys of ichthyoplankton around MPAs suitable techniques include towed net sampling and bottom fixed nets or traps.

For net sampling, the techniques have been described from Pacific and Atlantic surveys (particularly on pelagic species like pilchard) which generated a lot of publications and surveys of methods during the years 1960 and 1970 describing and comparing gear and their relative efficiency (Kramer *et al.*, 1972; Hempel, 1973) and in tropical waters namely by Leis, Smith and Kingsford. Recommended procedures for field operations as well as laboratory procedures are available in publications of international institutions (UNESCO, 1968, 1975, Smith and Richardson, 1977; FAO, 1967, 1974) or Wiebe *et al.* (1985) and Brander and Thompson (1989) in which sampling methods and theoretical considerations in terms of sampling strategy and data analysis are usefully detailed.

Various types of nets (with different meshes, length and opening surfaces) are used to collect ichthyoplankton. None will be specially recommended here, except to use a standard gear for comparison purposes (Bongo net, Longhurst-Hardy Plankton Recorder (LHPR), MOCNESS, Isaacs-Kidd, WP2 or Indian Ocean Standard net (IOSN). High speed towed nets (HSTN) do not seem to be suited for sampling near the shore and plankton pumps seem to be better suited for extensive surveys (Taggart and Leggett, 1984; Harris *et al.*, 1986). Opening and closing nets are recommended to study vertical distribution or to sample horizontally at different levels from the bottom to the surface, if the distribution of target species is already known. Such samples are usefully coupled with salinity and temperature profiles. If the vertical distribution is not known, vertical or oblique hauls are preferred in order to census all larvae present in the water column. A mesh of 300µ, 500µ is recommended to study the first stages, a mesh of 1 mm would be sufficient to study older larvae and postlarvae. Different types of collectors are used, and the usual equipment required in classical plankton studies: sieves, funnels, bowls, tweazers, pipettes, pill boxes, *etc.*

The plankton nets may be towed by a ship (active sampling). They may also be attached to the bow of a moored boat or fixed to the bottom (passive sampling). In any case, a measure of the filtered volume is necessary to compare the results (calculations of densities per volume): a flowmeter is installed in the middle of the net mouth. A time-depth recorder gives the depth of sampling and a depressor contributes to the stability of the gear. The duration of sampling may last from a few minutes to several hours, depending on the flux or the boat speed.

Other types of gear are especially well-suited to near-bottom sampling such as sleds, equipped with depth sounder, in order to know the distance of the gear from the bottom, or beam-trawls that are suitable for for settler sampling. Trawl surveys employing net systems, and in some areas acoustic signals and net systems (for juveniles of demersal and pelagic species) are more selective samplers. Near the shore, purse seines are used to capture pelagic juveniles (Murphy and Clutter, 1972). Qualitative samples may be usefully taken by small hand nets or push nets along the shore. To avoid disturbance to the seagrass and bottom in shallow waters a bow-mounted pushnet may be deployed from an outboard skiff (Olney and Boehlert, 1988). In addition to planktonic samples, semi-quantitative data on settling larvae may be obtained by trap sampling (buckets placed in the sediment for example) or light-traps (Doherty, 1987). The studies conducted on reef fishes in tropical waters give methods which might be adapted for Mediterranean shallow bottoms among them traps and visual observations by SCUBA diving (Leis, 1986; Boehlert, 1987).

Conservation, sorting and identification of fish eggs and larvae

Some recommendations are useful for conservation and fixation of eggs and larvae (Smith and Richardson, 1977). Generally a 3% to 5% neutralised phosphate-buffered formaldehyde solution is recommended but ethanol is preferable in the case of further otolithometric studies (Ahlström, 1976). After determination of the total volume of larvae sampled by displacement, fish larvae have to be sorted. Fractionating must be limited to samples containing exceptionally large numbers of eggs and/or larvae. The time needed to sort samples has to be previously correctly estimated; it is frequently underestimated.

The number of larvae is expressed as number of individuals per 10 m3 of water filtered or 10 m² of sea surface. Larvae are measured and identified to the lowest possible taxon, developmental stages of eggs and larvae may be specified. Larval identification is mainly based on morphological characteristics and pigmentation patterns. The morphology of young fishes may in fact be really different according to the different stages. Unfortunately, there is a lack of identification keys for larval stages and a lot of identification criteria are based on researchers' experience and are not available in the international literature.

The main guides which may be used to sort Mediterranean fish larvae are issued from Atlantic and Mediterranean studies. The reference for the north-western Mediterranean ichthyoplankton is the monograph n°38, known as " Fauna Flora Golfo Napoli " (D'Ancona *et al.*, 1931-1956). The identification sheets of CIEM are also useful. Some books on Atlantic species may be used, such as Ehrenbaum (1905-1909) and Russell (1976). Probably most of the other descriptions of fish larvae and juveniles have been published in local revues, CIESM records and academic works, and need to be collected to aid identification of Mediterranean fish larvae. Some publications are frequently cited and used: Aboussouan (1964), Arbault and Lacroix-Boutin (1969), Lee (1966), Marinaro (1971), Ré (1979), Sabatés (1990).

Other data

Density independent factors and effects of environmental factors have been demonstrated to be of prime importance for the survival of fish larvae (Lasker, 1984). Zoo- or phytoplankton sampling, meteorological conditions, hydrological or other abiotic factors have to be recorded and will contribute to the understanding of the distribution and variability of fish larvae. The main abiotic factor to be monitored is the current and studies of the nearshore circulation are needed to understand larval transport (Norcross and Shaw, 1984) and the direction of exportation of planktonic stages.

1.4.3. Problems and limitations

The main barriers to the successful execution of ichthyoplankton studies are : imprecise definition of survey objectives and sampling strategy, errors or imprecisions occurring during field surveys (estimations of depth of the gear, filtered volume, *etc.*), errors or imprecisions occurring in the laboratory (fractionating, sorting, definition of developmental stages), underestimation of the technical effort required (in time and personnel) from field operations to identification in the laboratory.

Precise estimates of the number of fish larvae require many carefully captured and sorted samples. The major biases are caused by escapeing, extrusion through the meshes (from nets) of nets used to capture embryos and early larvae, and visual avoidance of the approaching gear by older larvae, demonstrated from day/night differences in catches. Diurnal and nocturnal densities may be very different and nocturnal concentrations may be 10 or 100 times higher than diurnal values. The nature of these biases is discussed in numerous publications, among them Lasker (1984).

The design of the sampling strategy, the statistical analysis and the interpretation of data, must take into account that larvae of all sizes have a tendency to be aggregated. Patchiness has many origins, among them schooling of spawning adults, diurnal periodicity in spawning behaviour, local sites of high larval mortality, dynamic oceanic events such as convergence zones, and larval behaviour.

An understanding of larval distribution and mortality requires a thorough understanding of early life history traits such as parental factors of time and space of spawning, pattern of spawn distribution, egg size and yolk reserves. Some behavioural and physiological characteristics of marine fish larvae affect their survival and growth as feeding behaviour, vision capability, prey selection patterns, swimming capacity and searching behaviour, metabolism, growth rate, and time to the onset of schooling (Blaxter, 1986). These traits are interrelated and consequently form distinct life history strategies (Lasker, 1984). In the design of strategy and in data interpretation, this knowledge of parental factors and larval behaviour is vital.

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1.5. FISH VISUAL CENSUS METHODS FOR DETECTING GRADIENTS OF ABUNDANCE AND BIOMASS ACROSS BOUNDARIES OF MPAS

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1.5.1. Introduction

The emigration of subadult and adult fishes to surrounding non-protected areas would result in the recovery of exploited populations outside the limits of MPAs, thereby enhancing the yield of neighbouring fisheries. Indirect evidence of such a phenomenon could be obtained by detecting (i) a progressive increase over time of the mean density and biomass of these target species both inside and in the areas surrounding MPAs (Russ and Alcala, 1996), and (ii) a gradient of abundance and mean size of target species across the reserve boundaries, the slope of this gradient depending on the catchability and mobility of the target species, as well as the habitat structure both inside and outside the MPA (Rakitin and Kramer, 1996; Chapman and Kramer, 1999). To study the first evidence requires obtaining long-term (>10 yr.) data sets. The second option, based solely on spatial sampling designs, would seem more feasible -although much less conclusive. Quantification of fish assemblage structure (species composition, abundance, and biomass) can be done using fishing methods, and underwater visual census (UVC) techniques. The aim of this present section is to review UVC techniques that can be used for the study of biomass export from MPAs, and, on the basis of our experience, to recommend a protocol for the Mediterranean Sea.

1.5.2. Pros and cons of underwater visual census techniques

Since its first use in Hawaii (Brock, 1954) and the Bermudas (Bardach, 1959), fish UVC techniques using SCUBA diving have been increasingly utilized in all seas, including the Mediterranean (Harmelin-Vivien and Harmelin, 1975), as an alternative to traditional methods such as fishing gear, anaesthetics (quinaldine) or poisoning (rotenone) (Russel *et al.*, 1978). Other techniques have been used to lesser degrees, either because of their destructive effects on habitats (*e.g.* dynamite), or due to the difficulty of their use in the marine environment (*e.g.* electrofishing, see Davis and Anderson, 1989). Numerous authors (*e.g.* Russell *et al.*, 1978; GBRMPA, 1979; Harmelin-Vivien *et al.*, 1985; Thresher and Gunn, 1986; Bortone and Kimmel, 1991; Charbonnel *et al.*, 1997; Francour, 1999) have reviewed visual census techniques. From these reviews a range of advantages of UVC techniques emerge:

(a) they are relatively easy to learn;
(b) they can be rapidly performed, so that a

great amount of data can be recorded very efficiently;

(c) later lab-work is not needed;

(d) they are cheap (the diver himself is the "sampling device");

(e) they permit a great variety of variables (*e.g.* relative abundance, density, size structure, species composition, species richness, individual behaviour, shoaling behaviour, space use, *etc.*), with the possibility of calculating the accuracy and precision of the estimates;

(f) they do not alter natural mortality and size structure of the studied populations, therefore minimizing the impact of the interference of the observer's action on their temporal dynamics, so that they are especially well-suited to work in MPAs and artificial reefs;

(g) they are particularly suitable in the case of architecturally complex substrates, where other techniques (namely destructive ones) cannot be used;

(h) they are extremely flexible for any sampling design to be implemented in the field, so that a great variety of ecological questions can be dealt with .

Nevertheless, it is well known that, like every sampling technique, UVC involves a range of a series of methodological shortcomings, such as: constraints of SCUBA diving -limited time underwater, maximum attainable depth < 50 m (due to blood saturation with nitrogen, with the subsequent problems of decompression and narcoses, and the capacity of the air tanks), water temperature (lack of concentration due to the cold, risk of hypothermia), low underwater visibility (turbidity), waves, currents, presence of dangerous living organisms (at least at some latitudes); observer's experience (both diving and counting fish), and training level (visual identification of species, estimation of individual sizes, determination of sex, *etc.*). Other biases can be attributed to fish behaviour, such as their crypsis or hiding capabilities, temporal fluctuations of presence or abundance -either cyclical (day-night, lunar, seasonal) or non-rhythmic (variations of light intensity or turbidity, intensity of currents and waves, anthropic disturbances, *etc.*)-, and individual response of fishes (either attraction or escape) to the presence of divers. Because these features imply problems of accuracy of estimates (Christensen and Winterbottom, 1981; Sale and Douglas, 1981; Brock 1982), fish UVC techniques have been frequently criticized (Sale and Sharp, 1983; Fowler, 1987; McCormick and Choat, 1987; Lincoln Smith, 1988; Greene and Alevizon, 1989; St.John *et al.*, 1990; Watson *et al.*, 1995). From these studies we conclude that UVC techniques involve important biases when estimating absolute fish densities in a given area, but they allow proper comparisons among zones because of their consistency, by hedging errors that are similar from place to place and through time. Some works have compared different census methods (quadrats, transects, point counts, random walks, *etc.*), using different recording devices (*e.g.* underwater boards, audio, photography, video), by means of different methods to introduce the observer into the marine environment (*e.g.* snorkelling, SCUBA diving, ROVs or manned submersibles) (Brock, 1982; Bortone *et al.*, 1986, 1989, 1991; Thresher and Gunn, 1986; Greene and Alevizon, 1989; D'Anna *et al.*, 1999; Francour *et al.*, 1999). These studies highlight the need to combine several visual in situ methods when the purpose of the study is to obtain a complete image of the fish species composition at a given locality.

1.5.3. Techniques to estimate fish number and size underwater

Counting fish has been performed using a variety of methods, from trying to count directly the exact number of individuals, to using abundance classes (Harmelin-Vivien *et al.*, 1985). The first method induces a false sense of precision, especially if we consider that the human brain seems to have difficulty in visually counting groups made up of more than 20 objects (Bevan *et al.* in Charbonnel *et al.*, 1997). On the other hand, mixed techniques (*e.g.* counting small numbers accurately until a given limit, and then establishing abundance classes) have the disadvantage that the level of precision is, as in the above case, spurious, and also the degree of error is unknown, unequal and dependent on the means (i.e. the bigger the number to estimate, the greater the error we commit). Therefore, the solution of utilizing abundance classes seems to be more suitable (Harmelin, 1987). The statistical basis of their use is that, if the possible estimates of a real value (*e.g.* abundance) show a non-gaussian distribution with variance depending on the mean, then the distribution of their logarithms is gaussian and with constant variance (Frontier 1986). In our case, the fact that visual observers tend to underestimate the actual number of individuals (Sale and Sharp, 1983; Jennings and Polunin, 1995) approximates the distribution of estimated values of abundance to the lognormal. If we limit each observation to be assigned to one abundance class following a geometric series, then the geometric mean of each class corresponds to the arithmetic mean in log-scale. Thus, we commit the same error (i.e. the variance is homogeneous for every abundance estimation) when assigning an observation to a given class, this error being at most equal to 4s (where s^2 is the variance of class intervals in log-scale) (Frontier, 1986).

The estimation of size distribution of species is important, especially for the study of the effects of MPAs (Bortone and Mille, 1999). By knowing the size of individuals we can estimate their biomass, using the appropriate length-weight conversion equations. Actual size of observed individuals can be estimated very precisely (*e.g.* to the nearest 1 cm) (Bortone and Mille, 1999), something perhaps unrealistic when studying the whole fish assemblage. On the other hand, by assigning each observation to one of a few (3-5) predetermined size classes (*e.g.* Bayle-Sempere *et al.*, 1994), in addition to losing precision, we are adding difficulty to the task to be performed underwater, since we have to decide instantaneously if a given individual corresponds to a small, medium or large size class for species that are very different in average size, body shape, or mobility. On the basis of our experience, perhaps the best way to estimate fish size is by approximating it to the nearest 2 cm (García-Charton and Pérez-Ruzafa, 1998; Bayle-Sempere, 1999) while the alternative of using a greater number (8-10) of size classes is as difficult as the case of a few classes, and the obtained precision is similar to using 2-cm intervals. Other techniques based on triangulation methods (*e.g.* Swenson *et al.*, 1988; Van Rooij and Videler, 1996) are barely operative, since they require knowledge of the distance between the observer and the fish, and sometimes the use underwater of cumbersome equipment (*e.g.* stereoscopic cameras, calibrated mirrors, *etc.*). Finally, numerous authors (e.g. Bell *et al.*, 1985; Harmelin-Vivien *et al.*, 1985; Bortone and Mille, 1999) have shown how continuous training is essential, even for experienced observers, in order to validate visual censuses.

1.5.4. Recommended protocol for the Mediterranean

Bearing all the above considerations in mind, and on the basis of our previous experience, we propose the following sampling protocol as the most suitable for verifying the existence of spatial gradients of fish abundance and biomass. In order to facilitate the detection of such a gradient, coastal MPAs (rather than islands), and similar habitats both inside and in the vicinity of these MPAs, are to be preferred.

Size and shape of the sampling unit

50 x 5 m transects, although other transect sizes may be used depending on the size, mobility and home range of the species involved (*e.g.* 400 x 10 m for big fishes -groupers, jacks, bonitos, barracudas, *etc.*-, 20 x 1 m for small, sedentary fishes such as gobids, ble-

nids, callyonimids, gobioesocids, tripterygids, as well as scorpionfishes and juveniles of demersal species).

Sampling design

As the objective is to detect spatial gradients of abundance across the boundaries of a given MPA, sampling units should be placed along the coastal axis from inside to outside MPA (up and down the coast). Spatially nested designs are to be preferred in order to optimize the spatial comparisons and avoid confounding effects of other spatial factors (*e.g.* differences in habitat structure) (García-Charton *et al.*, *in press*). For a given sampling effort, performing random replicates (i.e. counting fishes only once per transect) is to be preferred to repeating counts several times on the same transect, so that the spatial, small-scale variability is better accounted for.

Fish counts

Assign each observation to 9 pre-determined abundance classes, following Harmelin (1987), whose limits (a,b) coincide with the terms of a base (approx.) 2 geometric series: 1 / 2-5 / 6-10 / 11-30 / 31-50 / 51-100 / 101-500 / >500. Geometric means (?ab) of each fish abundance class (1 / 3 / 8 / 18 / 39 / 71 / 142 / 317 / 500) should be used when constructing the data matrix, while we should use the log transformation for data analyses (García-Charton and Pérez-Ruzafa, 1998).

Size estimation

Measure the size of individuals to the nearest 2-cm, by comparison with a 50-cm plastic rule fixed to underwater slate. Continuing training and calibration sessions are needed to ensure the consistency of estimations.

Other considerations.

