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CHAPTER 6

HARD BOTTOMS

Contents

6.1 Introduction

The rocky coastal substrates, commonly referred to as hard bottoms, represent a quantitatively trivial fraction of the marine environment if compared to the spatial extent of the soft bottoms; nevertheless, they represent a scientific and economic value of comparable magnitude. This is no surprise since the hard bottom communities allow a number of ecological processes of general importance (competition, trophic cascades, habitat structure, etc.) to be studied, and represent a great biodiversity reservoir. The hard bottom heterogeneity is indeed much greater than that of soft bottoms, leading to a variety of conditions and differentiated assemblages that strongly contrast with the apparent uniformity of soft bottoms. Moreover, hard bottoms are often characterised by sessile organisms with modular structure (algae, sponges, cnidarians, bryozoans, tunicates), that have no correspondence in any other environments.

The great economical value of hard bottoms is due both to fisheries, as they host an alieutic fauna of great commercial value, and to their role in tourism, nautical and diving tourism in particular. It is not by chance that virtually all the marine protected areas are established in correspondence with rocky coasts. In recent years it has become clear that the impact of fisheries and tourism causes management problems for hard bottoms, and so a better understanding of their ecology is required.

Scientific and economic importance and the need to integrated management demand further and more accurate studies on spatio-temporal distribution patterns and community dynamics, as well as on the life strategies of the species they are composed of. The difficulty of such studies in a complex environment, as hard bottoms are, leads to the study methods being more numerous and less standardised than those used for soft bottoms. A further difficulty lies in the fact that in order for hard coastal bottoms to be studied efficiently, it is necessary to use scuba diving techniques, that only in recent years have become a common tool for marine biologists (Bianchi and Morri, 2000). In this chapter, some of the main methods will be presented, with particular regard to those traditionally employed in Italian seas (Pansini and Pronzato, 1982; Balduzzi *et al.*, 1986; Bianchi *et al.*, 1991). We will focus on surveys and sampling of a strictly biological kind, whereas the methods for measuring ecological parameters may be found in other papers (Colantoni, 1982; Mazzella *et al.*, 1986; Sgorbini *et al.*, 1988) and are treated in detail in Chapter 1 of this Manual.

6.2 General aspects of sampling by diving

The sampling design (randomness, replicates, etc.) has to be planned with the same general approach valid in all environments and with all methods (see Chapters 13 and 14 in this Manual); working with hard bottoms, however, involves peculiar aspects due to both their great complexity and heterogeneity, and to the problem of working underwater. In contrast to someone who works on land or from the surface, the diver has an always-limited field of view and can more easily be biased by some peculiar bottom feature. For the random choice of sampling stations, two systems are commonly used: 1) a plastified table with random numbers is brought underwater and the numbers are extracted on the bottom before the sampling starts; 2) routes and distances from a point on the bottom, which will be chosen as a convenient starting point, are previously randomly chosen.

Whichever the design criteria, they must combine scientific rigour and diving safety. Underwater sampling requires the scientific diver to be constantly fit for diving, especially when he has to work in environmentally hard conditions (cold water, scarce visibility, darkness, currents, etc.). A great water confidence and excellent skills with

gear and instruments are therefore needed when the diver's attention is addressed mainly to the work to be done, and less to the control of the dive itself: the latter is left rather to the automatisms developed through practice. For these reasons, dives must be rigorously and carefully planned (Colantoni and de Strobel, 1980).

When planning a dive, time must be taken into account not only with respect to the depth, but also to water temperature, as the scientific diver's activity is rather static and very susceptible to cold temperatures.

It should also be taken into account that work efficiency decreases with increasing depth even for skilled divers used to deep diving: below 30 m it is better to double the planned time for the same work to be done. Buddy diving is required and the two divers, who must operate in great harmony, should be equivalent both in terms of diving and scientific skills. In particular instances, for the sake of efficiency, the work could be carried out by one diver only, and the buddy would then act as stand-by diver. White plastic slates and graphite pencils are used for recording data underwater. Many materials are used for the slate: among the best suited, one can mention compressed polystyrene, which floats, and PVC, which does not. It is important that the slate surface is not too smooth or shining, otherwise the pencil will not write properly on it. The ideal slate size is approximately 20×30 cm or a little smaller, as that represents the best compromise between space availability and impediment (furthermore photocopies can be made on the common A4 format). The slate can be usefully pre-set following a scheme depending on the underwater work to be done, in order to make it as simple as possible (Fig. 1). Finally, a compass and/or a depth gauge, and a clinometer can be secured to the slate. At the end of the dive it is necessary to copy the data as soon as possible into a notebook or in the dive log, which should be purposely structured for scientific observations (Abbiati *et al.*, 1989). In some instances, tape recorders in a housing have been adopted to record the data from the diver's voice. This method is useful when the diver surveys along a track with autonomous or towed vehicles, as the use of a recorder leaves the hands free, so that it is therefore not necessary to stop and write. On the other hand this method presents some disadvantages: i) a full-face mask is required; ii) voice quality is often poor; iii) after the dive, it is time consuming to transcribe the tape; iv) water infiltration or flooding can occur, as for every other equipment in a housing.

Useful references for scientific diving planning and execution can also be found in Melegari (1969), Woods and Lithgoe (1971), Drew *et al.* (1976), Earll (1977), Colantoni (1980), Gamble (1984), Hiscock (1987), Flemming and Max (1996), NOAA (2002).

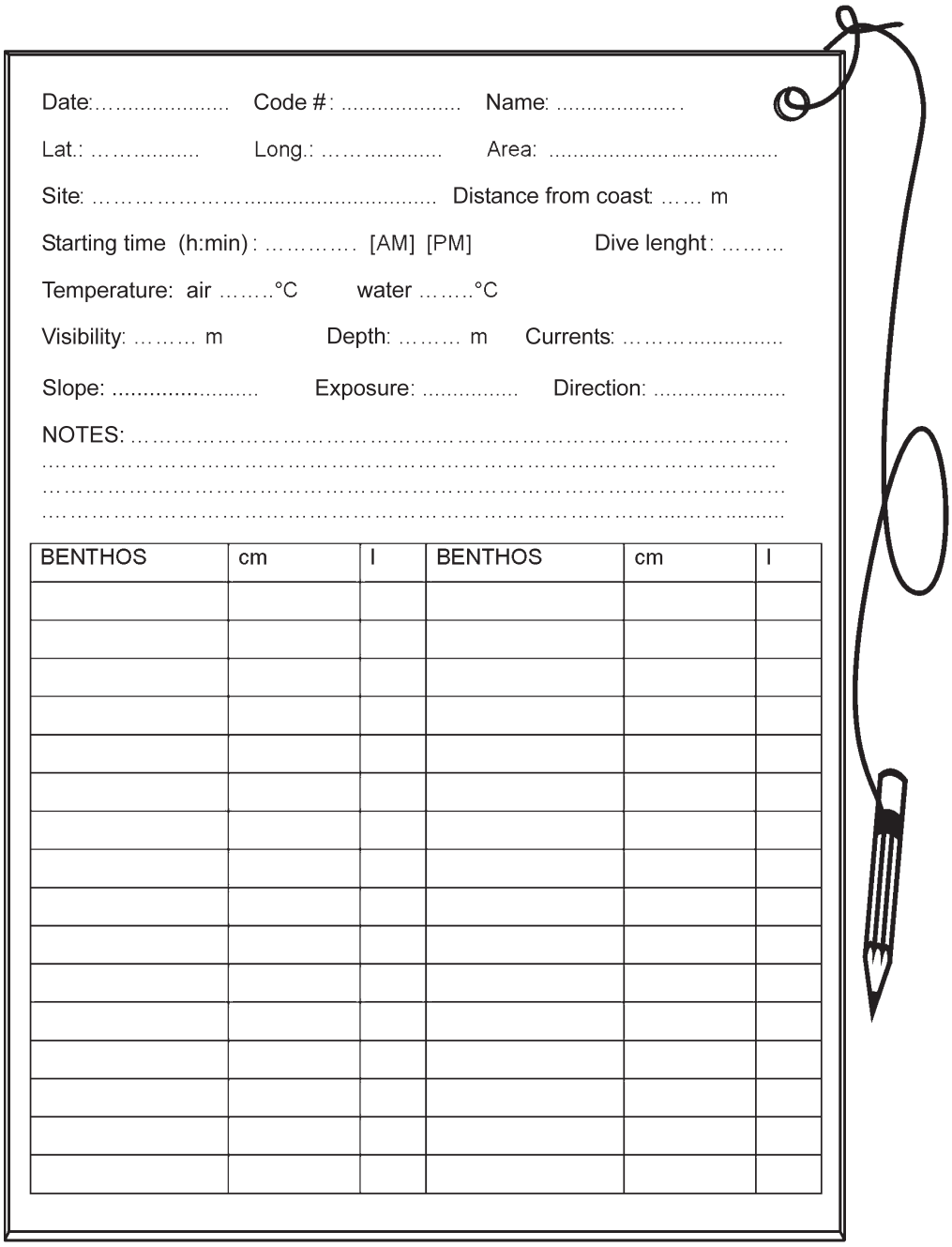
6.3 Representativity of sampling

The first goal of sampling is to obtain representative data for the measured variable. This means the capability of a set of measures of a variable to correctly estimate the parameters of the frequency distribution from which the variable arises (for example the average density or the variance of a population).