In order to minimize bias, additional criteria should be adopted, such as: perform UVC at the same moment of the day (*e.g.* between 10:00-14:00 h) and in the same season of the year; and avoid turbid waters (*e.g.* horizontal visibility < 8 m), strong currents and bad weather conditions.

Quantification of habitat structure

Small-scale variation in habitat structure -both complexity and heterogeneity (García Charton and Pérez Ruzafa, 1998) can potentially interfere with protection, either reinforcing or buffering its effects (Chapman and Kramer, 1999; García-Charton *et al.*, *in press*). Therefore, habitat descriptors should be measured to control for this interference (see Section 3.2 of this survey for a brief review on how to measure fish habitat structure).

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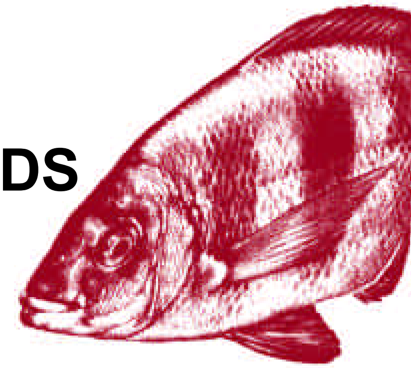

1.6. EXPERIMENTAL FISHING METHODS

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1.6.1. Introduction and objectives

Biomass export from MPAs or spillover effect must produce gradients of abundance across MPAs boundaries (Rakitin and Kramer, 1996; Chapman and Kramer, 1999) and, as a consequence, increase catches outside the protected area.

Catch per unit effort (CPUE) has been widely used as an index of abundance of exploited populations. This index assumes that catchability coefficient is a constant and that changes in CPUE accurately reflect changes in the abundance of fish. However there are many circumstances in which measures of CPUE are poor indices of stock abundance (King, 1995). Most of the problems in using CPUE data relate to the differences between the way fishing effort is measured (apparent effort) and the way in which actual or effective effort is changing. Hence, CPUE data of experimental fishing, as long as standard gear is used, are likely to provide good estimates of abundance over time and space (King, 1995) and may be applied to the study of abundance gradients off MPAs.

1.6.2. Recommended methods

Bottom trawl surveys are the most common type of experimental fishing since area sampled may be estimated. Otter trawls are the most widely used type of bottom trawl in commercial fisheries, and owe most of their success to the herding action of the trawl doors and sweeplines used. Some authors have shown that the trawl doors, sweeplines and mud or sand clouds they produce present auditory and visual stimuli that can actually herd fish or shellfish into the path of the oncoming net (Wardle, 1986). The reaction to the stimuli by the different targeted species vary widely and the actual sampled area is difficult to determine (Gunderson, 1993).

Beam trawls are the most ancient and simplest of trawl systems, still used in commercial fisheries. Professional versions of this gear are usually heavy (about 12 m wide), but the research applications employ relatively small beams (<6 m wide). The principal advantage of this gear in research applications is the well-defined area swept (Gunderson, 1993). Its primary use should be in sampling fish and invertebrates that are demersal and show only limited escape responses.

Both methods need to measure accurately the distance fished, which could easily be done with DGPS. The performance and efficiency of different otter or beam trawls must be determined in order to obtain comparable results. This means that intercalibration is needed if different teams use these gear in different zones.

Passive gear such as gill-nets, traps and long-lines have been used too for experimental fishing but their inconvenient is that they only provide relative data and it is difficult to obtain results related to surface or volume. While efforts have been made to develop a methodology for estimating the effective area sampled by such gear types (Eggers *et al.*, 1982) they have relied on somewhat simplistic assumptions and the inability to define the sampling area precludes direct estimation of abundance (Gunderson, 1993). Moreover, their selectivity is very high, and could be used only for a narrow range of demersal species. On the other hand, these gear could be used in non trawable areas (rocky bottoms) and for species that may not be properly sampled with trawling.

1.6.3. The use of experimental fishing to assess biomass export from MPAs

Experimental fishing could be useful in MPAs monitoring to test expected gradients of abundance in time and space (*e.g.* Rakitin and Kramer, 1996). However, in many MPAs it could be difficult or impossible to fish inside the protected area and the applicability of experimental methods will be reduced to monitoring abundance around MPAs. Ideally, it would be necessary to have a BACI (Before-after, Control-impact) design (Underwood, 1994). When no sampling has been done before the establishment of a MPA, inference could be done from spatial comparisons (Kingsford and Battershill, 1998).

Experimental fishing could be used also as an indicator of fisheries enhancement by MPAs. Since commercial fishing around MPAs is normally a multi-species, multi-gear small scale fishery, sampling total catch may require a major effort in terms of time and funds. In this situation, to use CPUE as an index of abundance effort must be standardized (*e.g.* Guerra and Sánchez Lizaso, 1998). In this case, experimental fishing with commercial gear may be a good indicator of commercial fishing yields. However it may be interesting to study the changes in effort distribution since a concentration of effort around MPAs is expected (*e.g.* McClanahan and Kaunda-Arara, 1996).

The biomass export from MPAs could be assessed by means of experimental fishing using artisanal commercial gear such as trammel nets or hook-lines. These gearusually target those species more directly affected by of protection. The sampling should be carried out using a predefined unit effort (meters of nets or number of hooks), and at different zones over a distance: from the limits of the MPA to several kilometres away. The sampling design should include a spatial replication, but the greatest sampling effort must be made on the temporal factor due the high variability of catches over time. In this sense, several temporal scales hierarchically arranged must be considered. Sampling design must consider some control zones in fished areas in order to compare the results from the protected zone.

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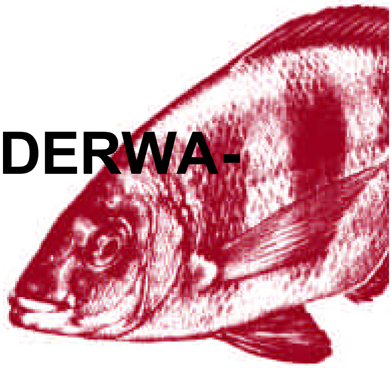

1.7. EXTERNAL TAGGING FOR UNDERWATER MONITORING

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1.7.1. Introduction and objectives

External tags are visible manmade marks applied externally on the organism's body . These types of tags are easily detectable and may carry individual codes and information to make data collection easier. Their use for identifying fishes (either individuals or groups) is an old and well established technique widely employed in studies of age determination, growth, mortality, abundance, migratory patterns and behaviour (Parker *et al.*, 1990; Nielsen, 1992).
In recent years, mark-recapture techniques using external tags have been employed for monitoring movements of fish and biomass export in marine protected areas (see Russ and Alcala, 1996). However, as the success of such techniques depends to a great extent on the catching and reporting by commercial and/or recreational fishermen, recently direct underwater mark-resighting methods have been developed to determine fish population parameters (Parker, 1990; Davis and Anderson 1989) and reef fish movements (Campbell, 1995; Matthews and Reavis, 1990; Trendall and Bell, 1989).

This section summarises the different types of external tags and deals with the methods involved in tagging experiments with particular reference to biomass export studies.

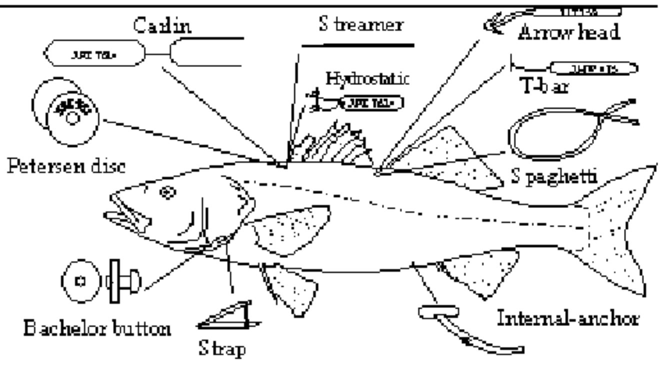
1.7.2. Classification of external tags

In grouping the vast number of tags and ways of marking, different criteria have been followed according to the nature of the mark (McFarlane *et al.*, 1990) or to the way they are attached to the animals (Nielsen, 1992), i.e., transbody (tag protrudes through both sides of the animal's body), dart-style (tag protrudes through only one surface of the animal's body) and internal-anchor tags (the anchor lies against the inside wall of the fish's body cavity).

The following table loosely groups external tags in three main categories. It also reports the way they are attached to the animal body and shows a schematic view of each tag.

Further details about external tags, tagging techniques and attaching procedures are also reported in Jakobsson (1970) and Jones (1979).

Tag type	Attachment of tag to the animal body
RIBBONS, THREADS, WIRES:	
Streamer tag	Transbody
Spaghetti tag	Transbody
PLATES, DISKS:	
Petersen disk/Atkins plate	Transbody
Bachelor button	Transbody
DANGLING TAGS:	
Carlin, hydrostatic, strap	Transbody
Dart/arrowhead tags	Dart-style
T-bar anchor	Dart-style
Internal anchor	Internal anchor



1.7.3. Pros and cons of the most common external tags

The suitability of a tag depends mainly on the objectives of the study and on the characteristics of the animal to be tagged. Knowledge of the advantages and disadvantages of the different tags available can help in choosing the most suitable type.

Transbody tags are used for marking crustaceans (streamer and spaghetti tags), juvenile fish (coded wires) and large sized fish (Petersen disk). They have a high retention rate but the attachment operations are time-consuming and the risk of entanglement is high.

Internal anchor tags have been widely used on large as well as small sized fish (Wallin *et al.*, 1997). They have high retention and survival rates (Ludwig *et al.*, 1990), but the tagging procedure is slow.

The dart style group, including dart tags and T-bar anchor tags, is the most common type of tag in use today.

Dart tags have been widely used for studying movements of lobster and of large sized nektobenthic and pelagic fish. They have been shown to have a high retention rate and a very low effect on growth and survival of juvenile fish (102-103 mm SL) (Winner *et al.*, 1999).

The T-bar anchor is probably the most used type of tag and it is suitable for a large range of demersal and pelagic species. They have been successfully employed in mark-recapture studies of sparids (Sheaves *et al.*, 1999; Buxton and Allen, 1989). The easy and fast tagging operation, by means of suitable guns, allows to mark a great number of individuals in a short time.

Very good performances similar to dart tags have been demonstrated for T-bar anchors (Winner *et al.*, 1999).

Despite these advantages, some limitations could negatively affect marking studies based on dart style tags. Firstly, the penetration of the gun needle through the skin causes an access route for infections. Secondly, the wound may be enlarged by the continuous drag of the tag protruding from the fish, and some individuals may loose the tag due to the enlarged hole. Thirdly, the overgrowth of algae on the tag, especially on those fishes living in vegetation-rich habitats, increases the drag and weight of the tag making swimming difficult. An increase of entangling in fishing nets and filamentous algae has also been observed.

1.7.4. Recommended method: underwater visual surveys using external tags

Some survey techniques based on scuba divers and external tags have been recently experimented with the aim of overcoming some limitations of traditional tag-recapture studies (stress of fish during the capture-release process, quality and consistency of recapture information, *etc.*).

Due to their fast tagging procedure and to high underwater visibility, dart style and T-bar anchor tags have been often used in underwater mark-resighting methods.

The T-bar anchor tag, modified by the addition of a numbered Petersen disc, has been successfully employed in a study on movement patterns of rockfish making use of underwater tagging and visual recapture (Matthews and Reavis, 1990). The use of this technique allowed a higher rate of fish resighting than in other studies based on traditional mark-recapture methods.

An underwater tagging technique using T-bar anchors tags with subsequent visual census has been applied for studying the movement of small serranids in an artificial reef area (Campbell, 1995).

Visual census and underwater marking with dart tags have been carried out to follow movements of groupers in marine protected areas (Chauvet *et al.*, 1991).

A combination of mark-recapture techniques employing dart tags, recapture by seine fishing and underwater visual census has been used for studying movements and distribution of jacks in a fisheries conservation zone (Holland *et al.*, 1993).

Other underwater tagging methods using external tags for underwater visual observations are reported by Adkison et al., (1995) and Gitschlag (1986). The results of these studies have proved that underwater tagging techniques (i) are useful especially for reef fish, (ii) reduce the capture-and-release stress and (iii) allow high visual recaptures providing more information on the behaviour and home range of fishes.

1.7.5. Problem and limitations

Generally speaking, an ideal external tag should not affect the animal in any way. In particular, a tag should neither increase the mortality of the tagged individual nor affect its growth. These assumptions are especially important for population dynamics studies based on mark-recapture surveys. On the other hand, behavioural changes of tagged specimens due to the tag might influence the results of movement and migration studies based on tagging experiments. Tag retention and tag recognition, depending on several factors (i.e., attachment mechanism of tag, species being tagged, visibility of tag, *etc.*), are two other important features of a valid tag.

Direct underwater observation of tagged fish may overcome some limitations of the conventional mark-recapture studies as well as help in collecting more detailed information on the short-scale movements of reef fishes.

In conclusion, since no tag meets all the requirements mentioned above, the choice of a valid external tag should be made in relation to the objective of the project, according to the features of the species being tagged and to the type of data being collected.

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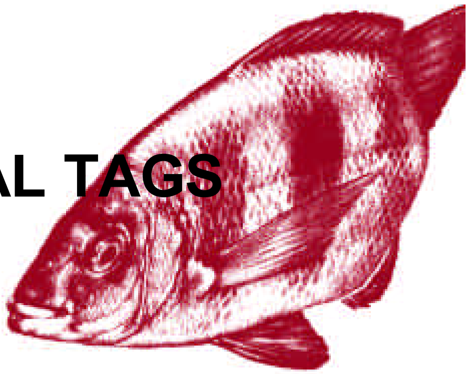

1.8. IDENTIFICATION OF NATURAL TAGS

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1.8.1. Introduction and objectives

The importance of fish movements, such as home range and home range relocation (Kramer and Chapman, 1999), in relation with MPAs was stressed after Russ and Alcala (1996) clearly confirmed that fish flux from MPAs to open fishing zones (the so-called "spillover effect") actually took place. To date , the most common methods to investigate fish movements are mark and recapture methods (Seber, 1982) and ultrasonic telemetry (Hilborn and Walters, 1992; Zeller and Russ 1997).
However mark and recapture experiments may involve more or less severe handling of fishes that can result in increased mortality rates, decreasedmovement capability or behavioural changes in tagged individuals (McFarlane and Beamish, 1990). Ultrasonic telemetry or radio tracking methods may also require the traumatic surgical implantation of transmitters on fishes, resulting in a high number of individuals being severely affected after implantation (Zeller, 1999). For this reason , a wide variety of internal microtags, subcutaneous paints, or freeze branding techniques, have tended to replace external tags of the anchor or dart tag type (Beukers *et al.*, 1995). Although it has never yet been used in fishes, individual identification from natural specific marks could be a useful alternative to artificial tagging, as it totally avoids manipulative processes and the associated undesirable consequences. Moreover, the recent rapid development of computer image treatment software allows the processing and storage of a great deal of of data on natural marks identifying large numbers of individuals (Mizroch *et al.*, 1990). Individual photo-identification can be an effective technique in accurately monitoring some sedentary highly vulnerable fish populations at diverse spatial and temporal scales; some potential uses can be summarised as follows :

· small-scale studies during short periods of time (i. e. one summer),
a given population can be assumed to be a closed population. On this assumption, individual identification can replace traditional mark recapture methods if the identified individuals are considered as "tagged" and the recognised ones "recaptured";

· large-scale studies (perhaps including protected and unprotected areas), carried out over a short time period,
it could be possible to establish whether the home range of some individuals actually straddles the boundaries of an MPA. This is one of the possible ways of fish biomass export from MPAs to open fishing areas, as

Zeller and Russ (1997) have already demonstrated for Plectropoma leopardus using ultrasonic telemetry;

· small-scale studies over a long time period, the level of annual renewal of a given population can be established by comparing the individuals that remain and those that disappear from a given zone over a fixed period of time (ranging from one to several years or seasons);

· long term studies involving large areas (including protected and unprotected marine areas), it seems feasible to establish whether home range relocation from MPAs to fished zones really occurs over the years. Though the opposite process could also take place, a possible net flux of biomass from MPAs to unprotected zones could be established by comparing which individuals formerly identified inside a MPA have been recognised in open fishing zones.

1.8.2. Recommended methods

Although individual identification is a desirable technique to carry out studies on fish movements inside the MPAs, its common use is seriously limited in most littoral fish populations. It is very difficult to distinguish clear individual marks even among the most sedentary, territorial and coloured littoral rocky reef fishes if they are too small or too mobile. Only certain species exhibit colour patterns that make each individual easily distinguishable from others merely on sight. Of these, only a few are big-sized and highly sedentary species. The Dusky grouper (*Epinephelus marginatus*) is one of the species that fulfils all these ideal criteria for individual identification in the Mediterranean Sea. Recomero© (Alazard, 1999; Garcia and Alazard, 1998) photo-identification software has been developed on the basis of individual recognition of the spots on the head (GEM, 1996; Lelong, 1999) and the scars in pelvic and caudal fins of grouper. Data on all these features, along with additional information (geographical position, zone, date, depth, size, colour pattern, sex, behaviour, etc) can be stored in a database. Further comparisons among individuals at whatever time or spatial scale can be established by contrasting the new data with previously stored information (Alazard, 1999). Currently restricted to individual photo-identification of dusky grouper, the software also offers the possibility of use for other related fish species (Louisy, 1998) after minor adjustments.