The representativity of a sample data set is explainable as a function of two features of the estimates it gives: accuracy and precision (see also Chapter 1 in this Manual).

6.3.1 Accuracy

The accuracy indicates how much the estimate value of the variable is close to the true value. The closer the estimate to the parameter the greater the accuracy. There



Date:..... Code #: ..... Name: .....

Lat.: ..... Long.: ..... Area: .....

Site: ..... Distance from coast: ..... m

Starting time (h:min): ..... [AM] [PM] Dive lenght: .....

Temperature: air .....°C water .....°C

Visibility: ..... m Depth: ..... m Currents: .....

Slope: ..... Exposure: ..... Direction: .....

NOTES: .....
.....
.....
.....

BENTHOS	cm	l	BENTHOS	cm	l

Fig. 1 - An example of a diving slate, illustrating the layout for transect data recording.

are different sources of inaccuracy in a sample data set. For example, a common goal of many ecological studies is to examine hypotheses about seasonal variations in the mean abundance of a population of organisms. Often the sampling is done on one date only per season, and "seasonality" is estimated comparing data from four sampling dates in a year. This approach is likely to provide inaccurate estimates (overestimates or underestimates) of the seasonal variation, because a single sampling date will not be representative of a whole season. A second source of inaccuracy is represented by errors in the sampling method. There can be errors in counting due to the experimenter, loss of organisms during the collecting and overestimates or underestimates of the sampled area. The accuracy of the density measurements of organisms is a relevant feature for studies based on absolute values, as in the case of natural population censuses.

6.3.2 Precision

The precision is the degree of concordance of a set of sample measurements made on the same population. A set of measurements similar to each other will be more precise than a set of discordant ones. The precision depends on the variability intrinsic to the measured variable and on the sampling effort. Increasing the precision of sample data will increase the probability of observing differences among treatments when they exist.

6.3.3 Diagnostics

Even in a simple sampling there are at least two levels of variability: variation of the sample data and variation of the sample estimate.

The first level measures variability among single observations (or replicates). If replicates are obtained from sampling a population of organisms in different points of the area, the variability of sample data reflects the spatial distribution of organisms. This kind of variation depends on the measurement of the sampling unit in relation to the degree of aggregation of the population. The variability of the sample data is measured by the variance of the sample or by the standard deviation (S) that is the square root of the variance. The latter has the advantage of being directly commensurate to the mean, while the variance is a squared measure.

The variability of the sample estimate is the variability of the mean estimated from the sample. This measure is the Standard Error (Tab. 1) and represents the standard deviation of a population of sample means. Let us imagine we repeat the sampling many times from the same population of organisms using the same number of replicates. From each sampling we obtain an estimate of the average density of the population. The mean will not be exactly the same even if data originate from the same population of organisms (then from the same frequency distribution of the variable), because the sampling is random. Each time we estimate the mean of the data, the set of obtained values describes a frequency distribution of sample means. The standard error is the standard deviation of this distribution. Rarely in practice do we have enough resources to generate distribution of sample means by re-sampling the same population many times. The standard error, instead, is estimated from the single sample and gives information about the variability around the sample mean. It is called "error" because it shows how much the mean of the sample ($\overline{x}$) is adequate to estimate the mean of the whole population (μ). High values of the standard error indicate that repeated sampling would generate very dissimilar (imprecise) means, so

that the mean estimated from each sample of data would not be a good estimate of the true mean of the population. If instead the standard error is low, it is likely that a repeated sampling will give similar values of $\bar{x}$ (high precision), so that every single estimate is close to the true value of the parameter. From that derives the fact that precise data are generally accurate too and that both the attributes can be improved by increasing the number of replicates.

The accuracy of a sample estimate, as the mean abundance of a population in a

Tab. 1 - Parameters commonly evaluated in ecological studies, and sampling statistics; x_i is the value of the i^{th} sample and n is the total number of samples.

Parameter	Statistics	Formula
Mean (μ)	$\bar{x}$	$\sum_{i=1}^{n} x_i/n$
Variance (σ^2)	S^2	$\sum_{i=1}^{n} (x_i-\bar{x})^2/(n-1)$
Standard deviation (σ)	S	$\sqrt{\sum_{i=1}^{n} (x_i-\bar{x})^2/(n-1)}$
Standard error of $\bar{x}$ ($\sigma_{\bar{x}}$)	$S_{\bar{x}}$	$S/\sqrt{n}$
95% confidence interval of μ		$\bar{x} - t_{0.05}(s/\sqrt{n}) \leq \mu \leq \bar{x} + t_{0.05}(s/\sqrt{n})$

given habitat or the variance in the abundance measurements at a particular spatial or temporal scale, can be expressed also as a confidence interval for the estimated parameter. In the case that the measured variable is normally distributed, the confidence interval can be calculated using the estimates of parameters obtained from the sample data and a frequency distribution of reference. For example, the confidence interval of a sample mean for a normally distributed variable can be calculated using the t distribution. By convention, we often refer to $t_{0.05}$, i.e. to the absolute values of the distribution that include 95% of observations. Other t values can be used for different confidence levels. The confidence interval of 99% can be obtained using $t_{0.01}$, that is the absolute values that include the 99% of the distribution values. See Burdick and Graybrill (1992) for more details. For variables that are not normally distributed, confidence intervals can be obtained using the procedure of repeated sampling (*bootstrap*) and Monte Carlo simulation (Manly, 1991).

6.4 Choice of the sampling unit

The shape and size of the sampling unit influence the accuracy and precision of the estimates, the perception of the ecological reality examined, and the efficiency of the study program.

The main criteria that influence the choice of the geometric shape of the sampling unit are based on the possible influence of the edge effects. Different geometric shapes have a different perimeter/area ratio according to the following order: circle < square < rectangle. The circular sampling unit minimises the edge effects due to the uncertainty of deciding whether an organism is inside or outside the sampling unit (Krebs, 1999). Nevertheless, the geometric shape most commonly used on hard substrate is the square.

The sampling unit size should be chosen with respect to the size of organisms being sampled, to their spatial distribution and to the costs for the realising the sampling program. Sampling units of too small size with respect to the organisms will give ecologically not significant data. In the extreme case, they would generate only

two possible values, 0 and 1, that would give information only about the presence or absence of the organism but not about its density; on the contrary, a size too big

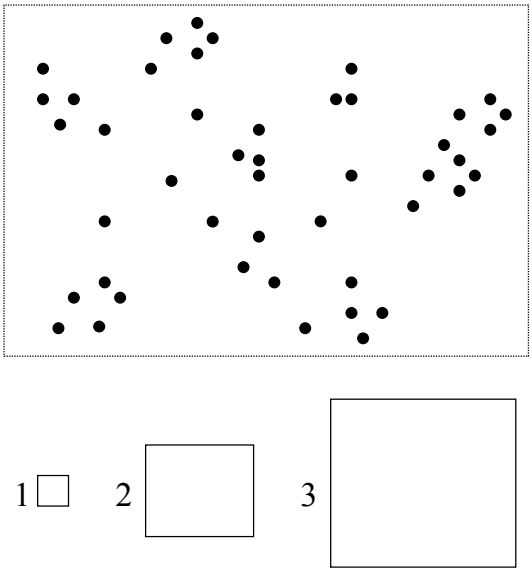


Fig. 2 - Relationship between sampling unit size and organism distribution. The scheme represents a hypothetical population in a habitat patch and three possible sampling unit sizes. Sampling with a quadrat of size 1 would generate only two possible density values: 0 and 1. Quadrat 3, on the other hand, is too big with respect to the aggregation scale of the organisms. Quadrat 2 proves to be of suitable size.

with respect to the aggregation scale of the organisms would hide the distribution pattern (Fig. 2).