1.8.3. Problems and limitations

Some environmental and specific difficulties can limit the potential use of photo-identification in spite of any improvements made:

· Murky waters and the particular behaviour patterns of the target species, avoiding divers in heavily fished zones, can constitute serious problems since close and clear images of fishes are required.
· Dusky grouper can exhibit up to 8 different colour patterns (Louisy, 1996; Zabala *et al.*, 1997a,b) and some of them may mask almost totally the head spots. However, the spots are clearly visible in the prevailing standard mottled or cryptic colour pattern the grouper exhibit most of the time.

The real value of the Recomero© software and of other software of this kind as tools to assess fish populations remains uncertain. Further improvement is required to make the software powerful enough to handle large volumes of data. Only then will the method of automatic photo-identification be able to replace or complement other techniques.

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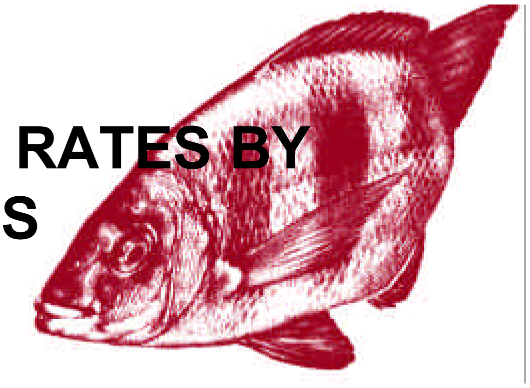

1.9. ESTIMATING MOVEMENT RATES BY MARK-RECAPTURE METHODS

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1.9.1. Introduction

Tag-recapture methods are often used to study movements of marine animals. Obtaining qualitative information on direction and range of movements is relatively straightforward in spite of the number of conditions that must be met for the method to work well (Jones, 1966; 1979). However, to estimate population size or movement rates the assumptions are more stringent and a good knowledge of the population dynamics and distribution of the studied species is necessary.

The aim of this section is to provide an overview of the application of mark-recapture methods for assessing biomass export from MPAs. The first assumption is that we have a population that is protected in a marine reserve in which the density is higher than in surrounding fished areas. It is further assumed that there will be mechanisms such as food limitation or density dependent movements as well as seasonal and ontogenetic migrations that may result in a net transport of individuals away from the marine reserve. In a study of dispersal of fish from a marine reserve, Attwood and Bennett (1994) distinguish migration (movement predictable in time and space which may be reversible) and dispersal (unpredictable and without return). In studies of biomass export from MPAs, net movement is of interest and movements, either directional or random, in and out of the marine reserve, must be considered.

1.9.2. Methodology

Estimates of rates of movement between reserve and no reserve areas from mark-recovery data rely on experimental design and statistical methods used to study movements of animals among spatial strata (*e.g.*, Hilborn, 1990; Anganuzzi *et al.*, 1994; Xiao, 1996). Such estimation is in principle possible if: (a) there is marking and recovery simultaneously in all spatial strata, (b) independent data are available on the probability of capture (or number of recoveries per unit effort), (c) the tag return rate (number of tags reported per number of tagged individuals recaptured) is known and (d) natural mortality rate is assumed constant in time and space (Anganuzzi *et al.*, 1994). In the context of biomass export studies from MPAs, the most obvious problem is that in areas without fishing effort - such as marine reserves - no tags are recovered. Experimental fishing or underwater observation in the reserve areas generally solves this problem and is often combined with the capture method needed to do the tagging. Since the estimates of movement rates are strongly affected by tag return rates, to design a mark-recovery experiment the number of recoveries needed to estimate movement rates within predetermined levels of precision and accuracy must be calculated (see Xiao, 1996 for an approach).

There is remarkably little statistical work on analysis of movement data applicable to fisheries (i.e., in which individuals are recaptured only once). From the initial analysis proposed by Schaefer *et al*., (1961) that consists of plotting the distance travelled versus the time since release and calculating the proportion of the recaptures as a function of time, different models have been developed (*e.g.* Hilborn, 1990; Schwarz *et al*., 1993; Anganuzzi *et al*., 1994). The general framework developed by Hilborn (1990) for the analysis of animal movement from tagging data may be used in biomass export studies. The framework has four elements:

1) a population dynamics and movement model for the survival and movement of tagged individuals: Includes natural mortality and movement as well as tagging mortality and tag shedding rates. The movement among areas is a Markovian exchange process. Given the number of tag releases, the fishing effort, the catchability coefficient in each area and the probability of movement from one area to another, the model predicts how many individuals will be alive from each tag group (tag releases in given area and time) at any time in each area.

2) an observational model: Specifies the relationship between the tagged specimens predicted to be in an area under model 1 above and the tagged organisms that are actually captured and reported. This model ideally includes non-reporting of tags, misreporting and rates of tag loss.

3) likelihood of recoveries: The above two models yield a matrix of predicted recoveries for each tag group and a matrix of observed recoveries. From them, the likelihood of the observed recoveries given the parameters of the studied population is calculated. A statistical likelihood or probability model is developed that calculates the likelihood of the number of recoveries actually being reported if the population dynamics and observed models are true.

4) non-linear function maximisation procedure: This function is needed to find the values of the population dynamics and observation models that maximize the likelihood of the observation given the parameters of the model.

There are a number of assumptions of the method and parameters of this framework that need to be estimated. Below we indicate the precautions to be taken and the approaches that may be followed for each one:

Random distribution of marked individuals throughout the population

To meet this assumption tagging should be done throughout the range of the population in the MPA and in the studied areas outside and in proportion to the local abundance (*e.g.* as a proportion of CPUE in experimental fishing). Marked individuals should be released in the same localities as captured and enough time should be allowed between marking and recapture for mixing.

Same probability of capture of the tagged and untagged individuals

Tagging and the tag may affect catchability, causing differences in the rate of fishing mortality between tagged and untagged populations. This fact would obviously produce an error in the estimated rate of exportation. Ideally this assumption should be tested when the tagging method is selected. Comparison of recapture rates of the group tagged with different types of tag (the tag under study and others normally less durable, *e.g.* marking with ink) or tagging method (*e.g.* capture and tagging versus underwater tagging) are some of the methods used to evaluate this assumption (*e.g.* Cormack, 1968; Chittleborough, 1970).

Tag loss and the tagging procedures do not increase mortality significantly

Tag induced mortality and tag loss reduces the size of the marked population. Failure to account for this may lead to biased results. Experiments under controlled conditions have been carried out to evaluate tag-induced mortality (immediately and overtime) and rate of tag loss (Waldman, 1991; Montgomery and Brett, 1996). Ideally the results of experiments have to be corroborated in the field. The most common method to estimate rates of tag loss in the field is by double tagging experiments (Bayliff and Mobrand, 1972; Kirkwood, 1982; Wetherall, 1982), which is independent of fishing mortality during the recovery period. This method has been developed employing different types of tag, the same type of tag inserted in different places or a combination of marking and tagging. The rate of tag loss is then estimated from the recaptures obtained over time. Because both the tagging induced mortality and the rate of tag loss could be different for the different segments of the population (*e.g.* in crustacean moulting individuals) it is recommended to evaluate them separately for different groups and for successive tagging operations.

Full reporting of recoveries

Since in many tagging studies professional or recreational fishermen do most of the recoveries, reporting will not be complete. The estimation of the reporting rate is of great importance and great effort should be made to optimise recovery information about rates of reporting, as well as about the position and date of recovery. Advertising campaigns, rewards or any other action aimed at improving reporting rates should be carefully planned. The tag reporting rate may be estimated by different methods (Green *et al.*, 1983; Pollock *et al.*, 1991, 1995) and their effectiveness depends to some extent on the socio-cultural characteristics of the fishermen. More recently Hearn et al. (1998) have developed a model to obtain reliable estimates of tag-reporting rates when tagging operations are done twice a year.

1.9.3. Problems and limitations

One of the greatest difficulties faced when carrying out mark-recapture experiments to study movement rates is the lack of knowledge of the spatio-temporal distribution of fishing effort. Another major problem with the analysis of tag-recapture data is that no account is taken of the probability of capture between the time of release and the location of recovery (Hilborn, 1990). That is, few fish may be caught in an area simply because there is a high probability of capture along the way. In the case of movements in and out of a marine reserve, the fishing that commonly takes place along its boundaries would reduce both exportation beyond them and the intensity of movement towards the marine reserve.

The recapture method must be controlled and specifically designed to ensure that the samples are representative of the study population. However outside the reserve the fishing methods are size selective and as a result the size distribution of tagged individuals captured by the commercial fisheries will be biased by the size selectivity of the gear used. In addition the recoveries of specimens smaller than the legal size will often not be reported.

Finally, one of the most basic impediments for the successful development of mark-recovery studies is the limited knowledge of the distribution, migration patterns and biology of the species of interest. Thus to design a proper experiment it is recommended to carry out preliminary studies to define the type and extent of movements of the species as a function of size, sex or other relevant factors.

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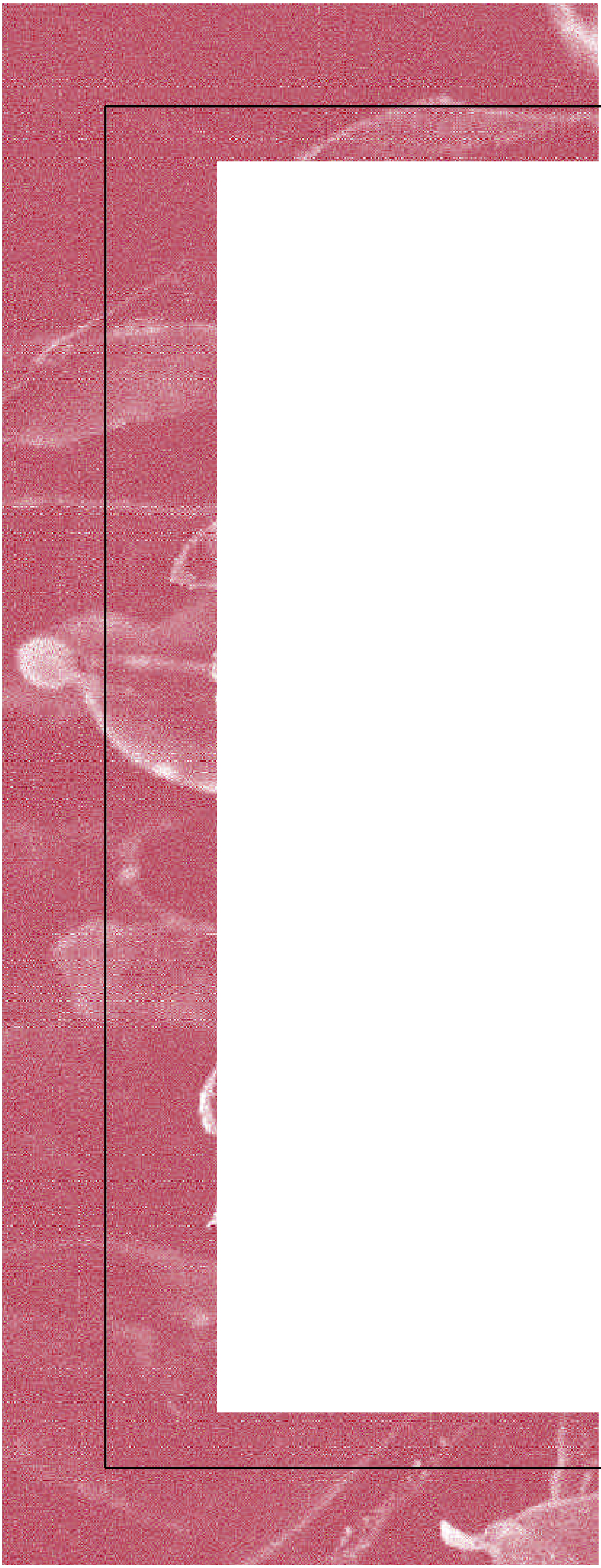
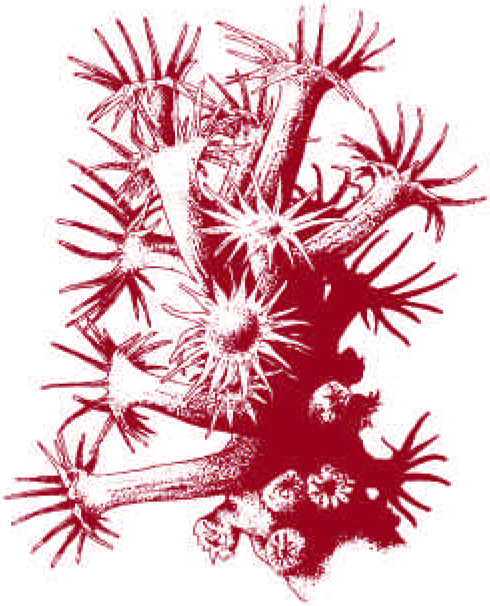
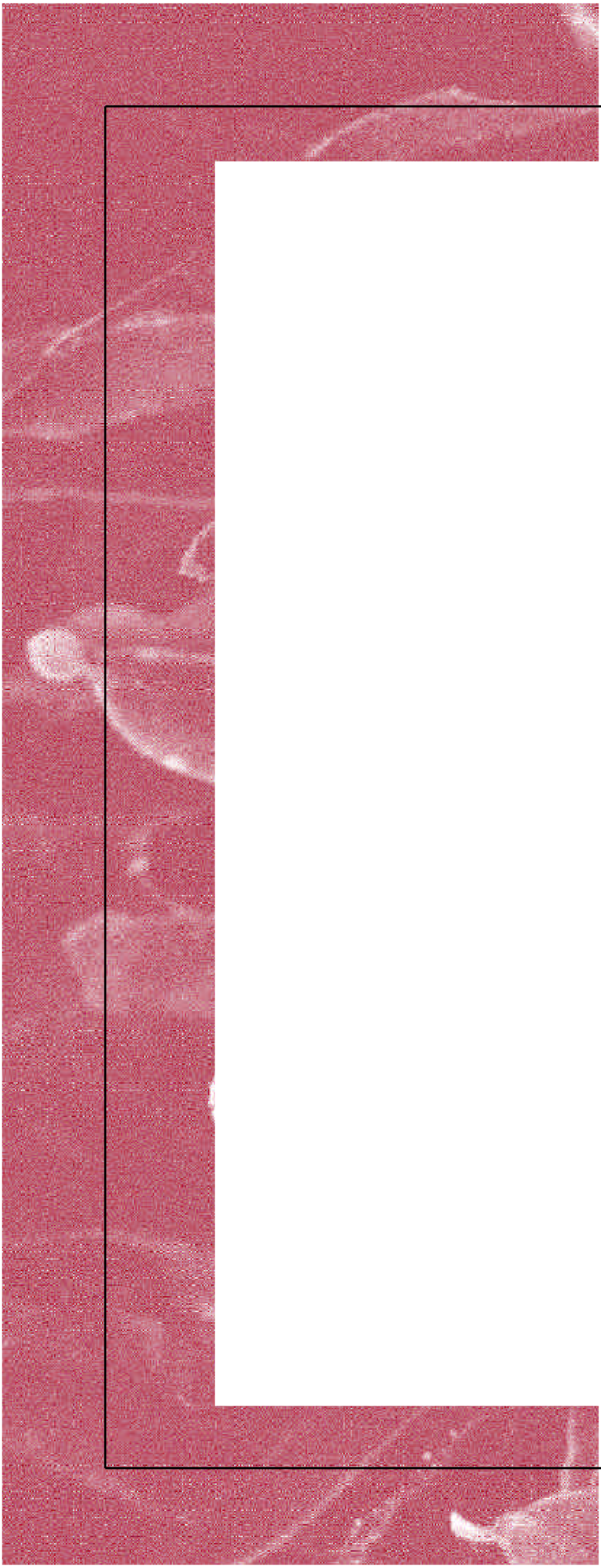
1. Methods for Assessing Biomass Export from Marine Protected Areas



CHAPTER 2.

METHODS FOR ASSESSING IMPACT OF HUMAN ACTIVITIES IN MARINE PROTECTED AREAS





INTRODUCTION

The Mediterranean marine protected areas (MPAs) are diverse in their jurisdictional features and offer a wide array of situations, ranging from large multiple use areas to small sanctuaries closed to human activities. However, the high aesthetic value of seascapes and landscapes in MPAs have triggered a steady development of tourism activities. These activities, *per se*, imply interactions between visitors and natural communities. MPA management consists then in (i) assessing the consequences of these interactions (positive, negative), and (ii) balancing human impact and protection of species and communities.

Mediterranean MPAs concern continental coasts or islands, except one recent offshore protected area between France, Corsica and Italy. Moreover, non-extractive human activities linked to 'aquatic tourism' are also carried out along the shore (trampling) or from boats, in shallow zones (swimming, snorkelling), or in deeper areas (diving). Consequently, the area of influence of the impact of these human activities ranges from the surface to around 40-50 m.

In the western Mediterranean, the main biocenoses present in this depth range are shallow rocky areas (supra and midlittoral zones), the soft-bottom areas (sublittoral zone), the seagrass beds (sublittoral zone), and the rocky boulders and coralligenous areas (sublittoral zones). The soft-bottom areas are slightly affected by no-take human activities and few scientific data are published on the stress level induced by these activities.

This chapter reviews published methods used to monitor and/or to study the interactions between visitors and three communities: the rocky shallow communities, the *Posidonia oceanica* seagrass beds, and the coralligenous communities. This review will give scientists or managers involved in MPA monitoring and management a broad overview of the most powerful techniques to describe communities and species, and to assess the extent of the impact of human recreational activities.