The size of the sampling unit also influences the measures of association among

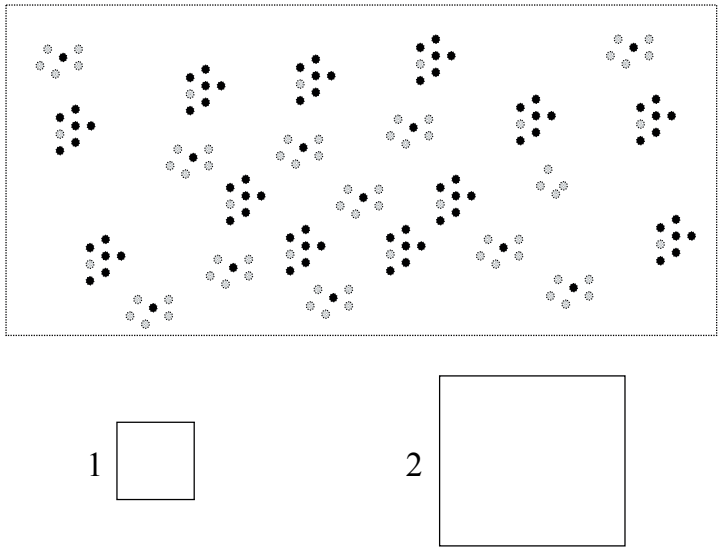


Fig. 3 - Relationship between sampling unit size and the association between two species. The illustration shows two hypothetical species in a habitat patch and two possible sampling unit sizes. The relationship between the two species is negative if sampled using size 1 unit, and positive if sampled using size 2 unit.

species, leading to opposite results: the relation could prove negative with too small sampling units or positive with too big ones (Fig. 3).

In general, considerations relative to the sizes of the organisms dictate the minimal level of the unit sample size. The knowledge of the life history of studied organisms is extremely important because it will give the basis for predicting the environmental grain to which the organisms answer. To identify the most appropriate sample size needs specific optimisation analyses. The aim is to define an efficient sampling procedure, where the efficiency is the cost (in terms of time or money) of obtaining the right level of precision.

6.4.1 Minimal area

A particular problem of the sample size concerns the relationship between number of species and sampled area. The smallest area able to contain a representative number of species of an assemblage can be evaluated through an examination of the species-area curves that define the number of species as a function of the sampled area. The sampling unit size over which an increase of area does not generate an increase of the number of species is indicated as the minimal area for the assemblage under examination.

The problem of the minimal area is of a practical nature and concerns again the cost-benefit analysis between the information retrieved and the sampling effort (Fig. 4): the latter can be expressed not only as area, but also as number of samples (Morri *et al.*, 1999) or in other ways (Bianchi, 2002). For what concerns the Mediterranean marine

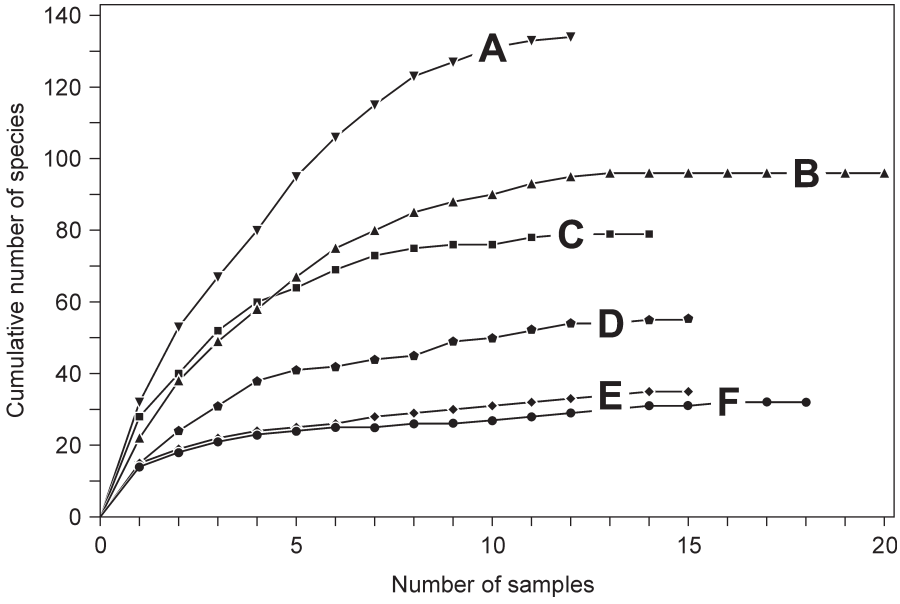


Fig. 4 - Example of minimal area estimate for a sessile epifaunal assemblage scraped off a rocky bottom in 6 different stations (A-F). In this example, the richest station (A) requires not less than 10 samples in order to obtain a representative sampling of the sessile epifauna diversity.

research, investigation on the minimal area was initially tackled for algal assemblages (Boudouresque and Belsher, 1979), by using two different methods: sampling on small equal and contiguous areas, or sampling on tiled areas of increasing size. Experiences on animal assemblages (Weinberg, 1978) has led to very different results in terms of minimal area, even if many Authors are oriented, at least in the case of destructive sampling, to quadrats of 20×20 cm (Bellan-Santini, 1969). In any case, a sample size that could be recommended universally does not exist. The sampling unit size depends on the nature and objectives of the study program and particularly on the magnitude of the effects to be measured, on the intrinsic variability of the populations under consideration and on the resources available.

6.5 Numerical descriptors

In the study of hard bottom assemblages, there are characteristic difficulties in estimating the quantitative importance of the organisms, mainly due to the difficulty of combining in a single scale and comparing numerical data relative to solitary and modular organisms (Morri and Bianchi, 1983; Bianchi *et al.*, 1989).

6.5.1 Qualitative and quantitative evaluations

In the simplest case it can be sufficient to evaluate the presence or absence of a particular species in the examined site. This is the case of qualitative survey, and the result will be a table in which the presence of a species is indicated by 1, the absence by 0.

It is often necessary, however, to support the presence of a species with an estimate of its quantity and so one will operate a quantitative survey. The quantity (or numerical value) of a species can be expressed with different parameters, in relation to the specific aims or to the intrinsic characteristics of the organisms.

6.5.1.1 Biomass and biovolume

Biomass is the quantity of organic matter per unit area; in certain applied contexts, the *standing crop* (for vegetal resources) or *standing stock* (for animal resources) can also be used. Biomass can be measured in many ways (fresh weight, dry weight, dry weight without ash, energetic content) but all need collection of organisms, lead to the loss of the sample, and are in general very laborious (Palmerini and Bianchi, 1994). The measure of the biomass is fundamental when the energetic flux of the community is of interest. When the aim is the characterisation of the assemblages from the point of view of the occupation of the substratum, the biovolume, i.e., the quantity of space occupied by the organisms, is preferred to biomass. The measurement of the biovolume in the field is laborious and not precise: it can be done with three-dimensional biometric measurements or with photos, then elaborated at the computer. Measurements in the laboratory need the collection of organisms, but offer many advantages: there is no loss of sample and, if done by immersion and shifting of the water level in graduated cylinders, they combine operative simplicity and precision. Biomass and biovolume allow solitary and modular organisms to be compared on the same scale (see also Chapter 10).

6.5.1.2 Abundance and density

The measurement of abundance implies counting all the individuals of a particular

species. When referred to an area of known surface it can be transformed into density, which represents the numbers of individuals per unit area. Counting is theoretically a very precise method and can be done with sufficient simplicity, but needs much time. Furthermore, it can be applied only to solitary organisms and not to algae and colonial organisms, which tend to form a more or less continuous turf: this limitation is crucial in the study of sessile benthos, in which algae and colonial fauna often represent the greater part of the assemblage. Even in the case of solitary organisms, however, counting of the individuals may prove impossible for gregarious and superabundant species (serpulids, barnacles, etc.), with individuals located one on each other in superimposed strata or in inextricable clusters. On the contrary, counting is the most suited method for the mobile fauna.

6.5.1.3 Coverage and cover

The terms coverage and cover are often used interchangeably, but in reality they are different. Coverage is a feature of the sea bottom and represents the portion of the substrate not bared, i.e., one covered by a biotic assemblage; by definition, it cannot be greater than 100%.

Cover is instead a feature of the organisms, and represents the portion of substrate covered by every species individually (Boudouresque, 1971). Cover is correctly used for algae and is applicable also to sessile solitary and colonial animals. It can be expressed as an absolute measure of area, but usually is expressed in percentage. It is not suited for the mobile fauna.

A limitation of cover is that it considers the percentage of the substratum occupied by the projection of the species but not its three-dimensional development. The fact that total cover can reach more than 100%, for example in the presence of multilayered assemblages or epibiosis (Boudouresque, 1971), solves this problem only partially.

6.5.1.4 Frequency

Another method that allows for the unification on a single scale of modular and solitary organisms is the calculation of the frequency of the species in a reference grid (Bianchi *et al.*, 1991).

For example, if a grid of 25 quadrats of 20×20 cm each is placed on the substrate, it can be rapidly counted in how many quadrats a particular species appears, independently of the quantity with which it is present in every single quadrat; if, for example, the species is present in 4 quadrats, it can be said that its frequency is 4/25, that is 0.16.

The measurement of frequency combines rapidity and precision of quantitative estimate and can be applied indifferently to solitary and modular sessile organisms: on the contrary, it is not suited for the mobile fauna. It is a fast and precise estimate, but it must be made clear that it is not comparable with abundance or cover (Fig. 5). When a very thick grid is used, with numerous small quadrats, the calculation of frequency tends, however, to converge towards a measurement of cover (Foster *et al.*, 1991; Meese and Tomich, 1992).

Frequency is a measurement applicable essentially to *in situ* sampling: as the spatial distribution of organisms is destroyed with the removal of the sample from the substrate, it proves impossible to calculate frequency *a posteriori*. Also in the case of frequency, the presence of organisms of large size and/or disposed on several layers make the measurement unsatisfactory.

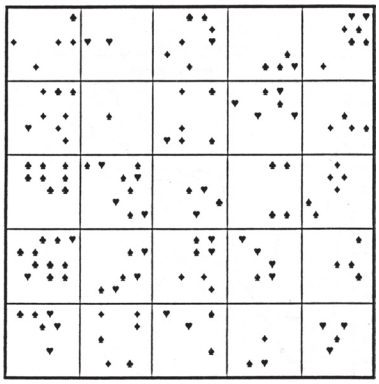


Fig.- 5 - Quantitative survey on hard bottoms using a 1 m^2 square quadrat.
♥, ♦, ♣, and ♠ are 4 sessile species whose quantities can be estimated as follows:

	Abundance	Density	Cover	Frequency
Species ♥	38	$38 \cdot m^{-2}$	2.5 %	0,68 (17/25)
Species ♦	32	$32 \cdot m^{-2}$	2.1 %	0,40 (10/25)
Species ♣	21	$21 \cdot m^{-2}$	1.4 %	0,52 (13/25)
Species ♠	46	$46 \cdot m^{-2}$	3.1 %	0,88 (22/25)

6.5.2. Semi-quantitative evaluations

Species quantity can be roughly estimated in a semi-quantitative way, for example using different indices (Boudouresque, 1971; Hiscock, 1987). The advantage of this method is speed, comparable to that of simply qualitative surveys. A semi-quantitative estimate is sometimes sufficient for the characterisation of an assemblage. Immediate descriptive attributes such as "scarce", "abundant", "very abundant" can be respectively transformed into indices with values of 1, 2, or 3 (absence is obviously 0): adequate statistics do exist to be applied to such indices and give fully satisfactory results.

6.6 Sampling methods

In their classic manual of Mediterranean benthic bionomics, Pérès and Picard (1964) state that diving allows a useful work to be accomplished underwater down to approximately 50 m depth, and that the main advantage of this technique is that of combining quick physiognomic surveys over relatively large areas with exhaustive punctual samplings, done with destructive (collections) or non destructive (visual and photographic) methods. Each of these methods presents advantages and disadvantages (Tab. 2) and it is only through their integration that complete results can be obtained (Zabala *et al.*, 1982; Ros and Gili, 1984; Morri *et al.*, 1999).

Tab. 2 - Comparison between three common methods of underwater sampling of hard bottom benthic assemblages.

DIRECT COLLECTION	
Advantages	Taxonomy accurate. Evaluations objective. Reference collections.
Disadvantages	High cost. Slowness. Laborious. Need of specialists. Small sampling area. Destructive impact on ecosystem
Use	Studies with a strong taxonomic component.
VIDEO OR PHOTO SURVEY	
Advantages	Evaluations objective. Repeatability. Reference collections. Could be automated. Fast underwater work. Large sampling areas. No impact on ecosystem.
Disadvantages	Scarce taxonomic precision. Problems may arise when reading and interpreting the image *a posteriori*.
Use	Life cycle or temporal variation studies. Deep water work.
VISUAL CENSUS	
Advantages	Low cost. Immediate results. Large sampling areas. Repeatability. No impact on ecosystem.
Disadvantages	Risks of subjectivity in taxonomy. Underwater work slow.
Use	Preliminary studies. Exploratory surveys. Analyses of differences. Bionomic studies.

The hereafter-described methods essentially regard the epibenthos. When appropriate, it will be indicated whether the explained techniques are more suitable for the sessile organisms, or for the small mobile fauna. For the large-sized mobile fauna (decapod crustaceans, many echinoderms) specific procedures should be applied: some examples can be found in Kingsford and Battershill (1998). The study of endobenthos boring requires definitely specific techniques, and a whole paragraph is therefore devoted to them in this chapter.

6.6.1 Direct methods of collection

Objectives

A shared characteristic of these direct sampling (destructive) methods is that of requiring the collection of organisms to be brought to the laboratory and analysed in greater detail. The main collection methods for hard bottom organisms are essentially two: scraping and air-lift. Both allow quantitative sampling on defined areas.

6.6.1.1 Scraping

Material and equipment

Tools and instruments used for the scraping procedures are generally very simple. Usually, depending on the kind of substrate and on the organisms to be sampled, knives, scrapers, small hatchets, and other utensils are used. The use of hammer and chisel is recommended for more accurate collection. Given the water density, the hammer should be of adequate weight (2-4 kg). The sampled material is collected in a cloth or plastic (polyethylene), or zooplancton net (with a mesh not larger than 400 μm) bag. A square metallic (generally aluminium) or PVC frame can be useful in delimiting the area to be sampled.

Sampling procedure

The technique requires all of the assemblage in the set sampling area to be removed. Typically, the co-operation of two scientific divers is needed for the scraping procedures. While one of them holds the bag, carefully driving the material falling off the surface into it, the other one scrapes the rock with hammer and chisel (Fig. 6). The latter should regulate his breathing rhythm along with the hammering

Fig. 6 - Sampling on a rocky wall by scraping with hammer and chisel (note the hand safe rubber grip on chisel); material detached from the rock is collected while it falls into a polyethylene bag.

rhythm, so that the hammer hits the chisel during expiration: in this way the action is more efficient. A useful trick too is to preliminarily delimit the sampling area with the chisel, in order to avoid the use of the reference frame, especially in the case of vertical or sub-vertical substrates.

Problems and practical tips

The scraping technique gives excellent results for the flora and the sessile or sedentary fauna, while the mobile fauna can easily escape (Abbiati, 1991). Scraping is easily done when operating below 3-4 m; above those depths, sampling is possible only with very calm seas, otherwise the loss of material would make the sampling useless.

Compressed air devices have also been developed (drills and chisels) in order to improve effectiveness with respect to the manual instruments, but they have not provided good results, except in particular cases, because they do not allow the necessary care and precision to be applied.

In order to avoid the escape of mobile fauna, devices that hold it under a sort of transparent cowl have been purposely built. The material detachment operation is done manually with attached gloves, or with a more elaborate aspiration method working with the air-lift principle (Finnish IBP-PM Group, 1969). A drawback of these devices is their being cumbersome and complicated, so that they are not of very common usage. If sampling in dark environments (such as caves or in the case of night sampling), speleological diving helmets should be worn along with lights, thus leaving hands free.

6.6.1.2 Air-lift (suction sampler)

Material and equipment

Square metal or PVC frame; PVC, Plexiglas or metal tube for air-lift; cloth bag, zooplancton net or nylon stockings; regulator, 1st stage connected to a plastic hose of adequate length (approximately 4-5 m), spare diving tank devoted to the air-lift functioning.