2.1. METHODS FOR STUDYING IMPACT ON *POSIDONIA OCEANICA* MEADOWS

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2.1.1. Introduction and objectives

One of the most important biogenic habitats for targeted species on soft bottoms are the seagrass meadows, *Posidonia oceanica* being the main species that form them in the Mediterranean. 20 to 25% of the known Mediterranean species are observed in the *Posidonia* meadows (Boudouresque *et al.*, 1994).

A variety of natural and anthropic disturbances may induce alterations in the factors that determine the natural development of *P. oceanica* meadows (dumping, coastal construction, run-offs, *etc.*). Moorings on *Posidonia* meadows can also have a negative impact which depends to a large extent on the vitality of the *Posidonia*: coverage, shoot density, baring *etc.* (García-Charton *et al.*, 1993; Francour, 1994; Charbonnel, 1996; Poulain, 1996; Francour *et al.*, 1997, 1998, 1999).

This implies a reduction in their abundance and changes in their spatial distribution (Boudouresque and Meinesz, 1982; Péres, 1984). So the spatial structure of the meadow represents an excellent indicator to determine its degree of development, quality and state of health, and to determine and quantify the effects of disturbances of different origins (West, 1990; Ruíz-Fernández, 1992).

To describe the spatial pattern of seagrasses from a hierarchical perspective (Robbins and Bell, 1994; Bell *et al.*, 1995; Vidondo *et al.*, 1997), we can distinguish between individual shoots (10^{-2} m), clumps (10^{-1}-10^{0} m), beds (10^{1}-10^{2} m) and meadows (10^{3}-10^{4} m), to encompass the different processes which affect seagrass dynamics over this continuum of scales. From a number of studies a striking diversity of spatial formations of Posidonia meadows has emerged, leading some authors (somewhat vainly) to attempt to inventory and classify them: different types of lower limits (Colantoni *et al.*, 1982), "hills" (Boudouresque *et al.*, 1985a), "barrier-reefs" (Boudouresque *et al.*, 1985b), "cordons" (Vetere and Pessani, 1989), "atolls" (Calvo and Fradà-Orestano, 1984), *etc.* Nevertheless, these "static" spatial structures are nothing but particular types of spatial pattern resulting from interactions among the factors of dynamics of seagrass populations (recruitment, clonal growth, mortality) and a variety of local environmental factors that control its abundance (Duarte and Sand-Jensen, 1990; Robbins and Bell, 1994;

Vidondo *et al.*, 1997), so that more fruitful results could be obtained by studying the seagrasses under such a hierarchical, multiscaled approach.

However, the majority of the studies on the spatial structure of *P. oceanica* meadows distinguish between two different scales: macro and microscale.

From the macroscale point of view, the meadow appears as a mosaic of patches of sediment, dead roots and, in some instances, rocks. The incidence of superficial hydrodynamism and sediment stability are the main factors limiting the growth of the meadow in the shallowest zones, and the light is the limiting factor in the deepest zones. At this scale, coverage is the most important descriptor of the degree of heterogeneity in the meadow (Giraud, 1977; Romero, 1985).

From the microscale point of view, inside the patches, shoot size, shoot density, average length or elevation above the sediments are the parameters that determine the microstructure of the meadow (Giraud, 1977; Panayotidis *et al.*, 1981, Romero, 1985).

Authors use, and often combine, methods involving direct counts by divers, aerial photography, submersibles, underwater photography and video, echosounders and side-scan sonars, to map the distribution of seagrasses and to estimate their state of health, based mainly on shoot density (*e.g.* Meinesz and Laurent, 1980; Boudouresque *et al.*, 1985b; Falconetti and Meinesz, 1989), sometimes utilising stochastic interpolation techniques such as "kriging" (Scardi *et al.*, 1989; Pergent, 1990).

More recently, image analysis, GIS and remote sensing techniques have been proposed to produce more accurate maps and characterisation of meadows (Ferguson and Korfmacher, 1997; Norris *et al.*, 1997; Robbins, 1997; Pasqualini *et al.*, 1999).

Estimation of the negative effect of anthropic factors (such as anchoring, coastal construction work, bottom trawling, sewage, *etc.*) on seagrass meadows has to be done by comparing the values attained by selected indicators in sites where these factors are in force, with natural variability of these descriptors. The range of variation of these descriptors depends on environmental factors such as depth, light penetration, hydrodynamism, sediment characteristics or bottom topography (Romero, 1985), and varies geographically. Therefore, comparisons between disturbed and intact meadows have to be based on local measurements, rather than using "universal" standards of variation (Ruiz Fernández *et al.*, 1999), and taking into consideration factors other than disturbance (*e.g.* depth, orientation in relation to waves and currents, or bottom topography) when locating the sampling units. Furthermore, the decision of what variables to use as indicators of impact on seagrasses should be based on the estimated scale of effects (for instance, the extent and grain of the effects of anchoring would not be the same as those of bottom trawling or the presence of a yacht harbour).

2.1.2. Recommended methods

Coverage

Coverage can be measured using linear transects randomly located at each sampling point, calculating it as the percentage of living meadows occupying the transect. A suitable length for these transects would be 10 metres.

Another method has been standardised since 1995, using a transparent PVC sheet (30x30 cm) subdivided into 9 squares (10x10 cm). The observer positioned 3 m above the bottom counts the number of squares occupied by Posidonia (Gravez *et al.*, 1995). The sum-up of those counts (?x / 9) helps to determine the percentage of the *Posidonia* meadow coverage on the bottom.

Shoot density

Shoot density can be measured by counting the total number of shoots present inside a 400 cm² quadrat. In this case, it is necessary to replicate the observation (at least 3 measures at each sampling point), due to the high variability of the data obtained. Giraud (1977) established a first scale of meadow classification depending on the shoot density (Tab I).

Tab I. : *Posidonia* meadows classification depending on shoot density (Giraud, 1977).

Density per m²	Meadows type
- more than 700 shoot/m²	Type I, very dense meadow
- from 400 to 700 shoot /m²	Type II, dense meadow
- from 300 to 400 shoot /m²	Type III, sparse meadow
- from 150 to 300 shoot /m²	Type IV, very sparse meadow
- from 50 to 150 shoot /m²	Type V , semi-meadow
- less than 50 shoot /m²	Isolated shoots

A second scale including the influence of depth on *Posidonia* density was established (Pergent-Martini, 1994; Pergent *et al.*, 1995) which determined four categories of meadow according to depth: abnormal, sub-normal inferior, normal, and sub-normal superior.
NB : the depth can be responsible for 54 % of the variability of the shoot density.

Global density

Romero (1989) described an index, known as the global density index, that integrated the two previous parameters and characterized in an appropriate way the degree of development of the meadow at a site

$$DG = (d/s)*(C/100)$$

where
DG = global density expressed in shoots by m²
d = number of shoots counted in the 400 cm² quadrats in the patches
s = surface of the quadrats in m²
C = coverage, expressed in %

Baring

P. oceanica rhizomes grow in a vertical (orthotropic rhizomes) or a horizontal plane (plagiotropic rhizomes) (Molinier and Picard, 1952; Caye, 1980). These intermingled rhizomes, dead or living, together with the sediment that fills the interstices, constitute one of the most characteristic structures known as "matte". This French term indicates the sea floor elevation produced by *Posidonia*. Rhizome baring generally expresses a sediment deficit for *Posidonia* meadows. Conventions for measuring rhizome baring were defined by Boudouresque *et al.* (1980) : - for plagiotropic rhizomes, baring is the distance between sediment and the inferior part of the rhizome ;
- for orthotropic rhizomes, baring is the distance between sediment and leaf bases, minus 2 cm.

Matte compactness

A reproducible method to measure matte compactness has been described for studies in Port-Cros National Park (Poulain, 1996): a lead weight (5 kg) slides along a metal stick and hits a blocker, pushing the lower part of the stick into the matte. The penetration depth of the stick provides a measurement of the matte's compactness.
The response of the *Posidonia* meadows to anchoring impact can depend on the matte compactness.

Fragmentation

Under the impact of disturbances (dumping, coastal construction, mooring, *etc.*) the *Posidonia* meadows boundaries are fragmen-

ting. Depending on the gravity of the disturbance, boundary fragmentation will initially be perceptible as undulations, then in a subsequent stage as invaginations of the boundaries, and finally as a real fragmentation of the meadows into small patches . Their size will depend on the proximity of the source of disturbance (Charbonnel *et al.*, 1995).
Meadow fragmentation can be measured using linear 10 m long transects, randomly located at each sampling point, and measuring the part of living meadows crossed by the graduated tape. This method is based on the line intercept method (Forgeard, 1987).

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2.2. METHODS FOR STUDYING THE IMPACT OF TRAMPLING ON ROCKY SHALLOW AREAS

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2.2.1. Introduction

Environmental conservation is achieved by setting up protected areas, and managing them for conservation purposes. The effectiveness of management should be constantly assessed by a monitoring program to determine potential human impact, to evaluate its effects and therefore improve conservation measures. Besides pollution, the main impact occurring on marine rocky shallow areas (i.e. intertidal and littoral fringe) are due to human frequentation.

Humans can act on the biota by trampling (Woodland and Hooper, 1977; Beauchamp and Gowing, 1982; Kay and Liddle, 1989; Povey and Keough, 1991; Brosnan and Crumrine, 1994; Keough and Quinn, 1998; Schiel and Taylor, 1999) or collecting marine organisms for food and/or bait (see Moreno *et al.*, 1984; Castilla and Bustamante 1989; Underwood and Kennelly, 1990; Kingsford *et al.*, 1991; Adessi, 1994). This impact is more significant within marine protected areas (MPAs) owing to the risk of unregulated overfrequentation of the shoreline. Hence, Mediterranean marine protected areas should be monitored regularly for evaluation of management measures, the impact of visitor frequentation and the direct and indirect effects of protection.

In this chapter we address the effects of trampling (people walking over rocky shores) and hereafter we summarise the main sampling methods available in the literature and used to test these effects on rocky shallow areas.

There is evidence that the impact of human trampling on rocky shallow areas can be very substantial and may represent a severe threat to the diversity of the entire community (Brown and Taylor, 1999). The physical removal of certain species through dislodgement, crushing or weakening its attachment to the substrate (see Brosnan and Crumrine, 1994), may affect not only its population size but also, via competition, predation or habitat forming, the abundance and species richness of the other species of the community (Brown and Taylor, 1999). Stepping on erected algae on rocky shores may cause a rapid decrease in algal cover, canopy and biomass, leaving the community in a less complex state dominated by turf forms and low profile algae (Brosnan and Crumrine, 1994). This problem together with other possible effects on animal species (Povey and Keough, 1991; Brosnan and Crumrine, 1994; Brown and Taylor, 1999) is of great concern to marine ecologists, conservation biologists, and managers of marine protected areas.

Empirical evidence has demonstrated in several cases that the vulnerability (or, conversely, resistance) to human trampling depends mainly on the nature and morphology of marine organisms. Generally, branching and foliaceous living corals are more susceptible than encrusting and low caespitose forms (Liddle and Kay, 1987; Kay and Liddle, 1989; Liddle, 1991).

Experiments over a range of intensities revealed that algal mats (i.e. *Hormosira* spp.) were more vulnerable both to low and high intensity of trampling, whereas coralline mats were affected only by high number of tramples per area (Povey and Keough, 1991; Schiel and Taylor, 1999).

Thus, determining which kind of effect and intensity of human pressure may affect community structure and composition can help managers fine tune their conservation actions.

2.2.2. What to monitor in Mediterranean MPAs?

In the Mediterranean Sea at present, no experimental study has been designed to quantify the effects of human trampling on rocky shallow areas. The limited knowledge of the ecology of most organisms of the rocky shore is probably a major constraint for reliable assessment of the responses to human physical disturbance.

For this reason, much research is required to single out the indicator species to be monitored given that the numerically 'dominant' ones are not necessarily the most instrumental in critical ecosystem functioning (Safriel *et al.*, 1995).

Some authors have identified two pre-requisites for an indicator, though not many species possess them (see Brosnan *et al.*, 1994; Safriel and Ramos-Esplá, 1995):

a) it should be common at the study site, easy to detect, identify and count, preferably in a non-destructive manner;

b) its biology should be relatively well known, at least to the extent that the species is expected to have a high degree of interconnection within its ecosystem.

The first pre-requisite guarantees the reliability and persistence of the monitoring program, even when resources and expertise are scarce; whereas the second guarantees that monitoring results are indeed indicative of the positive effects of conservation. In contrast to this, when a 'diffuse' conservation effort dissipates through the ecosystem, it may affect many species in direct and indirect ways. Hence, a 'well-connected' species should pick up the effect, and produce a detectable signal.

In the Mediterranean sea the two zones that may be strongly affected by human trampling are the midlittoral and littoral fringe. Very likely, due to the narrow tidal range occurring in most parts of the basin, human trampling on Mediterranean rocky shallow areas has a more limited but 'concentrated' extent than those highlighted in other marine regions. There is little doubt, however, that the increasing number of visitors, that is particularly apparent within Mediterranean MPAs (see review by Badalamenti *et al.*, 2000), may lead to a considerable alteration of the state of these communities in the near future.

2.2.3. Description of large-scale characteristics: the communities

The spatial arrangement of rocky shallow areas varies along the Mediterranean coasts, depending on the geology, geomorphology, topography, hydrology and hydrodynamics at local scale. Mediterranean rocky shores are generally formed by boulders of varying degrees of architectural complexity (Garcia Charton *et al.*, 2000) and, at different scales of observation, by 'habitat formers' (sensu Jones and Andrew, 1992) that may enhance the structural complexity of coastal rocky bottoms. For the description of the two zones potentially exposed to human trampling (midlittoral and littoral fringe zones), we recommend the publications by Pérès and Picard (1964), Pérès (1967), Ros *et al.* (1984) and Bellan-Santini *et al.* (1994).

2.2.4. Description of small-scale characteristics: the species

Three major components of the midlittoral and littoral fringe could be easily monitored at small spatial scale, the erect macroalgae (i.e. *Cystoseira* spp., *Laurencia* spp., *Corallina* spp. and others), the limpets (*Patella* spp.), and the bioconstruction formations (i.e. vermetids and *Lithophyllum* rims).

Table I summarises these potential descriptors of human trampling effects on Mediterranean rocky shallow areas and the main sampling methods to test these effects on the basis of the available literature. However, much research is required for identification of the most appropriate indicators, which may be either species, groups of species or communities.

Erect macroalgae

In the Mediterranean sea the assemblages of large erect macroalgae are very complex and diverse, and generally *Cystoseira* species represent the main algal component (Ros *et al.*, 1984). In exposed areas, two brown algae (*Cystoseira mediterranea* and *C. amentacea*) reveal the higher coverage on horizontal or sub-horizontal rocky substrates forming a bushy stratum up to 25-30 cm in height. Other macroalgal species may be present or replace the *Cystoseira* belts on the littoral fringe. In this group, along with other *Cystoseira* species,

Table - 1. Potential descriptors of the effects of human trampling on Mediterranean rocky shallow area and the main sampling methods to test these effects on the basis of the available literature.

Taxa	Communities	Variables	Sampling methods	Literature
Erect macroalgae	Littoral fringe	%cover biomass %canopy cover	Block design Transect Quadrat Photosampling	Povey & Keogh, 1991 Brosnan & Crumrine, 199 Schiel & Taylor, 1999 Chemello, this volume
Limpets	Midlittoral	Density size structure	Quadrat Visual census	Povey and Keogh, 1991
Vermetid formations	Littoral fringe	%cover dead/alive	Quadrat Transect	
Lithophyllum formations	Midlittoral	%Cover	Quadrat Transect	

there are the rhodophyceans *Laurencia* spp. and *Hypnea* spp., and the phaeophyceans *Sargassum vulgare*, *Halopteris scoparia* and *Padina pavonica*.

As already highlighted in several non-Mediterranean studies (see above) erect macroalgae may reveal a high susceptibility to human physical disturbance and generally may suffer significant declines (i.e. in biomass, %cover and %canopy cover) even shortly after trampling started. This may shift the whole community to an alternate state dominated by low profile algae and turf forms (see Airoldi, 1998). Therefore, due to the spatial heterogeneity of the physical disturbance, caution should be used in adopting this species as a uniform indicator of environmental change (Keough and Quinn, 1998).

Limpets (*Patella* spp.)

Although some studies outside the Mediterranean revealed no effect of human physical disturbance on limpets (Povey and Keough,1991), more recently some authors considered them as good bioindicators both for trampling and harvesting effects within Mediterranean MPAs (Safriel *et al.*, 1995). The most common species in the Mediterranean midlittoral zone are *Patella rustica* (upper midlittoral zone), *P. aspera* and *P. coerulea* (both inhabiting the lower midlittoral zone).

Bioconstruction formations

a) The algal-vermetids formations (*Dendropoma petraeum*)

Vermetid biogenic formations are located on rocky shores at intertidal level (Pérès and Picard, 1964; Pérès, 1967; Safriel, 1975; Laborel, 1986; Antonioli *et al.*, 1999). Their skeletal structure results from the association of the tubular shells of the gastropod *Dendropoma petraeum* and the rigid structure of the coralline alga *Neogoniolithon notarisii* and the foraminifer *Miniacina miniacea* (Laborel, 1986; Bellan-Santini *et al.*, 1994). The relative proportions of the main components are highly variable, following the degree of exposure to surf. Vermetid formations are not very common in the Mediterranean shores being restricted to the southern part of the basin (i.e. warmer areas) mainly on carbonate shores (Chemello *et al.*, 1990). Their susceptibility to erosion by human trampling is very likely.

b) The *Lithophyllum* rim

The distribution of *Lithophyllum* formations is limited to the lower midlittoral zone mainly in the western Mediterranean basin (excluding the warmer part) and generally is well-developed on exposed coasts. The morphology of the rim is highly variable, from a simple coating to a wide step-like structure. It grows on vertical or subvertical rocky substrates, and may attain 2 m in breadth and 1 m in height. The most important species is *Lithophyllum lichenoides* that produces small cushion masses with a lamellar arrangement (Pérès, 1967; Laborel, 1986). Another calcareous alga (*Dermatolithon confinis*) encrusts the crevices of this formation (Ros *et al.*, 1984).

2.2.5. Sampling strategy

A major constraint in detecting human impact in marine habitats is the difficulty of separating anthropic effects from natural spatio-temporal variability (see Stewart-Oaten *et al.*, 1986; Schmitt and Osenberg, 1996). As a result, field experiments before and after trampling (the recovery of disturbed populations) and possibly a wide range of intensities and spatial scales is recommended (see Underwood, 1994).

Thus, long-term monitoring programs at large spatial scale are efficient to detect intra- and interannual variations in population size (abundance, coverage, *etc.*) and size-distribution, minimising natural variability in time (i.e. recruitment success, mortality rates and size-dependent mortality) and space. Moreover, the effects over a range of intensity are of particular interest since human trampling on rocky shores is not constant during the year (and this is particularly so in the Mediterranean sea), being concentrated in certain periods (i.e. late spring, summer, and early autumn) and localised areas.

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2.3. METHODS FOR STUDYING THE IMPACT OF DIVER FREQUENTATION AND MOORING ON CORALLIGENOUS COMMUNITIES

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2.3.1. Introduction and objectives

The human activities linked to 'aquatic tourism' in coastal areas are mainly SCUBA diving, boating and fishing. The use of an anchor to immobilise a boat is common to all these activities and can thus have an impact on one of the main biocenoses of littoral areas, the coralligenous community. Boat mooring can affect (i) the coralligenous structure or its architectural complexity (i.e. at large scale level) or (ii) can induce damage of species inhabiting coralligenous areas (abrasion, 'uprooting' or breaking). These two levels of impact are studied according to two kinds of method: the first concerns large scale level (the community) and the second small scale level (the species). We summarize here the main sampling methods reported in the literature for studies at both scale levels.

2.3.2. Limits of the community

Coralligeneous concretions constitute an important type of Mediterranean calcareous deposit extending mainly from 40 m depth down to 120 m (Pérès and Picard, 1964) and is the richest community in term of both species and organisation among the Mediterranean benthic communities (Ros *et al.*, 1985). These bioconstructions are mainly built by Corallinaceae algae and some invertebrates which strengthen several meter thick rims and banks (Laborel, 1961). Such species have fragile skeletons and a low turn-over rates (i.e. low P:B values). Several organisms are responsible for the destruction of coralligeneous concretions (Laubier, 1966) and the echinoids, like *Paracentrotus lividus* and *Sphaerechinus granularis* are known to erode them (Laubier, 1966; Sartoretto and Francour, 1997).

2.3.3. Description of large-scale characteristics: the community

The main large-scale characteristic of coralligeneous concretions is the rugosity linked to the architectural complexity of the biotopes. Several authors have attempted to define the structural complexity of rocky substrate. Luckhurst and Luckhurst (1978) measured structural complexity by the ratio of developed length to linear length. This method is hardly suitable if the rocky bottom consists mainly of blocks or boulders. Francour *et al*. (1995) and Harmelin *et al.* (1995) use respectively scales from 0 to 3 and 1 to 4 to quantify the structural complexity. The estimated complexity measures are well suited to a local study managed by the same team, but could hardly be comparable between authors or between locations. For complex bottoms, mainly composed of blocks or boulder assemblages, Ruitton *et al.* (*in press*) developed a cavity index according to the method of Molles (1978). The structural complexity is characterised by: (i) mean size (A) of the external face of blocks (mean of all a measurements), (ii) mean size (B) of cavity aperture between blocks (mean of all b measurements). The cavity index (Cav), expressed in percent, is calculated as follows:

$$Cav = B^2.100/(A^2+B^2)$$

On each randomly selected block and cavity two measurements, one parallel to the sea surface and the other perpendicularly corresponding to the block or cavity median are taken (Fig. 1).

A new approach for architectural complexity was proposed by Garrabou *et al.* (1998a) following landscape theory developed in terrestrial ecology. Underwater photographs are taken using a Nikonos V underwater camera, equipped with a 28 mm and a close-up lens, two synchonized strobes to reduce shadows, and a fixed frame. Each slide was processed in the laboratory to underline the limits of each taxon or group covering the substrate. A special landscape software allows calculation of landscape indices which characterize the studied community.

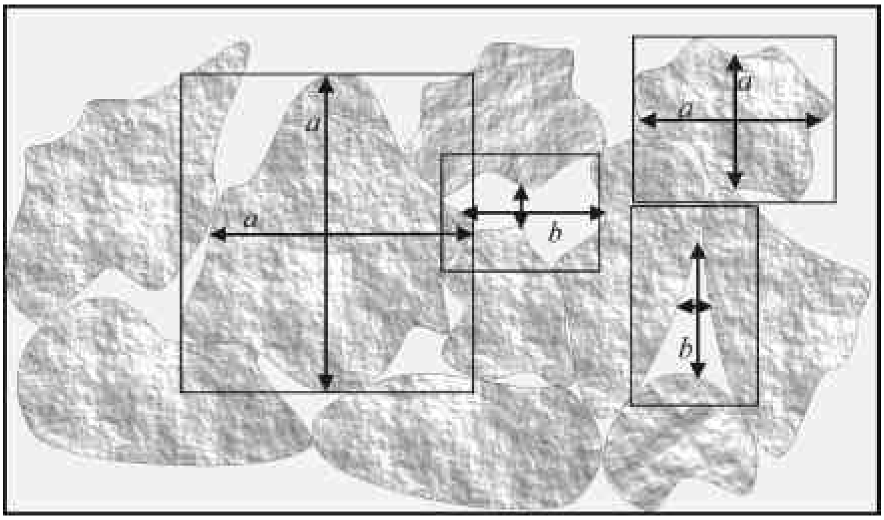

Figure 1: Measurement of (a) block external face and (b) cavity aperture in blocks or boulder assemblages to calculate a cavity index. (redrawn from Ruitton *et al*., 2000).

2.3.4. Description of small scale characteristics: the species

Three major components of the coralligeneous concretions could be monitored at small scale, the red coral (*Corallium rubrum*), the seafans (*Paramuricea clavata*, *Eunicella* spp., *Lophogorgia ceratophyta*), and the large erect bryozoan (*e.g. Pentapora fascialis*).

Red Coral (*Corallium rubrum*)

Monitoring the whole assemblage of red coral colonies in an area could imply the use of a high-tech sampling device (such as remote operated vehicle, video recording, deep dive with mixed gas, *etc.*) due to the bathymetric range of this species. In the upper part of this bathymetric range (down to 50 m in depth), an estimation of the demographic structure of the larger (or higher) colonies makes it possible to to well characterize the part of the assemblage most sensitive to erosion (Harmelin *et* *al.*,1999). About 30 colonies are measured in situ by a SCUBA diver who randomly selects the highest colonies. The maximum height, the maximum span and the diameter of colony foot are measured on selected colonies with a small graduated ruler and a divider.

Seafans

The characteristics of the demographic structure, and biometry of a seafan population could be studied with 4 parameters: (i) density of seafan populations, (ii) the maximum height of seafan (defined as the distance from the colony base to the end of the longest branch), (iii) proportion of cankered (or injured) seafans, (iv) the cover of necrosis on individual cankered seafan (Harmelin and Marinopoulos, 1994; Harmelin *et al.*,1999).

The density of seafans is usually estimated on a 1 m² frame (or sometimes 0.5 m²). From the practical point of view, to avoid damage to erected seafans, the 1-m² surface can be delimited with a rope or two 1 m solid rods linked by two 1 m ropes. If the density of the seafan population is low or if the depth is too great (more than 40 m), the density can be measured along 5 x 2 m² transects. They are delimited by a rope laid on the bottom with a 2 m rod at one end and a weight on the other end.

At shallow depth (10 to 25 m), the mean height of seafan and the proportion of cankered seafans can be measured on the same quadrat (1 m² or 0.5 m² in dense seafan populations). In deeper zones, the dive time is limited and a random method is often more efficient (Harmelin and Marinopoulos, 1994; Francour, 1998). The proportion of cankered seafans in a population is visually estimated by randomly counting the number of healthy seafans (no necrosis) and the number of cankered seafans (more than 5% of the seafan surface covered by necrosis). These random counts allow a rapid evaluation of parameters in large areas (Francour, 1998).

The cover of necrosis on individual cankered seafan is made easier by comparison with an underwater model showing different percentages of necrosis (Fig. 2). These models can be used in shallow and deep areas.

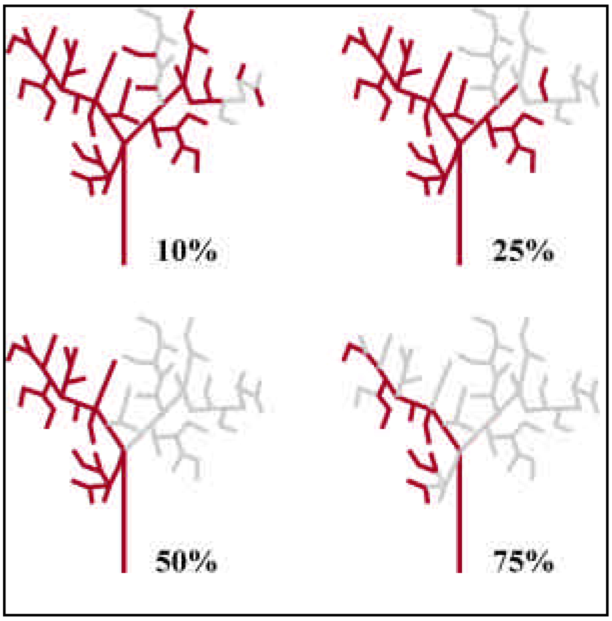

Figure 2: Underwater model of seafan showing different percentages of necrosis (grey parts) *vs* non-injured coenenchym.
(redrawn from Harmelin *et al*., 1999).

In some cases, the age of injuries on seafan colonies could be a relevant parameter to estimate. A rapid method was proposed by Harmelin and Marinopoulos (1994). The injuries are considered as recent if there is no apparent fouling and old for fouled seafans. A more accurate method, only usable after harvesting a colony, has been proposed by Mistri and Ceccherelli (1993) for *Lophogorgia ceratophyta*, according to Grigg's work (1974).

Bryozoans

Frames of 1m² are randomly positioned, and all bryozoan colonies are measured for diameter, height, and degree of exposure (Sala *et al.*, 1996). Generally 20 to 50 replicates are done to smooth variations in density. The diameter and height of all bryozoan colonies are measured to the nearest centimetre by a SCUBA diver using a plastic ruler. Some authors analyse biological parameters (*e.g.* colony size, shape, height) on underwater photographstaken in quadrats (*e.g.* Cocito *et al*., 1998). To evaluate the degree of exposure of colonies to abrasion, the colonies are classified into one of five categories, according to decreasing (maximum to minimum) degree of exposure (1: as epibionts; 2: on convex surface; 3: on flat surface; 4: in concavity; 5: under overhang; Fig. 3).

2.3.5. Sampling strategy

The assessment of human impact has generated a healthy debate and it has been suggested that 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 after the impact and more than one (sampling) location per each of two groups (control and impact) (Underwood, 1990). However, some authors considered more than one sampling time before and after the impact and a greater number of locations (control-impact) to be more efficient (designs called "beyond BACI"; see Underwood, 1994).

The main coralligenous species (*Corallium rubrum*, seafans, large erect bryozoans) have a long life span and do not exhibit great seasonal fluctuations in biomass or volume, even if some variations of biological parameter (i.e. as feeding) are observed over a year cycle (Ribes *et al.*, 1999). Consequently, only one survey per year is generally sufficient to quantify the structure of populations (Garrabou *et al.*, 1998b).

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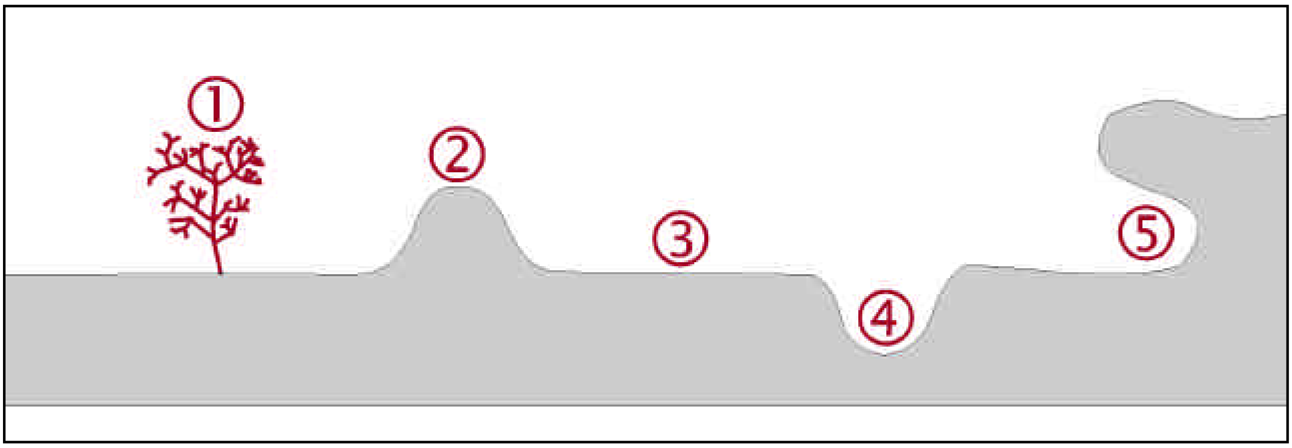

Figure 3: Degree of exposure of bryozoan colonies as epibionts (1), on convex surfaces (2), on flat surfaces (3), in concavities (4), and under overhangs (5) (redrawn from Sala *et al.*, 1996).

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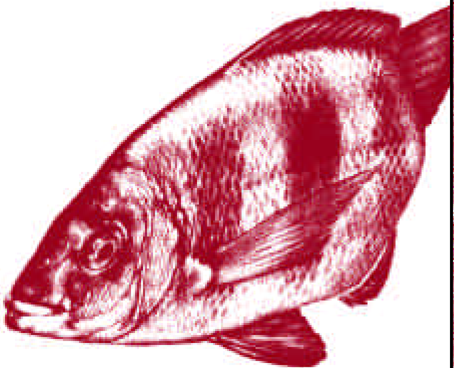
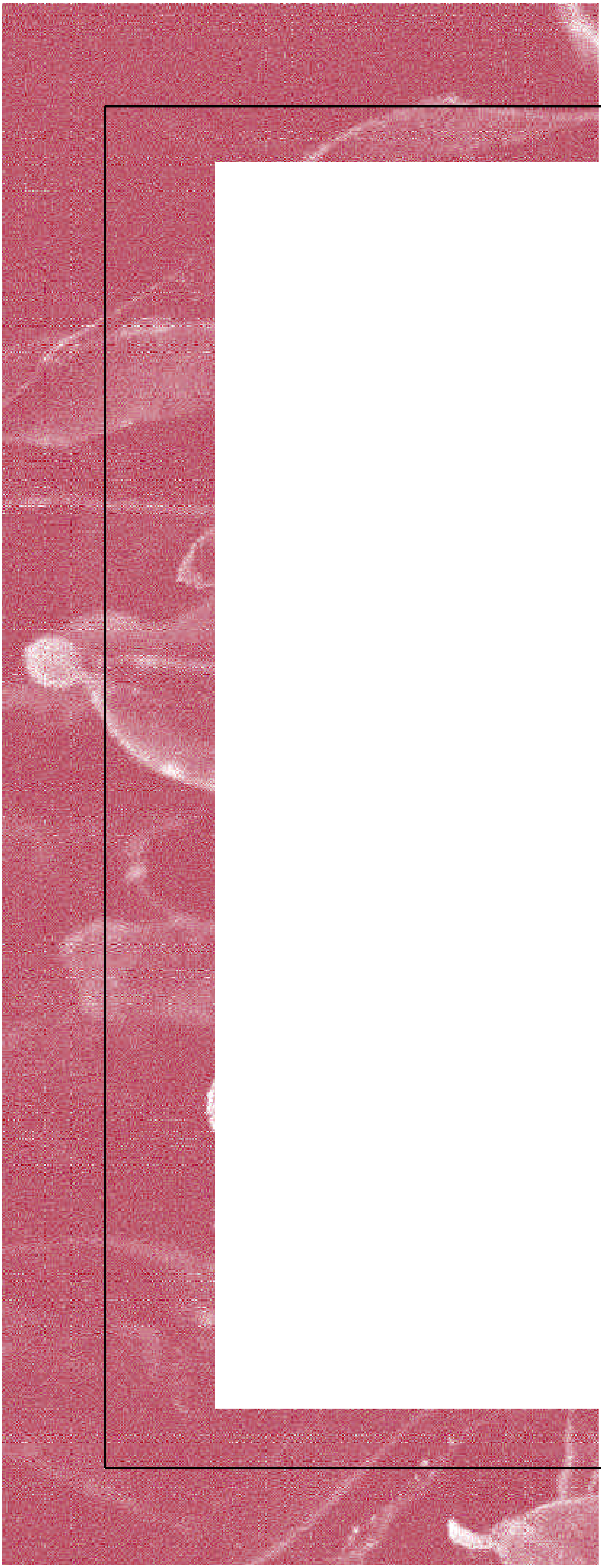
CHAPTER 3.