Sampling procedure

The air-lift is made of a rigid tube, usually of PVC or Plexiglas (less frequently stainless steel), connected to a compressed air tank through a hose and a pressure reducer (Fig. 7). Diameter and length of the air-lift may vary depending on the substrate to be sampled and on the organisms to be collected, but it is usually about 0.80-1 m long and 5-8 cm in diameter (Benson, 1989). Giangrande *et al.* (1986) proposed a very handy and suitable instrument, designed for hard bottoms but also used for seagrass meadows (see Chapter 5 in this Manual). Compressed air enters the air-lift through a nozzle, commonly a "duck nozzle", jointed to the first stage of a regulator on a compressed air tank by a high-pressure hose (set at 3-4 bar). Opposite the sucking end a nylon net bag is tied; the mesh of this net varies depending on the kind of organisms to be sampled (generally 400 μm).

The instrument works in a rather simple way and takes advantage of the depression created in the air-lift by the vertical traction of the air expanding towards the surface. This depression drags within the tube most of the small highly mobile phytal fauna which is held in the bag on the top of the device (Fig. 8).

This method is commonly used on hard substrate with high vegetation cover, and requires the delimitation of the sample area using a quadrat of known area; then the air-lift is positioned and passed over the whole vegetation. Attention must be paid

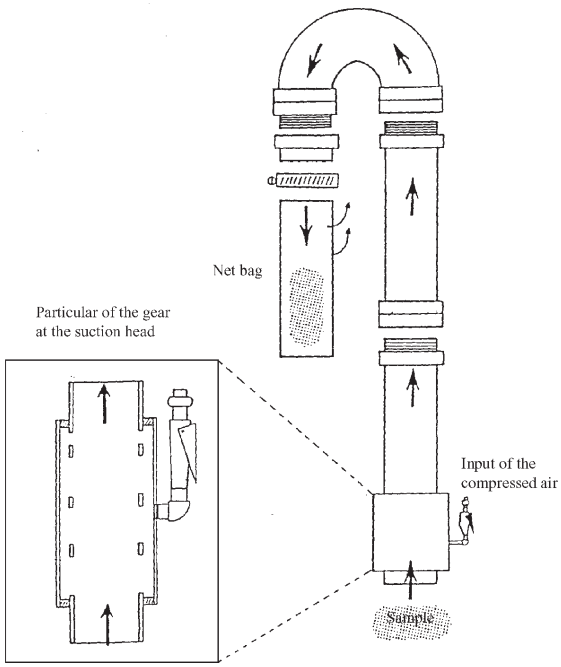


Fig. 7 - Schematic layout of a air-lift, suction pump (modified from Benson, 1989).

to start the air flux away from the sample area in order to avoid reflux. After this first phase, macrophytes are removed (they can be kept in a separate bag), and another air-lift passage is done to collect the mobile fauna that has fallen from the algae onto the substrate.

Problems and practical tips

The sorbona is a generally effective instrument over horizontal or sub-horizontal substrates (maximum inclination around 45-50°) and at depths greater than its length. Among the disadvantages, slow execution and cumbersome equipment, requiring a tank in addition to the air-lift, should be remembered. Among the advantages, a more effective collection of mobile fauna should be emphasised (Giangrande *et al.*, 1986; Gambi *et al.*, 2003).

Air-lift and scraping can be integrated to collect both the sessile and the mobile component. The air-lift sample is done first, then macroalgae are removed (paying attention not to damage the holdfast of each individual useful for identification purposes) until the substrate is bare. A

Fig. 8 - Using the air-lift for sampling on a rocky wall.

complete scraping of the biological cover with hammer and chisel down to the bare rock will follow, eventually sampling the endobenthic forms. A final passage with the air-lift allows the collection of all the scraped material, even the finest, and the remaining animals.

In case the work should be conducted on vertical or sub-vertical surfaces, it is suggested to tie the air-lift and its tank to a line as long as the sampling depth. Also for the air-lift, in the case of night or cave environments the use of a speleological helmet with lights is advisable.

6.6.1.3 Processing of samples in the lab

Both scraping and air-lift samples have to be sorted in the laboratory, the organisms being separated at the species level, or, at least preliminarily, at higher taxa level. This operation, when possible, should be carried out on fresh material, before fixation. It is important to do the sorting on live organisms especially for the mobile fauna. The sample is put in a container filled with seawater and left undisturbed for some hours, even for a whole night. While the water quality decreases (the container can be eventually covered to accelerate the hypoxia process), the mobile fauna hidden in the interstices of the substrate fragments, or among the algae, emerges, so that it can be easily collected with tweezers, or rinsing the sample and filtering it with a fine mesh sieve (possibly not larger than 400 μm). The material is then put into tubes or jars.

When working with fresh material is not possible, samples should be fixed in 10% formalin in neutralised sea water, with borax for example: using non neutralised formalin can damage the exoskeletal structures often necessary for the organism's identification. The sample to be sorted should be placed in a basin and rinsed in running water, allowing the mobile fauna to be swept away by the water flux falling on a sieve underneath (possibly with mesh not larger than 400 μm). The material collected in the sieve is then put in a small basin. The sample basin and the mobile fauna small basin are examined under a table light lens or under a dissection microscope, manually separating and collecting every solitary organism. The mobile fauna should be carefully collected from bioconcretion blocks, if present, and from the algal blades.

During the analysis of the sample, whether fresh or preserved, algae and sessile fauna (including the epiphytes) should be carefully detached from the substrate using lancets and small cutters. In the case of encrusting species, it is sometimes necessary to keep part of the substrate since the detachment could destroy the organism.

The different species (or higher taxa) that have been sorted should be kept in adequately sized containers and in the proper liquid (70% alcohol for calcareous organisms, neutralised formalin for soft-bodied organisms) until they have been identified and numbered. Reference collections can be kept in alcohol. The above-mentioned procedures are described in greater detail in Chapter 4 of this Manual.

6.6.2 Photographic and visual methods

Objectives

These methods are defined non destructive since they do not require the removal of organisms: samples are represented by photos or video records, or by inventories and counts done directly underwater in the case of visual censuses. Photography claims a longer standing tradition of use and a much greater operational standardisation

than video, which has come into use more recently and more for documentary and exploratory purposes than for proper sampling (George *et al.*, 1985). Visual census requires the recognising of organisms directly underwater and can therefore be conducted only by experienced scientific divers.

6.6.2.1 Photographic surveys

Material and equipment

Waterproof or in-housing photocamera; underwater flashes; close-up or wide-angle lenses depending on the case; reference frames or quadrats; film rolls; videocamera; videotapes.

Sampling procedure

The photographic sampling of hard bottom assemblages consists in photographing a defined area, usually delimited by a frame which allows the film to be parallel to the bottom using a rigid spacer. In the case of small areas, extension tubes are used (macrophotography). Instead, in the case of areas between 70 cm^2 and 400 cm^2, close-up systems are used, coupled with additional lenses: the Nikonos system, unfortunately now out of production, is still the most commonly used (Tab. 3). For larger areas, up

Lens	Sampled area
UW Nikkor 80 mm	7 × 10 cm
Nikkor 35 mm	13 × 19 cm
Nikkor 28 mm	16 × 24 cm

Tab. 3 - Surface areas that can be surveyed, using different lenses, with a Nikonos camera and the original close-up kit.

to approximately 1 m^2 (Fig. 9), a wide-angle lens is to be preferred; in this case, the flash must be equipped with a diffuser to avoid illumination discrepancies (and different readability) between the centre and the edge of the photo. The use of two removable underwater flashes on a stirrup, one of which in slave mode, is always

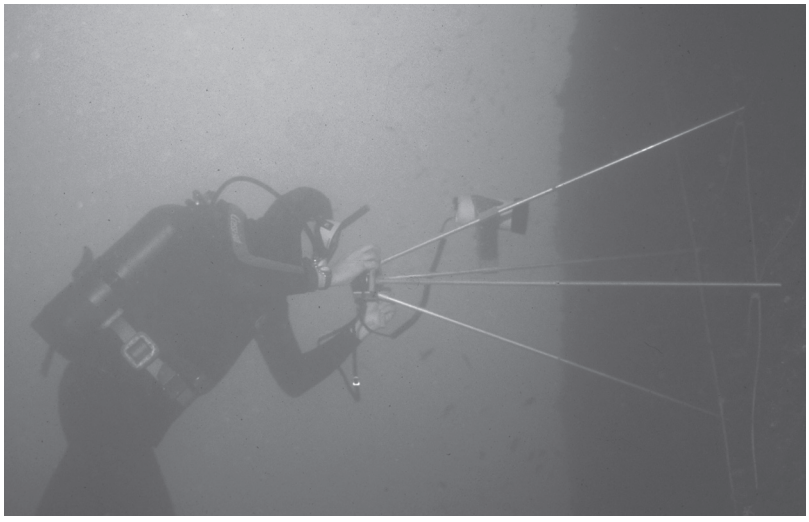


Fig. 9 - Time-lapse underwater photographic apparatus.

advisable. For photographs taken at approximately 1 m of distance, the spherical distortion arising from the use of a wide-angle lens is negligible (Sgorbini *et al.*, 1996).