METHODS FOR MONITORING AND ASSESSING COMMUNITY LEVEL EFFECTS OF PROTECTION: FISH-URCHIN-ALGAE INTERACTIONS





INTRODUCTION

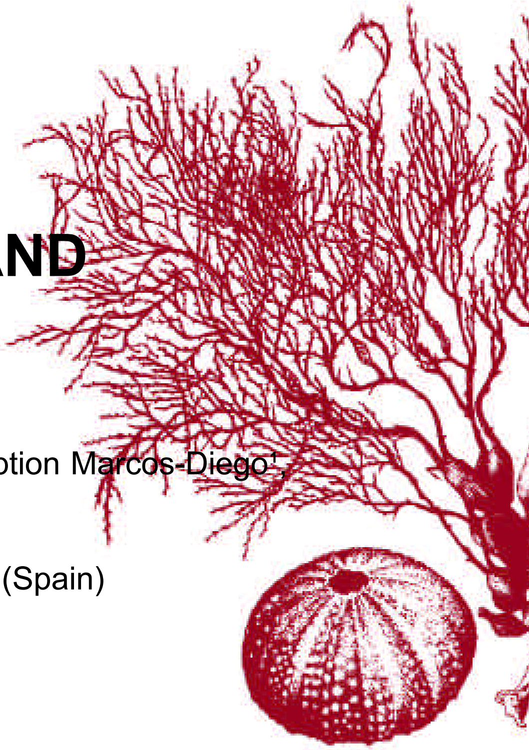
Sea urchins play an important role in determining both the composition and structure of sublittoral communities. As the main herbivore along Mediterranean coasts, the sea urchin *Paracentrotus lividus* can produce severe effects on algal communities by over-grazing erect algae and inducing the formation of coralline barrens. At the same time, it has been hypothesized that the abundance of sea urchin populations is controlled by predation, especially by fish.

Thus, one of the more conspicuous results of the cascade effect observed when comparing protected and non-protected marine areas is the relationship between fishes, urchins and algae. Fishes eat urchins and urchins graze on algae. The absence of fishes which feed on sea-urchins leads to a proliferation of these echinoderms and to an almost complete loss of algal cover. In contrast, in marine reserves, the top-down control asserted by fishes permits a well-developed and structured vegetation. Consequently, when studying the cascade effect researchers look for direct relationships between fish abundance, urchin density and algal recovery.

This chapter will review methods for studying the three individual components of the fish-urchin-algae interaction. First, methods for studying the main aspects of sea urchin population dynamics (density, size structure, recruitment and mortality) and biology (reproduction and diet) will be reviewed. Secondly, methods for studying both fish assemblages and benthic algae are presented with reference to the fish-urchin-algae interaction.

Successively, the importance of habitat structure in fish-urchin-algae interaction will be dealt with and ways of measuring habitat structure in Mediterranean littoral habitats reviewed. Grazing and predation are the objects of the section dedicated to the study of the processes involved in fish-urchin-algae interaction. The most common methods of investigation utilised will be reviewed, highlighting weaknesses and strengths.

The last section of this chapter will deal with sampling strategies. Here the aim will be to give the reader a general idea of field experimental sampling design in both descriptive and manipulative studies.



3.1 SAMPLING TECHNIQUES

3.1.A. POPULATION DYNAMICS AND BIOLOGY OF SEA-URCHINS

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3.1.A.1. Introduction

Sea-urchin density and the success of a population depend not only on the pressure of depredation and food availability, but also on factors controlling fecundity and reproductive success and recruitment. Echinoderm reproduction is controlled by a range of environmental and endogenous factors such as photoperiod and temperature (Pearse *et al.*, 1986) and also by quality, quantity and availability of food resources, that affect seasonality (Tyler and Gage, 1984), fecundity and gonad yield (Hagen, 1998).

Furthermore, trophic status can also affect the grazing pressure of sea urchins on algae. Eutrophic conditions involve changes in algal assemblage composition favouring the development of species with different palatability (as *Enteromorpha* spp. or *Ulva* spp. in front of *Cystoseira* spp., *etc.*). Regular echinoids have a wide spectrum of food types including soft-bodied organisms (plants and animals), hard surfaces (rock with encrusting or boring plants and animals) or hard animals (coral, bivalves) and soft substrata (De Ridder and Lawrence, 1982) and, basically, they are opportunistic in their feeding. A species may be functionally herbivorous at a particular place or time due to the availability of plant material but this does not make it compulsorily a herbivore (Lawrence, 1975). In fact, suspended materials such as drift algae or seagrasses and zooplankton can play an important role as food resource. Debris are captured by spines and tube-feet, carried to the mouth and eaten (De Ridder and Lawrence, 1982).

In the Mediterranean, it is not in all marine protected areas (where the absence of overfishing increases the pressure of predators) that there has been a decrease in sea urchin abundance, and the also opposite applies, that in all non-protected areas there is lesser abundance. Currently, the influence of other processes such as the potential capacity for reproduction, dispersal, availability of shelter or recruitment is being discussed.

To improve our understanding on *Paracentrotus lividus* and other dominant sea-urchin species population dynamics is a strong priority. Furthermore, as undisputed key-species, P.lividus population structure can provide meaningful information about the state of the whole littoral zone (Sala *et al.*, 1998).

Density and size structure are the first parameters for understanding sea urchin populations. These simple parameters give easily collected but important ecological information about the species and about the marine zone in general (Turon *et al.*, 1995; Sala *et al.*, 1998, Estes and Duggins, 1988). Because of their high density and the conspicuousness of sea urchins, these two descriptors can be easily studied *in situ*, by means of underwater visual censuses, without destructive methods. Moreover, this non-destructive method allows easy replicative protocols for the monitoring of natural populations over time.

Post-settled *P. lividus* population dynamics has been largely neglected even if their earliest phases as benthic components can be a key bottleneck for the success of each cohort. Thus recruitment and survival rates of post-settled cohorts can play an important role in determining the magnitude of adult populations (Rowley, 1989; Ebert, 1983; Verlaque, 1987; Azzolina, 1988; Lozano *et al.*, 1995; Sala and Zabala, 1997; Lopez *et al.*, 1998).

Furthermore, because of these complex interactions it is also important to monitor both feeding strategies and reproductive activity and fecundity when studying the cascade effect through fish-urchin-algae interactions.

3.1.A.2. Evaluating populations

All methods to determine density and size structure consist in delimiting a minimum size area and counting all sea urchins located there. Some authors use circular 10 m² plots (Pearse and Hynes, 1987) or randomly repeated 0.25 to 1 m² quadrats (Carpenter, 1986; Rowley, 1989; Kenner, 1992; Watanabe and Harrold, 1991); but in the Mediterranean area the most widely used method to study these two populational parameters is by way of linear transects, 25 to 50 m long x 1 m wide (Sala, 1996; Sala and Zabala, 1996; Sala *et al.*, 1998; Harmelin *et al.*, 1980; Turon *et al.*, 1995). In order to estimate the size-structure population, all sea urchins >1 cm diameter inside the transect are counted, and their diameter (test without spines) measured with calipers. Alternatively, when densities are extremely high, only the first 100 individuals have to be measured (Sala *et al.*, 1998).

When attempting to study post-settled sea urchin populations destructive methods (scraping) are unavoidable because of the impossibility of detecting accurate numbers of small post-settlers by visual censuses (Pearse and Hynes, 1987). Scraping is destructive and expensive (time consuming) but it is useful to describe the firsts steps of natural sea urchin populations in the field. It is also feasible to monitor natural survival over time, and to use population models to calculate mortality rates (Rowley, 1990; Kenner, 1992; López *et al.*, 1998).

Scraped samples have proved to be useful tools for the study of sea urchin recruitment (López *et al.*, 1998, Sala and Zabala, 1997; Pearse and Hines, 1987). Alternatively, artificial collectors are useful tools and they must be preferred for the study of the recent arrival of new individuals among adult populations: collectors represent a non-destructive sampling method, faster and easier to process than scraped samples. Moreover, supposedly there is no natural mortality (or less than in its natural habitat), and all individuals found in the collectors come from the collector running schedule.

Different types of collector have been proposed: oyster shells (Loosanov, 1964), rigid plastics (Tegner, 1989), plastic light diffusers (Bak, 1985; Harrold *et al.*, 1991), artificial grass (Ebert *et al.*, 1991) and brushes (Ebert *et al.*, 1994). Brush collectors seem to have major advantages over other sea urchin collectors in not requiring special fabrication, being cheaper and providing convenient replications of collector surfaces.

Artificial collectors can be easily sampled and replaced in very short time periods, allowing long time series monitoring, and providing very up-to-date information about recruitment processes (Ebert *et al.*, 1994; Harrold *et al.*, 1991).

Problems and limitations

Visual census seems to be the most easy and direct method to estimate sea urchin populations. The only restriction of this kind of sampling is the impossibility of studying post-recruits and early juvenile individuals because of the probable high error of in situ counting of sea urchins smaller than 1 cm (Sala *et al.*, 1998; Pearse and Hynes, 1987). Alternatively, scraping methods permit the study of recruits and post-settled phases, but apart from its destructive and time-consuming nature, there are several non-controlled factors (spatial heterogeneity, depth, algal cover, *etc.*) and natural processes (mortality, accumulation of oldest individuals, *etc.*) that could introduce error in our estimations. All these factors can be minimised with artificial collectors, that are useful for estimating the recruitment rate but not for natural processes occurring in natural substrata.

3.1.A.3. Studying the reproductive biology in sea urchins

Reproductive biology and strategies in marine invertebrates are studied on the basis of gonad and maturity indices, absolute and relative fecundity and the analysis of oocyte size/frequency data (Grant and Tyler, 1983 a and b). Gametogenic cycles in echinoderms are generally well understood and relatively easy to determine from gonad indices and histological examination. Gonad and maturity indices give us information on the reproductive periodicity and seasonality and on the size/age of first maturity. Fecundity and the analysis of oocyte size/frequency data give more accurate and powerful information on reproductive cycles and reproductive effort.

Collection and Preparation of the Material

When assessing gonadal activity and reproduction, animals of different sizes are sampled. Five to 10 subjective classes and 30 to 50 individuals (5 to 10 by size class) are usually used. Regular sampling, at intervals of one month, should cover a period of at least one year to include one annual reproductive cycle. However, in the context of the cascade-effect study it could be enough to include the reproductive season (maturation and spawning) of the involved species. Anyway, it must be considered that the reproductive season can be different in the distinct geographical areas included in the study because of differences in water temperature and climate.

After collecting, echinoids must be fixed in 8% seawater formaline and subsequently transferred to 70% alcohol. Prior to dissection, the whole animal must be damp dried and weighed and the test diameter and height measured. Gonads are dissected, damp dried and weighed.

Sea temperature, quantity and quality of nutrients and chlorophyll and benthic algal community composition and biomass must be recorded at each site.

Gonad index

The simplest indicator to estimate the reproductive state is the gonad index (G.I.). It is calculated with the equation:

G.I. (%)=(gonad weight/total body weight)* 100

It may be computed on a basis of wet weight or dry weight. In the latter case we avoid problems related to the variability of the water content of the animals.

Gonad histology and size/frequency data of oocytes

Sections of gonads can be studied in order to obtain quantitative data relating to the reproductive events. The microscopic study of the gonads is necessary as the G.I. by itself doesn't explain reproductive cycles, gonadal growth, cytological differentiation, spawning and/ or fecundity. With this additional study we can also estimate the cause/s of such gonadal growth, gametogenesis, maturation rate, *etc.*

For histology, one of the gonads of each individual must be fixed in Bouin´s fluid, rinsed in distilled water, dehydrated in an ethanol series, embedded in paraffin and sectioned. Serial cross- sections (7-8 mm thick) cut through the centre of a gonad can be stained with haematoxylin and eosin, PAS, Sudan Black B, or alcoholic tolouidine blue (Tyler and Gage, 1984). The gametogenic condition was categorised into five maturity stages based on the morphological criteria used by King *et al.* (1984) and analysed using a microscopy.

For the on-location comparison during breeding, the gametogenic status of the ovaries will be examined in detail and recorded photographically.

In females, the sizes of oocytes can be measured using a microscope and image analysis software. Only those oocytes sectioned through the nucleus must be measured (up to 100 oocytes). The range of oocyte sizes found is divided up into sizes classed according to maturity stages. As a precaution against heterogeneity of development within an ovary, it is wise to measure oocyte size in several sections and along two perpendicular axes on each section (Grant and Tyler, 1983 a).

Fecundity

Reproductive effort can be estimated by means of the total number of eggs produced by one individual. Absolute fecundity (total number of eggs by individual) and relative fecundity (number of eggs produced by unit of body weight) can be obtained by counting the number of mature eggs in an aliquot of gonad referred to the total volume.

3.1.A.4. Study of diet in sea urchins

Sea urchins exert a profound influence on the structure of macroscopic algal communities in temperate marine ecosystems. Their grazing can determine directly the distribution, abundance and species composition of the algae present. In some areas of the Mediterranean, urchin populations can become so large that overgrazing results and all macroscopic algae are removed, changing such habitats into a low productivity system termed coralline barrens.

The feeding preferences of sea urchins and their effects on benthic communities have been extensively studied in several habitats. Urchins can adopt two principal types of diet, grazing over the substrate or absorbing dissolved organic material directly from seawater. Some investigators have observed cannibalism among urchins under conditions of food deprivation or when food resources were drastically reduced.

In crust-dominated communities, urchins can persist for several years because they can adopt different strategies such as reducing metabolic activity (resulting in reduced body and gonad growth), reabsorb portions of their bodies, eat suspended materials and/or absorb dissolved organic material. Additionally the can prey on benthic diatoms, coralline algae, filamentous algae, detritus and carrion. In the Mediterranean, *Arbacia lixula* and *Paracentrotus lividus* appear to be mainly her-

bivorous (Frantzis *et al.*,1988). They display different feeding strategies. *A. lixula* is a selective browser feeding mainly on the fleshy algal turfs and the encrusting strata, while *Paracentrotus lividus* is a grazer that feeds on the erect algae strata and particulary *Padina pavonica* and *Cystoseira* spp. However very little is known about the trophic plasticity of these species under different trophic conditions and their further repercussion in the cascade effect. There are various hypotheses about such strategies for both urchins:
- they acquire different feeding strategies depending on competence and resource availability: filter-feeding, omnivorous- herbivorous, cannibalism;
- each species can graze over different kinds of algae and show preferences so that competition is not a problem;
- they feed on the same resources but they avoid competition living in different micro-habitats/depths (exclusion).

Qualitative and quantitative analysis of gut contents in sea urchins can provide information about the behaviour and kind of diet (Verlaque and Nédelec, 1983), palatability (Anderson and Velimirov, 1982), preference (Hay *et al.*, 1986), feeding rates and also the relationship between food ration and reproductive effort (Thompson, 1982) in function of the trophic status of the environment.

The gut contents of the captured specimens can be examined and identified through the microscope. Quantification of food items can be made after centrifugation to concentrate the solid material, drying at 80ºC for at least 72 h, weighing and ashing at 500ºC for at least 7 h and reweighing (Black *et al.*, 1984) or by volumetric methods (Frantzis *et al.*, 1988). Comparisons must be made between individuals with similar test diameter. The different species found can be classified into the different morphotypes (fauna, planktonic algae, erect vegetation, turf and crust). It is also usual to assess the preferential food by estimating the main species digested in function of their frequency of occurrence or relative abundance in the gut contents (Verlaque and Nédelec, 1983; Verlaque, 1984).

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3.1.B. SAMPLING TECHNIQUES : FISH ASSEMBLAGES

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3.1.B.1. Introduction and objectives

Despite its recent introduction, visual census has been employed in many studies to survey littoral fish assemblages. This method allows suitable space-time monitoring of the observable portion (sensu Harmelin-Vivien and Francour, 1992) of the fish assemblage and its methodological aspects have been studied widely (Keast and Harker, 1977; Jones and Thompson, 1978; Christensen and Winterbottom, 1980; Sale and Douglas, 1981; Sale and Sharp, 1983; Thresher and Gunn, 1986; McCormick and Choat, 1987; Lincoln-Smith, 1989; Buckley and Hueckel, 1985; St-John *et al.*, 1990), even compared with other techniques (Bortone *et al.*, 1986; Bergstedt and Anderson, 1990; Harmelin-Vivien and Francour, 1992; Michalopoulos *et al.*, 1994; Parker *et al.*, 1994) and its application in different situations has been discussed (Kimmel, 1985; Sanderson and Solonsky, 1986; Bohnsack and Bannerot, 1986; Bortone and Kimmel, 1991; Bortone *et al.*, 1991). For a review of different aspects see Harmelin-Vivien *et al.*, (1985).

3.1.B.2. Recommended methods

The most suitable visual census methodology to obtain quantitative data is the strip-transect, carried out along a defined distance and with known width. The most used sample unit is the 50 x 5 m strip transect, although this can be adapted according to the home-range of the studied species. During the survey, the diver records the number of individuals of each species noted on a logarithmic scales with predetermined abundance (GBRMPA, 1978), and estimates the size of each individual in order to define the size structure of the fish assemblage and estimated biomass. Some techniques can be used to estimate the fish length but the most useful and easiest is that carried out using a ruler attached to the end of a stick (Bohnsack and Bannerot, 1986). Training helps to increase accuracy in fish length estimates (Bell *et al.*, 1985). If the sampling is done by several researchers, it is absolutely necessary that the divers train together by doing some visual counts. A comparison of the mean and the size estimated for different species will make it possible to determine the accuracy and to compare the reliability of the results between divers.