In case one wants to build an *ad hoc* spacer, the *a* and *b* sides of the framed field (in the 24 × 36 mm format) can be calculated with the following formula:

$$a = 0.56 \times 2d \times \mathrm{tg}\alpha/2 \qquad b = 0.83 \times 2d \times \mathrm{tg}\alpha/2,$$

where d = distance measured from the film level, and α = lens underwater angle (for Nikkor lenses: 35 mm → 46.5°; 28 mm → 59°; 15 mm → 94°).

The focal distance must be set on infinite mode or on the minimum, depending on the instrument (close-up systems, additional lenses, extension tubes), and the brand. It is advisable to work in automatic mode so that the flash intensity is proportional to the light reflected by the subject. The lens diaphragm should be set on the minimal opening possible in relation to the strength of the lighting means (generally between 16 and 22).

Problems and practical tips

Besides being used for documentary applications, underwater photography is widely applied in studies of spatial patterns and/or temporal evolution of populations and assemblages (Pronzato, 1997). In cases of spatial pattern analysis, the photographic survey allows the collection of single species densities, as well as community structure data. Instead, in the case of temporal series, the chrono-photographic series can be planned on fixed sites: the information obtained ranges from individual variations (life cycles, evolution of diseases) to population dynamics, and to substrate colonisation and community development. In the case of very short time series, when the phenomena of interest take place in the span of hours or show

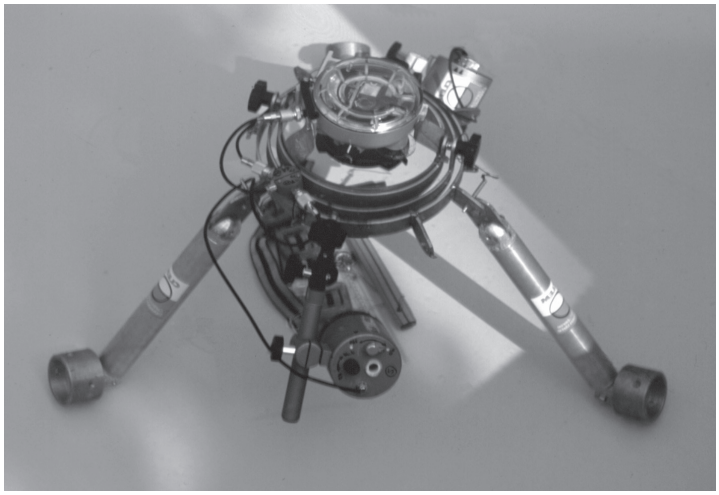


Fig. 10 - Photographic survey of a rocky wall assemblage using purposely made spacer and framer which allows sampling 1-m^2 of substrate for each shot.

circadian rhythms, time-lapse cameras are useful (Fig. 10). The photographic device of Cicogna and Pronzato (1985) represents a simple, cheap and versatile instrument, suited to work automatically and autonomously in different environments.

The photographic sampling equipment can be very different depending on the aims of the study, on the water turbidity, and on the degree of vertical development of the assemblage to be studied. Nowadays, reliable and efficient digital cameras (and videos) are available. Most of the applications documented in the literature, however, regard the use of 35-mm cameras, waterproofed or in housing, equipped with 50 to 100 ISO films for colour slides.

In case the same film roll is used to sample more than one site, the use of a single blank shot as a separation between sites is suggested. The separation shot can be done on the diving slate where all the sampling data are recorded.

Slides can be analysed in the laboratory under the microscope, using a support that allows the slides to overlap a regular transparent grid. Alternatively, the slide can be projected on a screen, and the grid superimposed. In particular instances (although longer and more laborious procedures are required), image analysis software can be used such as, for example, NIH-image, downloadable free from the web at <http://rsb.info.nih.gov/nih-image/> (Macintosh version) or at <http://www.scioncorp.com/> (Windows version).

6.6.2.2 Visual censuses

When sampling using the visual census techniques the scientific diver typically turns his attention to the so-called conspicuous species. These are non-cryptic species, big enough to be easily recognised and identified underwater, and physiognomically remarkable, in terms of abundance and/or biomass and/or cover. It should be borne in mind that the list of conspicuous species that can be observed during a dive should never be considered as an alternative to the floro-faunistic inventory which can be obtained only through an adequate number of direct samples: rather, it could be considered as complementary.

Visual census techniques can be subdivided into two main categories: 1) survey along a defined path (transect); 2) point sampling in a specific and well-defined reference area (quadrat). Both methods come from terrestrial ecology from which they have been adapted, without major modifications, to the marine environment.

6.6.2.2.1 Transects

The term transect comes from the Latin words *trans*- and *secare* (cut through), and has the twofold meaning of a reference line of fixed length and of a sampling method which uses such a line.

The reference line for marine benthos studies consists of a marked line, a Fiberglas tape, or a chain, positioned on the bottom, along which the organisms under the line, or within a belt at both sides of it, are counted and/or measured. The transect can also b e used as a simple reference line along which other sampling devices are positioned, for example quadrats; in the sampling methods described below, however, the transect represents both the line along which the operator moves to collect data, and the instrument used to collect it (Krebs, 1999).

The direction of the transect with respect to the coastline is an important element to consider according to the type of study that will be carried out. A transect perpendicular to the coastline (Fig. 11), commonly called “depth transect”, maximises

Fig. 11 - Depth transect.

the environmental variability and is well suited for bionomic studies aiming at describing zonation patterns (Bianchi *et al.*, 1991). On the other hand, a transect parallel to the coastline, hence at constant depth, minimises the environmental variability and allows the quali-quantitative composition of a specific assemblage to be surveyed (Loya, 1978).

The line, or any other instrument used to define the transect (chain, Fiberglas tape, etc.) should be placed at the fixed depth following the substrate surface as much as possible. Both line ends should be firmly secured to the substrate. It is desirable to avoid, or limit, any movement of the line from the original position, due to the current or to other water movements. Metal hooks, small lines and weights at the line ends can be useful for achieving this purpose. While one operator is positioning the line, the other should record depth, substrate inclination (degrees referred to horizontal), substrate orientation (compass degrees), transect direction (compass degrees) and any other relevant information on the diving slate. Four different transect sampling methods are here described (Fig. 12 and Tab. 4), each with different purposes and characteristics (Tab. 5).

6.6.2.2.1.1 Line Intercept Transect (LIT)

Material and equipment

Marked line, or Fiberglas measuring tape; depth gauge; compass; clinometer; diving slate; pencil; lines, hooks, weights.

Sampling procedure

The intercept to the nearest centimetre corresponding to the point where the organism, or the substrate, changes under the line is recorded on the slate (Fig. 12a). Transect length can be defined depending on the study objectives and on the kind of

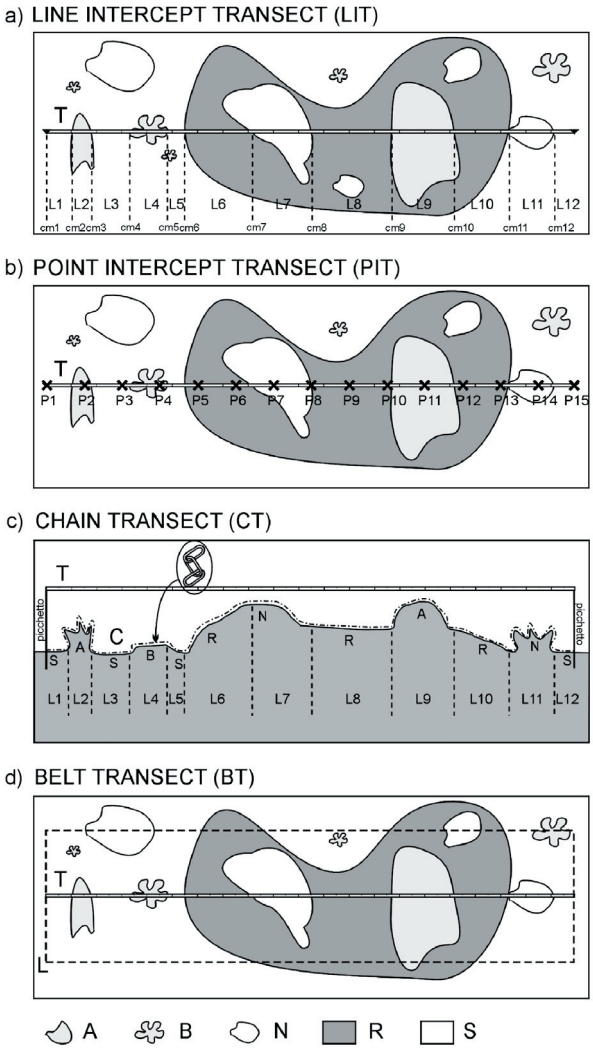


Fig. 12 - Schematic illustration of different transects. a) LIT (Line Intercept Transect); b) PIT (Point Intercept Transect); c) CT (Chain Transect); d) BT (Belt Transect). T = transect; C = chain; W = BT belt width. A, B, N = species or growth form; R = rock; S = sand. L = length of a segment of line (LIT) or chain (CT) covering an organism or substrate trait; cm = intercept of the new organism or substrate type under the LIT line; P = PIT progressive points.

organism, or community, to be surveyed. Commonly used transect lengths are 10 m and 20 m (Rogers *et al.*, 1994; English *et al.*, 1997).