In order to study the fish assemblage in with regard to the fish-sea urchin-algae connection

framework, the best strategy is the use of the 50 x 5 m strip transect over infralittoral rocky bottoms, counting all fishes observed.
The sampling design must be able to inform about variability from spatial and temporal sources. The ideal is thus to include several hierarchized spatial (site, locality, zone) and temporal (day, month, season) scales. The desirable minimum number of samples in each level of replication should be 3 in order to achieve suitable statistical power.
The effects of protection should be assessed comparing several protected and unprotected zones. If only one protected zone is available, the comparison can be done between this one and several unprotected zones (assymetric designs).
The fish data from the 50 x 5 m strip transect could be complemented by carrying out other sized strip transects for species with different life-histories: *e.g.*, small 10 x 1 m strip transects could be carried out for small cryptic species such as Blenniidae, Gobiidae, Tripterigiidae; or larger (*e.g.*, 400 x 10 m) strip transects could be done to record big highly mobile species such as Sparidae, Serranidae such as *Epinephelus* spp. or Carangidae.

3.1.B.3. Problems and limitations

This method could be limited by poor visibility, bad weather or high currents. Moreover, the training level of observers can affect the quality of data owing to underestimates of number of individuals and misidentification of species. Other problems with this technique are related to the possibility of counting repeatedly the same individuals, or the low sensitiveness of this technique for recording cryptic high sedentary species. See Harmelin-Vivien *et al.* (1985) for a review of limitations and constraints.

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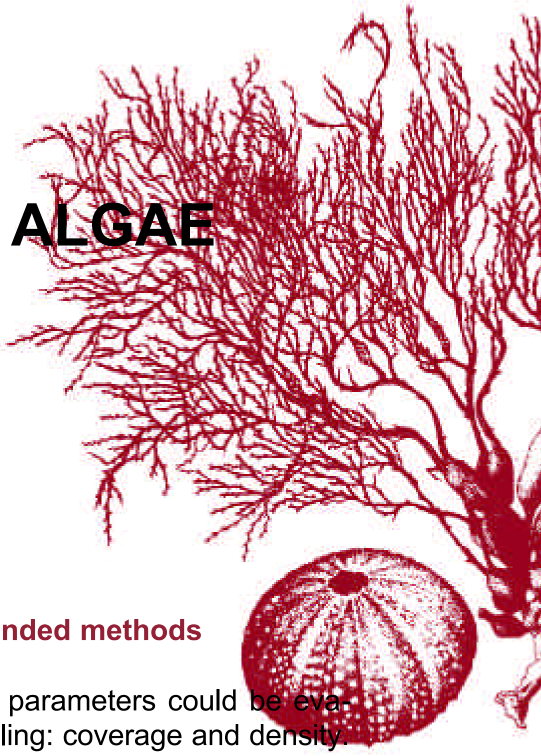

3.1.C. SAMPLING TECHNIQUES : ALGAE

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3.1.C.1. Introduction and objectives

The study of benthic phytocoenoses in the Mediterranean sea has maintained a purely descriptive approach for decades. The high heterogeneity of Mediterranean marine habitats, and in particular of benthic phytocoenoses, is probably a major constraint for the correct evaluation of ecological processes within Mediterranean marine protected areas.

Only recently, the direct and indirect roles of fishes in the organisation of the algal benthic community were elucidated through experimental investigations using exclusion cages (Sala and Boudouresque, 1997), whereas specific problems related to sea urchins and limpets grazing on algae were dealt with using manipulative experiments (*e.g.* Benedetti-Cecchi and Cinelli, 1995).

Three different approaches may be used for the evaluation of the effects of protection at community level: 1 - evaluation of algal biodiversity as a whole; 2 - evaluation of reserve effects on target-species; 3 - evaluation of changes induced by protection on ecological processes. Cases n° 2 and 3 could be approached using the same method.

3.1.C.2. Recommended methods

Two phytocoenotic parameters could be evaluated before sampling: coverage and density. The first was well described by Boudouresque (1971) who differentiated between covering (the surface percentage of the substratum covered by algae without any distinction among species and strata) and coverage (the part of a sampled area covered by a single algal species, expressed as surface percentage).

Covering can be estimated using quadrats of standardized surface (from 100 to 2500 cm², less frequently to 1 m²) in which the total surface covered by algae is estimated and referred to 1 m².

Coverage can be estimated by the projection of each algal species on the sampling surface. Total coverage is then different from covering because it represents the sum of the specific coverages, divided in strata, and often it could exceed 100%.

A similar technique can be used to assess density (the mean number of algal stands per square meter) for each species. In this case, the number of stands for each algal species is counted in a statistical number of quadrats (random replicates) of known surface and reported as mean number of stands per square meter.

1 - For the complete evaluation of the whole algal diversity, collection of sample scrapings off algae from the substratum is recommended. In this case, we should first define the minimum sampling surface using the rarefaction curve of the area/species ratio. The problem of minimum sampling area in Mediterranean phytobenthos was exhaustively discussed by Boudouresque (1971) and Ballesteros (1986). In these papers, areas between 140 and 600 cm² were taken into consideration and evaluated.

Algal samples should be collected on surfaces homogeneously carpeted by the same algal species (the dominant one in terms of surface covered), avoiding discontinuities, with the use of chisel and hammer to lever the holdfast's attachment to the substratum. In the laboratory, samples are sorted and identified at species level.

This destructive method was first proposed for the Mediterranean sea by Bellan-Santini (1969) and Boudouresque (1971). More recently, this technique was also used by Sala and Boudouresque (1997) studying the role of fishes in organising Mediterranean benthic communities: collection of samples was performed by scraping off all the algae from the substratum on a surface of 250 cm². This surface encompasses in their opinion the whole range of variation due to the extreme miniaturisation of phytocoenoses into the Mediterranean Sea.

As for the shape of samples, Littler and Littler (1985) suggested using rectangular quadrats, with long axis parallel to the axis of environmental gradient, because they tend to incorporate a greater diversity than square quadrats but there is no consensus among researchers on this assumption.

2 - No-impact sampling procedures may be used for the evaluation of ecological processes involving algal assemblages or the reserve effect on target-species. In these cases underwater photos or visual censuses are commonly used. The photogrammetric method is a no-impact sampling technique involving permanently marked locations. This technique is generally used to obtain detailed and quantitative data (coverage, frequency and density) for a given algal species (case 2) or the whole assemblage (case 3). The advantages of the use of underwater photos to study benthic phytocenoses are multiple: precision (i.e. fixed quadrats), reproducibility (different spatial scales), ethical considerations (easily feasible within no-take areas).

3 - More recently, Garrabou (1997) correctly reported the problems arising in algal-dominated benthic communities due to the complexity of the assemblages and proposed an alternative method for the accurate evaluation of the algal assemblages' diversity as a whole. He conducted a detailed analysis by scraping off areas of 400 cm² in order to define the composition and structure of the phytocenoses and to improve the results of photo-sampling. With this integrated technique the dynamic of 15 different algal species has been identified and monitored over time.

3.1.C.3. Problems and limitations

A general problem, common to the three methods, is the objective difficulty of identifying algae. There are few researchers who are specialized in benthic algae and not many of them are able to identify algae from a photo alone. This kind of problem could bias description of the composition of algal assemblages and their biodiversity value.

A limitation in the use of coverage estimation derives from geometric problems: algae with a regular shape or crustose are easily evaluated but an arborescent shape (as *Cystoseira* or

Sargassum) is difficult to measure, requiring other more complicated methods (Ballesteros, 1986).

1 - There are also problems associated with the use of the destructive sampling. The first is ethical: in no-take areas, this kind of sampling must be banned because of its heavy impact on benthic communities. The reversibility time is unclear but probably could be more than two years. A second limitation concerns the definition of the right dimensions for the choice of a minimum sampling area. This area could vary considerably between assemblages, depending on size and density of dominant algae, presence or absence of erect macroalgae, depth, pollution, light, *etc.*

2-3 - There is strong evidence that the low level of resolution in rich phytocenoses may be a major problem for the use of photogrammetric techniques or visual censuses. Erect macroalgae or in general upright forms canopy may conceal the amounts of underlying low profile species or turfs, detracting from the reliability of the assessment of the entire algal community. To overcome this problem of incomplete knowledge of the whole algal assemblage, some authors suggest cutting or temporally dislodging the upper macroalgal canopy to allow identification of the lower-level species (Littler and Littler, 1985). This method met with little success since it was simply not feasible in the field.

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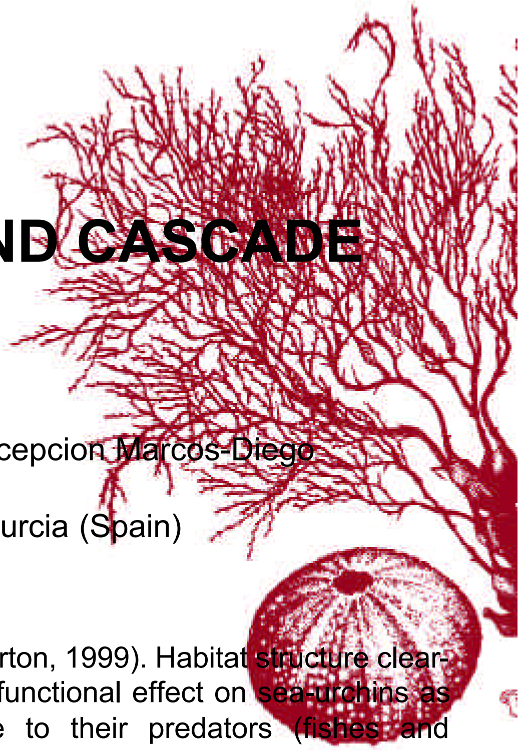
3.2. HABITAT STRUCTURE AND CASCADE EFFECTS



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3.2.1. Introduction

The importance of spatial variations of habitat structure for the understanding of the dynamics of marine coastal populations is increasingly acknowledged (Sebens, 1991). Variation in habitat structure can interfere with predation by facilitating predator avoidance (*e.g.* Beukers and Jones, 1997) or with competition by facilitating co-existence (*e.g.* Ives, 1995). If we consider habitat structure from a functional perspective, by including in this category any physical or biological, spatially recognizable attribute of the environment offering some kind of resource (food, shelter, orientation, *etc.*) to the organisms concerned at a given spatial scale (McCoy and Bell, 1991), then the problem involves ascertaining what features of this habitat are relevant to these organisms, what resources these features offer them, and how the organisms respond to spatial and temporal variations of habitat (García-Charton *et al.*, *in press*).

The influence of habitat on fish assemblages has been established in temperate as well as tropical areas (see references in García-Charton *et al.*, *in press*). In an analogous way, Mediterranean littoral fishes respond to small-to-medium scale variations of habitat structure (García-Charton and Pérez-Ruzafa, 1998; García-Charton, 1999). Habitat structure clearly exerts a functional effect on sea-urchins as a response to their predators (fishes and macroinvertebrates), so that they can be present in sites with strong predation pressure, but where shelters are available (Sala, 1996; Sala and Zabala, 1996). This relationship between abundance of sea-urchins and habitat structure in the presence of predators have long been recognized in other seas (*e.g.* Bernstein *et al.*, 1983; Mann, 1985; Andrew, 1993). The impact of grazers (mainly gastropods and sea-urchins), governing the dynamics of macroalgae, causes a shift from erect algal facies to coralline barrens, both in Mediterranean (Verlaque, 1984; Sala, 1996) and non-Mediterranean regions (Levitan, 1988; Morrison, 1988; Andrew, 1991). Habitat structure favours this effect by providing shelter for grazers against natural predators, so that the occurrence of barren areas is possible even in sites where predators are abundant (Sala, 1996).

Following Jones and Andrew (1992), when considering the biological component of habitat we can make the distinction between 'habitat formers' -those species that characterize a habitat, which are subject to an array of ecological processes governing their distribution and persistence (Kingsford and Battershill, 1998)- and 'habitat determiners' -those species capable of influencing the distribution of habitat formers, usually through predation or grazing. In the case of Mediterranean rocky substrates, algae, sponges, bryozoans, cnidarians, or vermetids can be considered as habitat formers, while fishes or sea-urchins are habitat determiners (García-Charton *et al.*, *in press*). Trophic cascades (*e.g.* fish-sea urchins-algae) are typical examples of the effect of habitat determiners (fish and sea-urchins) on habitat formers (algae).

3.2.2. How to measure habitat structure in Mediterranean littoral habitats?

When measuring habitat structure it seems worthwhile to distinguish between heterogeneity and complexity of habitat, defined by McCoy and Bell (1991) as the relative abundance of different structural components, and the absolute abundance of individual structural components, respectively.

Habitat heterogeneity

A kind of habitat heterogeneity at large spatial scale is that determined by the mosaic of different bottom types (soft bottoms, seagrasses, rock) forming the seascape (García Charton *et al.*, *in press*). At smaller scale, taking the rocky substrate as example, habitat heterogeneity would be the proportion of patches of sand and clumps of *Posidonia oceanica* embedded in the rocky matrix (Sebens, 1991; García-Charton *et al.*, *in press*), or even the difference between coralline barrens and foliose algal facies, determined by variability in grazing pressure or other physical disturbances.

Percentage cover by different bottom types inside a given sampling unit can be measured using more or less "objective" methods -photography, video, image processing, 'point-quadrat', 'intersected-length', *etc.*- (Foster *et al.*, 1991; Leonard and Clark, 1993; Garrabou, 1997; Robbins, 1997; Kingsford and Battershill, 1998; Pasqualini *et al.*, 1999), or by estimating visually the relative surface covered by each type (*e.g.* García Charton and Pérez Ruzafa, 1998). Although the latter method can be biased due to the observer's subjectivity (Baker and Little, 1989), its accuracy has been advocated when the observer is well trained (Dethier *et al.*, 1993), as well as being much simpler and faster, both useful attributes in underwater field work (García Charton and Pérez Ruzafa, 1998).

Habitat complexity

Habitat complexity is the physical topography, bottom relief, and number and size of fissures, crevices and holes, considered at different, hierarchically arranged spatial scales, due to the fractal geometry of coastal habitats (Bradbury and Reichelt, 1983, Bradbury *et al.*, 1984; Williamson and Lawton, 1991).

Different descriptors of complexity should be used at each spatial scale (García Charton *et al.*, *in press*). For large spatial scales (?10^2 m), descriptors such as verticality (García Charton, 1999) or vertical diversity (Helvey and Smith, 1985) are suitable variables. At intermediate scales (10^0-10^1 m), counting the number of rocky boulders classified by size (from pebbles to large boulders) has been shown to be useful for quantifying the relationship between fishes and complexity of Mediterranean rocky habitats (García Charton and Pérez Ruzafa, 1998, in prep.), and can be a way to approach the number and size of crevices opening to the surface - analogously to that modelled by Barry and Wickins (1992) and Wickins and Barker (1996) - when direct measurement is impractical. At smaller spatial scales (<10^0 m), rugosity has often been used as a measure of complexity (Kingsford and Battershill, 1998). Basically two methods have been performed: 'rope-and-chain' (*e.g.* Luckhurst and Luckhurst, 1978; Leum and Choat, 1980; Grigg, 1994; García Charton and Pérez Ruzafa, 1998), and 'profile-gauge' (*e.g.*

McCormick, 1994). It has been frequently shown that organisms responded to variations of different descriptors according to their body size and life-characteristics (García Charton *et al.*, *in press*).

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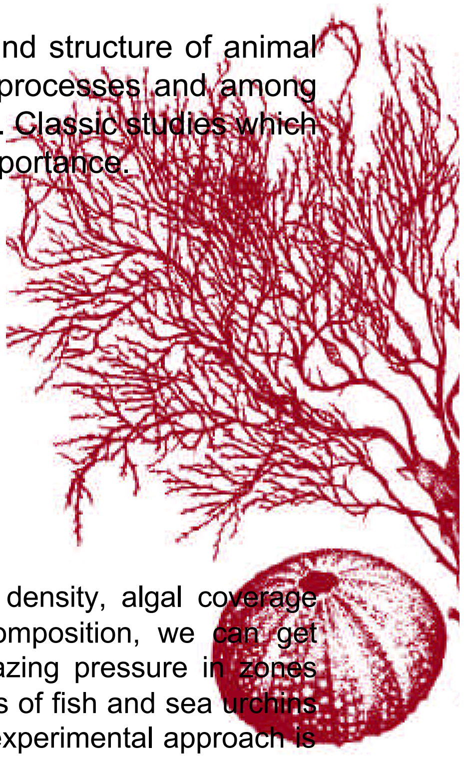
3.3. PROCESSES

The establishment of MPAs leads to changes in the composition and structure of animal and vegetal assemblages. Changes may be mediated by several processes and among them predation is probably the most important and well documented. Classic studies which refer to the fish-sea urchin-algae connection are of fundamental importance.



3.3.A. GRAZING

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3.3.A.1. Introduction and objectives

Fishing bans in MPAs potentially can produce changes in the phytobenthos, either in abundance, species composition and/or ecological processes (Hay, 1984; McClanahan and Shafir, 1990; McClanahan *et al.*, 1994; Sala, 1996, Sala and Boudouresque, 1997). The increase in numbers of fishes may cause changes in grazing pressure, directly or by cascading effects, by reducing sea urchin populations or by changing their behaviour.
Fish herbivory has a major role in tropical seas (*e.g.* Carpenter, 1986), but in temperate seas (and in the Mediteranean area) fish are not considered to be the principal herbivores: most studies suggest they have a negligible effect on algal abundance and diversity (Lundberg, 1980; Verlaque, 1990; Lundberg and Golani, 1995), but little attempt has been made to evaluate this effect experimentally (Sala and Boudouresque, 1997).