Problems and practical tips

The length of each organism (L) is the distance occurring between two recorded intercepts, and it is calculated by subtraction. To obtain the percent cover (r %) of the species or growth form (x) along a transect of length (T), the following formula should be used

$$r_x\,\% = L_x/T \times 100$$

To calculate the abundance of each organism, it is useful to record when the

Tab. 4 - Data collected for the different transects as in the schematic illustrations of Fig. 12.

LIT	Intercept	Lenght	Abundance
A	cm2 - cm3; cm9 - cm10	L2; L9	2
B	cm4 - cm5	L4	1
N	cm7 - cm8; cm11 - cm12	L7; L11	2
S	cm1 - cm2; cm3 - cm4; cm5 - cm6; cm12-T	L1; L3; L5; L12	1
R	cm6 - cm7; cm8 - cm9; cm10 - cm11	L6; L8; L10	1
PIT	**Points**	**-**	**-**
A	P2, P11	-	-
B	P4	-	-
N	P7, P8, P14	-	-
S	P1, P3, P15	-	-
R	P5, P6, P9, P10, P12, P13	-	-
CT	**Chain links**	**Lenght**	**Abundance**
A	n° anelli L2; n° anelli L9	L2; L9	2
B	n° anelli L4	L4	1
N	n° anelli L7; n° anelli L11	L7; L11	2
S	n° anelli L1; n° anelli L3; n° anelli L5; n° anelli L12	L1; L3; L5; L12	1
R	n° anelli L6; n° anelli L8; n° anelli L10	L6; L8; L10	1
BT	**-**	**-**	**Abundance**
A	-	-	2
B	-	-	4
N	-	-	4
S	-	-	1
R	-	-	1

Tab. 5 - Main characteristics of the different transect methods.

	Data	Advantages	Disadvantages
LIT	Abundance Percent cover Sequence	Low cost Repeatable	Slow
PIT	Frequency Percent cover	Fast Low cost Repeatable	No information on organisms' abundance, sequence and size
CT	Abundance Frequency Percent cover Sequence Substrate "rugosity" index	Low cost Repeatable The only method that can give information on substrate rugosity	Laborious. Potentially destructive. Not suitable for small organisms (smaller than half of the sampling unit, i.e. the chain link). Not suitable for fragile and/or fan shaped or branching organisms perpendicular to the substrate.
BT	Abundance Density	Low cost Repeatable Suitable for fragile and/or fan shaped or branching organisms perpendicular to the substrate	No information on organisms' percentage cover. Not suitable for organisms with aggregated distributions over large areas. Training procedure needed to standardise the operator estimate of belt width.

line covers the same object more than once along the same transect, indicating the different intercepts with a sequential number.

6.6.2.2.1.2 Point Intercept Transect (PIT)

Material and equipment

Marked line, or Fiberglas measuring tape; depth gauge; compass; clinometer; diving slate; pencil; lines, hooks, weights.

Sampling procedure

This method differs from the previous one where data is continuously recorded, in that identification data on the organism, or the substrate, is collected only at previously fixed points along the transect line (Fig. 12b).

Problems and practical tips

Percent cover of x (r_x%) is obtained dividing the number of points where x was found (P_x) by the total number of points along the transect (P_{tot}):

$$r_x\% = P_x/P_{tot} \times 100$$

The total number of points should be defined based on a good compromise between sampling speed and sample representativity, besides, obviously, the line length. Distances between two sampling points commonly used and reported in the literature are 20-50 cm (Rogers *et al.*, 1994).

6.6.2.2.1.3 Chain Transect (CT)

Material and equipment

Marked line, or Fiberglas measuring tape; pegs; galvanised chain; depth gauge; compass; clinometer; diving slate; pencil; lines, hooks, weights.

Sampling procedure

In this case the line is not laid on the bottom, but held hanging by two pegs fixed in the substrate. The chain is laid down on the bottom along the whole transect length, following the substrate and organisms contour with the chain links as far as possible (Fig. 12c). The use of light chains is preferred, and the chain should be, obviously, longer than the transect line.

Problems and practical tips

Percent cover, abundance, and sequence data can be obtained for each organism counting the number of links of known length along the transect. A spatial index (SI) can also be calculated giving information on the structural complexity, or "rugosity" of the substrate, using the ratio of the chain (C) and line length (T).

$$SI = C/T$$

Percent cover of x (r_x%) is obtained dividing

the number of links covering x (A_x) by the total number of links in the chain used to cover the transect (A_{tot}):

$$r_x\% = A_x/A_{tot} \times 100$$

6.6.2.2.1.4 Belt Transect (BT)

Material and equipment

Marked line, or Fiberglas measuring tape; fixed length stick; depth gauge; compass; clinometer; diving slate; pencil; lines, hooks, weights.

Sampling procedure

The line is used as reference and organisms are counted within a belt of fixed width W at both sides of it (Fig. 12d). The total belt width is usually 2 m to 5 m, the length of the line (T) 50 m. The BT allows the density of an organism to be estimated on the basis of its abundance in the surveyed area, which is W×T.

Problems and practical tips

As a training procedure at least, it can be useful to perform the BT using an object (a stick, for example) as long as the belt width (W), or half of it, to become acquainted with the distances across the transect (Fig. 13).

Fig. 13 - Visual census along a belt transect using a 1-m-long rod (secured to the slate) perpendicular to the transect line.

6.6.2.2.2 Quadrats

Material and equipment

A square frame made of metal (usually aluminium) or, even better, plastic material (PVC); depth gauge; compass; clinometer; diving slate; pencil; lines and hooks.

Sampling procedure

This method requires a square frame to be put onto the substrate (using small hooks and lines if necessary) in order to survey the sessile organisms found within the

frame. The frame can be of variable size depending on the study: a 0.5-1 m^2 quadrat represents a good compromise between underwater handling control and sampling representativity. Using a grid of lines, the quadrat can be usefully subdivided into smaller quadrats (for example 25 small quadrats 20-cm side length) that can be used as reference for quantitative surveys. The frame can be made of different materials. Metal frames are negatively buoyant and hard to handle underwater. The best solution is represented by a plastic tube (PVC for example) with holes allowing water to enter: in this way the frame weight is negligible on land, and the water entering the holes makes it slightly negative, but still very easy to handle, underwater. In addition, the structure is very stable both on flat and vertical substrates (Fig. 14).

Fig. 14 - Visual census of a rocky substrate sessile assemblage using a 1-m^2 quadrat, subdivided into a grid of 25 smaller squares.

Problems and practical tips

The quadrat method allows the following to be carried out (Fig. 5): i) counts of individuals (thus directly obtaining the density per m^2); ii) estimate of percent cover (estimating the cover within each small quadrat by eye and then adding up to the total); iii) frequency evaluations (counting the number of small quadrats where the species is present, and referring it to the total number of small quadrats); iv) obviously, presence/absence data can always be collected. Substrate depth, inclination (degrees referred to horizontal) and orientation (compass degrees) data must be collected for each survey (Tab. 6).

Tab. 6 - Quantitative bionomic surveys on rocky substrate by means of 1 m^2 quadrats at four different stations. Stations have different depth, substrate inclination (α), and orientation. Frequency was computed for each species (number of single quadrats of 20 cm^2 where the species is present out of 25).