3.3.A.2. Recommended methods

There is an initial descriptive step for estimating the grazing pressure by fish and sea urchins in sublittoral communities. By assessing fish, sea urchin density, algal coverage and algal species composition, we can get some idea of the grazing pressure in zones with different densities of fish and sea urchins (Sala, 1996); but an experimental approach is needed to record and quantify the relative importance of each agent in the overall effect. Changes in both structure and composition of algal communities in caging experiments make it possible to distinguish between the effect of fish and sea urchins (Lewis, 1985; Carpenter, 1986; Foster, 1987; Morrison, 1988). Due to their restricted mobility, sea urchins are easily handled inside small enclosures consisting of plastic nets (just 20 cm high) sealed to the bottom. In contrast, only big enclosures, which are enormously expensive and time consuming to maintain, can provide reliable data on the grazing pressure of fish populations. On the other hand, removals are also used to remove species for a limited amount of time and thus facilitate studies of grazer competition (Sammarco, 1982; Lessios *et al.*, 1984; Carpenter, 1990a,b; Robertson, 1991).
An alternative group of methods, which have been successfully used in tropical seas (and recently tested in the Mediterranean), is herbivory assays (Hay, 1981). By providing a num-

ber of controlled (species, size, *etc.*) prey to predators, by means of baited long lines or inside selected patches, these methods make it possible both to identify the nature of grazers and quantify the losses in biomass with considerable sensitivity.
A more complete experiment consists in combining caging experiments with herbivory assays, which makes it possible to quantify the separate effect of fishes and sea urchins (Lewis, 1986; McClanahan *et al.*, 1994), and also microherbivores (Carpenter, 1986).

3.3.A.3. Problems and limitations

Descriptive methods are useful for studying zones with different densities of herbivores, but avoiding possible biogeographical differences, for more complicate designs (*e.g.* combining the effect of fish and sea urchins) could involve serious limitations in the availability of experimental situations.
Caging experiments suit more complete designs and numbers of species within and outside cages can be regulated to determine their impact. However caging designs are expensive, produce caging effects, are frequently small scale and are rarely replicated.
Herbivory assays may involve different prey preferences, and can complicate detection of total herbivory rates when only one or a few prey species are used for feed in the assay.

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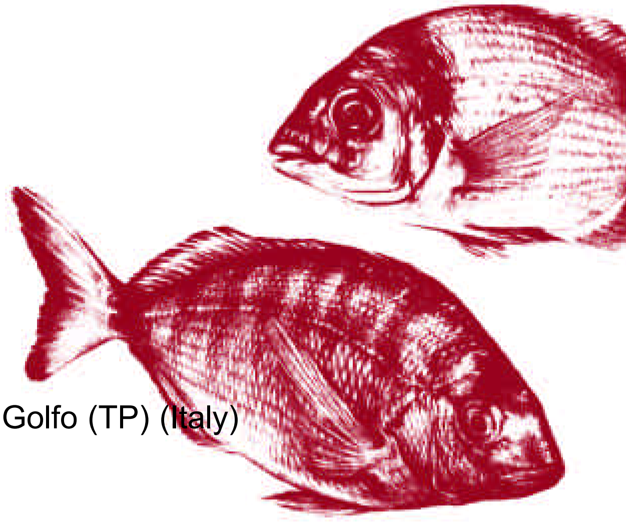

3.3.B. PREDATION

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3.3.B.1. Introduction and objectives

Why are predators of sea urchins important?

The absence of sea urchin predators (i.e. fish, star fish, decapods, gastropods and mammals, including humans) can cause an strong increase in sea urchin population and an overall increase in grazing intensity in the concerned area (Estes and Duggins, 1995; Mann, 1982; McClanahan and Kurtis, 1991; Sala *et al.*, 1998). As a result, in areas with dense sea urchin populations the seascape is characterised by large barrens dominated by coralline algae (Mann, 1982; Sala and Zabala, 1996). In contrast, when sea urchins are not plentiful, that is when their predators are present, luxuriant vegetation is generally found (McClanahan, 1999; Sala and Zabala, 1996;). Sea urchin predators are thus important because they maintain a balance within the overall community between their preferred prey and species that are competitively dominated by these prey species (Barkai and McQuaid, 1988; Sala and Zabala, 1996). The interaction between sea urchins, their prey (algae) and predators generates a three-trophic-level cascade (Estes and Duggins, 1995). The removal of a powerful grazer such as the sea urchin from a community can result in a radical change in the community structure (Mann, 1982). This gives many sea urchin species the role of key species, able to control the dynamics of seaweed and seagrasses (Sala and Zabala, 1996) and thus control marine benthic assemblages in shallow waters.

What are the most common sea urchin predators in the Mediterranean Sea?

In the Mediterranean Sea there are at least two important sea urchin species which can take part in the cascading process: *Arbacia lixula* and *Paracentrotus lividus*. Most research has been carried out on these species, particularly on *P. lividus* (Sala *et al.*, 1998). Among the predators of these sea urchin species we can list a number of fish, including Labridae, Blenniidae, Gobiidae and Sparidae species, which consume sea urchins mainly at the juvenile stage (Sala, 1997; Sala *et al.*, 1998), consuming the prey whole. Some fish species, such as the large-sized *Diplodus* species, *Sparus aurata* and sometimes *Dentex dentex* and *Pagrus pagrus* together with *Balistes carolinensis*, are able to feed on large-sized sea urchins. Some gastropods such as *Truncylariopsis trunculus* also feed on sea urchins, perforating the shell (Sala and Zabala, 1996) to suck the internal fluids and gonads.

Martasterias glacialis among starfish and *Eriphia spinifrons*, *Maja squinado* and *Palinurus vulgaris* decapod crustaceans are other sea urchin predators in the Mediterranean.

In some areas of the Mediterranean humans are another important predator of *P. lividus* adults, selectively consuming large-sized individuals (Sala *et al.*, 1998).

3.3.B.2. Recommended methods

There are a series of methods that have been used to assess the impact of predators on sea urchins. We can distinguish roughly between direct, indirect and manipulative methods. This distinction is useful for schematic purposes, but in reality most of these methods are used together. Before any method is used it is essential that both the sea urchin population structure and those of its potential predators are analysed following appropriate methods.

Direct methods

These methods probably represent the most basic and straightforward approach. They are based on the direct observation of sea urchin and their potential predators (McClanahan and Muthiga, 1992). It is possible to observe and compare areas with higher and lower sea urchin density, making correlations based on the presence, abundance and feeding pressure of predators. Observation can be carried out either by divers or using an underwater camera. In some cases it is also possible to observe and document the predatory activity of some species (McClanahan, 1998; Sala, 1997). These observations are generally successful when large-sized predators are observed. However, the availability of underwater digital cameras equipped with high resolution and magnification now also allows the observation of smaller predators.

Visual observation is also used to assess the results of manipulation such as the removal of predators from the environment, or to follow the activity of tagged predators (Cowen, 1983; McClanahan, 1995)

Indirect methods

The most common technique is that of examining the stomach contents of all the potential sea urchin predators. In this way it is possible to ascertain whether or not a certain species of a certain size feeds on sea urchin. The size of the sea urchin prey consumed can also be determined by measuring certain components of its shell, provided that the relationship between such parts and the size of the sea urchin species are known. This procedure is not possible for those species which do not consume urchin whole but break the shell and consume the eggs. An exhaustive review of studies on gut contents of sea urchin predators is in Sala *et al.* (1998)

Once the sea urchin, or a part of it, is identified in the stomach contents it is possible to assess its importance in the diet of the predator. The most common methods classify prey importance by assigning either a value of occurrence or of importance such as number, weight or volume percentage of the entire stomach contents (see Hyslop, 1980; Rosecchi and Nouaze, 1987; Tirasin and Jorgensen, 1999; for a review). Lastly, it is possible to assess the preference for sea urchin of the predator, provided that the availability of the prey in the environment is known, using one of the prey selection indices available from the literature, such as the Ivlev index of electivity, the selection indices of Strauss or that of Pearsson (Unger and Lewis, 1983; Wootton, 1998). The same indices can be applied to discover whether a particular prey size class is selected.

Manipulative experiments

Manipulation is among the most widely used techniques for assessing the "algae - sea urchin - sea urchin predators" dynamic. Fish predators and scavengers of sea urchin have been investigated by removing small urchin from their natural site and placing them in an area observed by divers (McClanahan, 1995; Sala, 1997). Using this method it has been possible to identify predators, to assess their guild, the way they attack and eat prey and the prey size selection of each predator.

The rate of sea urchin predation has been estimated by McClanahan (1998; 1999) in a series of tethering experiments using several sea urchins. Sea urchin individuals were pierced with a hypodermic needle and threaded with monofilament line. A series of these threaded urchins was used in experiments which consisted mainly of visiting the study area and counting the number of dead urchins to calculate the predation rate.

The rate of herbivory by sea urchin and fish has also been studied by collecting a number of fleshy, unbitten algae species as assays and placing them in control areas. The assays were then regularly visited to assess their integrity or count the number of bites, the latter then being classified as either fish or sea urchin bites (McClanahan, 1999).

In some cases predators are removed completely from a certain area and sea urchin number and microhabitat utilisation can subsequently be monitored for several months using fixed transects (Cowen, 1983).

Caging has been used to examine the effects of potential sea urchin predators on the survival of juveniles and small adult sea urchins (Scheibling and Hamm, 1991). A number of sea urchins are put in a cage together with some individuals of one of the potential predator species. Sea urchin survival is checked over time. Cages can be put on different substrata (e. g. with or without suitable refuges) in order to assess the effects of rugosity, presence of refuges and the like on sea urchin survival. To examine the role of refuges, in some cases transplantation experiments have been carried out (Scheibling and Hamm, 1991). In these cases a natural and complex substratum (e. g. a large boulder with holes and crevices) is transplanted onto a simpler substratum (sand) to examine the effect of more complex substrata as refuge.

Finally another class of methods involve laboratory experiments. They are generally aimed at assessing behavioural responses (*e.g.* aggregation, flight, hiding) of sea urchin to predators (Scheibling and Hamm, 1991).

3.3.B.3. Problems and limitations

It is important to stress that manipulation, conducted either by altering the natural conditions of the environment or through experiments in aquaria, can give misleading results. This is because the data produced can either be a consequence of environmental factors or simply a result of the manipulation itself (*e.g.* caging, tanks, removal of organism, and the presence of the observer and the like). There are a number of precautions to take in order to ensure the proper design of experiments and avoid misleading results (see McClanahan and Muthiga, 1992; Underwood, 1997).

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3.4. SAMPLING STRATEGY AND DATA ANALYSIS

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3.4.1. Introduction

When studying trophic cascades, the hypothesis to be tested is that overfishing removes top predators (fishes), leading to an increase in abundance of sea-urchins, that in turn causes large erect algae to deplete due to intense grazing. Therefore, the main factor to be investigated is the abundance of predators (which is equivalent, to some extent, to the level of protection from fisheries). This hypothesis is based on previous studies by Sala (1996) on the Catalan coast. However, other environmental factors can also affect the population dynamics of the organisms involved in the cascade (Sala *et al.*, 1998). An additional problem is the fact that these species (fishes, sea-urchins, algae) operate at different spatial scales. This problem can be studied using sampling strategies that are complementary (see previous sections). Numerous authors have reviewed different aspects of the design of sampling strategies (*e.g.* Green, 1979; Frontier, 1983; Andrew and Mapstone, 1987; Underwood, 1990, 1997; Legendre and Legendre, 1994; Schmitt and Osenberg, 1996; Kingsford and Battershill, 1998; Krebs, 1999). Generally, we can distinguish between experimental and observational studies, based on the capability of the observer to control the factors under investigation (Eberhardt and Thomas, 1991). A study is said to be experimental when the factor is manipulated and replicated, so that causal relationships can be established (Underwood, 1990, 1997). If the factor cannot be controlled, then we have to undertake descriptive observational studies, oriented to characterize a pattern of change in space and/or time of the variable of interest, and to infer a catalogue of possible explanations of the observed pattern. A kind of intermediate situation can be found in the case of unreplicated events, either under the experimenter's control (unreplicated experiments) or not (intervention analysis, impact assessment field studies).

3.4.2. Descriptive studies: field experimental sampling designs

A way of studying a complex situation such as the cascade effects of protection is via mensurative experiments (*sensu* Hurlbert, 1984), where the factors are spatially localized in the field, profiting from the variation of environmental conditions throughout the Mediterranean coast. It is worth considering adopting hierarchical sampling designs, aimed at measuring spatial variability at a variety of scales. Then, data can be analysed using hierarchical (nested) analysis of variance (Underwood, 1997). This procedure allows the components of variation to be partitioned among spatial scales, and then the scales that contributed most to the total variation to be identified (Morrisey *et al.*, 1992, Lindegarth *et al.*, 1995). Testing of hypotheses implies a <u>statistical decision</u> of rejecting or accepting a <u>null hypothesis H_0</u> establishing that there is not a significant effect of a given treatment when compared with a <u>control</u>, by determining the probability that the detected average difference among treatments is actually due to chance. When this probability is lower than a given level ? (usually ?=0.05) we reject H_0, thus concluding that the factor under investigation exerts an effect on the variable of interest. In this case, H_0 is that there is no spatial variation of abundance, except that due to chance. The error committed when we reject a true H_0 (Type I error) is determined by ?, so that by diminishing its value (*e.g.* ?=0.01) we make the analysis conservative. But minimizing Type I error is done at the expense of maximizing Type II error, determined by the probability ? of accepting a false H_0. The power (1-?) of the analysis, for a given level of precision, has to be established prior to sampling (Peterman, 1990; Eberhardt and Thomas, 1991; Fairweather, 1991; Shrader-Frechette and McCoy, 1992; Osenberg *et al.*, 1994; Underwood, 1994, 1997; Kingsford, 1998).

Example.- Consider both PROTECTION STATUS (MPA vs. NRA), replicated (several LOCALITIES under each PROTECTION STATUS). In each LOCALITY (protected or not) we randomly select 3 SECTORS separated by thousands of meters. For each SECTOR we select (again randomly) 3 ZONES situated hundreds of metres apart, and in each ZONE we perform 3 TRANSECTS (50 x 5 m) for fish UVC. We introduce the constraint that the ZONES have to be located on "pure" rocky bottoms, composed of rocky boulders, and situated 8-10 m deep. Along each TRANSECT we place 5 RECTANGLES (5 x 1 m) for counting and measuring sea urchins (*Arbacia lixula* and *Paracentrotus lividus*), and inside each RECTANGLE we place 4 QUADRATS (50 x 50 cm) for algal % cover (algal species grouped by ecomorphotypes). Then, the data can be explored using nested ANOVA, considering the following factors: PROTECTION STATUS P, LOCALITIES nested in P L(P), SECTORS S(L(P)), ZONES Z(S(L(P))), and TRANSECTS T as residual term. Variability of habitat structure can govern a substantial part of the observed small-to-medium scale spatial variability (see Section 3.2). Therefore, habitat descriptors should be measured, and their effect quantified in order to correctly interpret data. In this example, in the case of fish data the mixed analysis of variance would be as follows:

Source of variation	Type	# levels	Df	F vs.
P	Fixed	*p*	(p - 1)	L (P)
L (P)	Random	*l*	p(l - 1)	S (L (P))
S (L (P))	Random	*s*	pl(s - 1)	Z (S (L (P)))
Z (S (L (P)))	Random	*z*	pls(z - 1)	Residual
T, residual	Random	*n*	plsz(n - 1)	
Total			plszn - 1	

3.4.3. Descriptive studies: correlative evidence

Further to describing the spatial variation of the different variables considered, one may be interested in exploring the observed relationship among these variables. This can be achieved by performing regression analyses (Jongman *et al.*, 1987, Underwood, 1997) between abundance of organisms (fish-urchins, or urchins-algae), or among these abundance and habitat structure descriptors (see Section 3.2). The framework for this type of analysis is the theory of Generalized Linear Models (GLM), which comprises a series of different techniques (*e.g.* linear, log-linear, Poisson, probit, logistic, *etc.*) (Dobson, 1983; McCullagh and Nelder, 1989).

3.4.4. Manipulative experiments

Small-scale processes can be investigated through manipulative (*sensu* Hurlbert, 1984) experiments, including caging, removal/additions, transplants or tethering (Kingsford and Battershill, 1998) (see Section 3.3). A recurrent problem in such an approach is the possibility that manipulation induces artifacts on the investigated process (Peterson and Black, 1994). Usually, the data arising from these experiments are to be analyzed using analysis of variance (Underwood, 1981, 1997). When proportions are to be used for comparing two qualitative factors, contingency tables ($?^2$ test) are more suitable (Zar, 1996). Some hypotheses require comparisons of the same sampling units through time, that have to be analyzed performing repeated measures ANOVA (Green, 1993). A particular case of predation experiments is that of multi-choice feeding experiments, in which consumption of one type is not independent of consumption of other types held together (*e.g.* simultaneously offering to predators juveniles of urchins on vegetated and unvegetated substrates). This kind of design cannot be analyzed by usual procedures (Peterson and Renaud, 1989), and, although several authors have chosen to present their data without statistical analysis (Hay *et al.*, 1986), specific statistical techniques can be applied, such as one-sample Hotelling's T2 (Roa, 1992; Manly, 1993; Lockwood, 1998; Giménez-Casalduero *et al.*, 1999).

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MAST - III

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