Station **A**: 10 m, α = 80°, 270° N			Station B: 6.5 m, α = 0°	
Acanthella acuta	1		*Acetabularia acetabulum*	4
Amphiroa rigida	8		*Amphiroa rigida*	1
Cladophora prolifera	3		*Codium bursa*	2
Cladostephus spongiosus	1		*Dasycladus vermicularis*	17
Codium bursa	3		*Flabellia petiolata*	3
Dasycladus vermicularis	5		*Jania rubens*	7
Flabellia petiolata	16		*Lithophyllum incrustans*	1
Halimeda tuna	9		*Padina pavonica*	11
Halopteris filicina	2		*Penicillus capitatus*	2
Lithophyllum stictaeforme	2		*Stypocaulon scoparium*	3
Mesophyllum lichenoides	7			
Padina pavonica	22		Station **D**: 7 m, α = 55°, 290° N	
Peyssonnelia sp. 1	14		*Amphiroa rigida*	9
Peyssonnelia sp. 2	1		*Phorbas ficticius*	2
Phorbas ficticius	1		*Codium bursa*	6
			Dictyota dichotoma	5
Station **C**: 9.5 m, α = 65°, 330° N			*Eudendrium* sp.	2
Amphiroa rigida	10		*Flabellia petiolata*	9
Chondrosia reniformis	2		*Halimeda tuna*	5
Codium bursa	1		*Ircinia oros*	2
Corallina elongata	5		*Ircinia variabilis*	2
Flabellia petiolata	22		*Lithophyllum stictaeforme*	5
Halimeda tuna	3		*Mesophyllum lichenoides*	12
Halocynthia papillosa	1		*Padina pavonica*	21
Lithophyllum stictaeforme	7		*Petrosia ficiformis*	4
Mesophyllum lichenoides	5		*Peyssonnelia* sp. 1	12
Padina pavonica	17		*Peyssonnelia* sp. 2	5
Peyssonnelia sp. 1	23		*Stypocaulon scoparium*	1
Reteporella septentrionalis	2		*Tricleocarpa fragilis*	1

The percent cover visual survey is the measure preferred by many researchers nowadays. Fraschetti *et al.* (2001) proposed the following method. A 0 to 4 score is assigned to each species within each small quadrat: 0 if absent, 1 if covering approximately ¼ of the small quadrat area; 2 if covering approximately ½ of the small quadrat area; 3 if covering approximately ¾; 4 if the species occupies the whole small quadrat. The symbol «+» (usually assigned a conventional value of 0.5 when processing the data) is used to indicate the presence of a species with negligible cover (smaller than ¼). Values are finally summed up for all the small quadrats and converted into a percentage.

It is sometimes useful, for safety reasons, that each quadrat be sampled by a couple of scientific divers, both having excellent floro-faunistic knowledge, at least of conspicuous species. In theory, the two surveys should be identical, but slight differences always occur due to counting error, different visual angle, etc. The two surveys can then be averaged when processing the data into an integrated (hence more precise) survey, or can be kept separate, to obtain an indirect estimate of the error due to the observer. Different studies have proved that visual sampling is very

robust with respect to operator-induced error (Dethier *et al.*, 1993; Benedetti-Cecchi *et al.*, 1996).

6.7 Endobenthos boring

Besides the sessile or mobile organisms that live on the substrate (epibenthos), the hard bottoms benthos is also comprised of species living within the rock (endobenthos). The latter can be simply living in pre-existing cavities (cavitary species) or are able to actively perforate the substrate: this is usually observed in calcareous substrates. The boring of calcareous substrates is a phenomenon of great ecological relevance representing a phase in the turnover of limestone structures, and an intermediate disturbance agent promoting habitat three-dimensionality and diversity. Borers can be subdivided into micro- and macroborers. The former include cyanobacteria, cyanophytes, chlorophytes, rhodophytes and fungi, the latter sponges, bivalves, sipunculids, polychaetes and cirripeds. Bioperforation is accomplished through two different, but often not mutually exclusive, dissolution processes: chemical and mechanical (Hutchings, 1986).

6.7.1 Quantitative analysis of bioerosion

6.7.1.1 Indirect and direct methods

Substrate bioerosion can be indirectly estimated by evaluating the surface of structures such as holes, papillae, and siphons, which allow the bioborer to access the external environment. It is possible to obtain the volume or the biomass of the boring organisms using the appropriate equations, whose coefficients should be calculated experimentally for each species (Schönberg, 2001).

Direct quantitative estimates are based on the evaluation of the bioborer biomass or biovolume. Such variables must be calculated taking into account the existence of mineral structures typical of many boring organisms (spicules, shells, tubes, etc.). Different techniques are available to estimate both variables (Peyrot-Clausade *et al.*, 1995; Becker and Reaka-Kudla, 1997; Schönberg, 2001).

6.7.1.1.1 Biomass

Infested substrate portions are weighed before and after the digestion of tissue, which is achieved by leaving the sample in a 50% H_2O_2 solution for 2 weeks. Alternatively, small limestone substrate cubes can be completely decalcified to obtain the borer's tissue, especially in the case of microborers (a good decalcifying solution that leaves the tissue undamaged is the Perenyi solution, consisting of 0.05% chromic acid, 10% nitric acid, and 90% alcohol, in the proportion of 30:40:30). The remaining material, made up of boring organisms, is collected filtering the decalcifying solution. The decalcified residual can be used for a qualitative study of the involved organisms. See also Chapter 8 on Microphytobenthos in this Manual.

6.7.1.1.2 Biovolume

The total bore volume can be calculated in different ways, four of which are here described. The first three methods require the substrate to be cut, a procedure that makes the sample unsuitable for further analysis. The instrument used to cut the samples cuts at least 2-3 mm of substrate for each section, and the procedure

can crumble the sample when erosion is strong. The fourth method, on the contrary, leaves the sample intact, and hence usable for further analyses.

1) The substrate is cut in 0.4-1 cm thick sections. Cavities surface is measured either directly or on photographs of the section using a graphic table. Alternatively, it can be estimated using a millimetre scaled grid, and counting the number of quadrats over the eroded areas. The mean area is then calculated on measurements on adjacent sections, and this number is in turn multiplied by the section thickness to obtain the desired volume.
2) Thin substrate sections, fixed and included in resin, can be observed at the microscope or at the scanning electron microscope to obtain quantitative estimates of microborer (algae, fungi) erosion.
3) Substrate sections, devoid of organic matter through immersion in H_2O_2 or NaClO for a long time, are X-rayed (using 5-20 sec exposures at 45-65 kV depending on the substrate). Eroded areas are measured directly on the radiographic slab placed on a light board, or black and white prints, using the slabs as negatives. Different erosion tracks left by the numerous organisms (sponges, bivalves, worms, etc.) are then identified. Cavities bored by sipunculids and polychaetes are not easily distinguished, and, for this reason, are commonly considered together. X-ray analysis provides good results in case of less porous substrates. Thin erosion areas are more difficult to tell from substrate porosity in very porous substrates, such as scleractinian corals.
4) Substrate samples are dried and undergo tomography (120 kV and 60 mA). Each boring organism group can be identified with different colours on the print. Bioerosion areas are estimated by means of computer image analysis. Similarly to the X-ray method, the distinction between small bioerosions and natural substrate porosity is often very difficult.

6.7.1.2 Estimating bioerosion rates and modes

The use of artificial substrates allows the boring rates and modes of different organisms to be estimated with precision (Peyrot-Clausade *et al.,* 1995; Neumann, 1966). Regular blocks are cut (for example 8×4×4 cm, or 2-4 cm^2 by 0.5-1 cm) from a calcareous substrate. Before use, the blocks should be cleaned in H_2O_2 or $NaClO$, rinsed in distilled water for some days in order to remove any trace of organic material, or other undesired materials, and then dried and weighed. The blocks are put *in situ*, and then removed at regular time intervals (6 months, 2, 5, and 10 years) to evaluate the evolution of the boring communities.

The blocks can be secured with steel lines to natural substrates affected by boring organisms. Subsequently, the blocks with borers can be put, along with blocks without borers acting as control, in the natural environment or in an aquarium for different periods of time. The use of Iceland Spar, a transparent carbonate, allows development of the boring to be observed.

6.7.2 Morphological study of the bores

The morphological study of the boreholes is usually done using casts. In case of microborers (algae, fungi), substrate fragments are sampled and cut to obtain small blocks (3×3×8 mm); these are then fixed in 4 % glutaraldehyde and subsequently post-fixed in 1% osmium tetroxide (Golubic *et al*., 1970). After the post-fixation process, a buffer solution and distilled water rinse follows, then the samples are dehydrated

through a series of acetone solutions with increasing concentration, starting from a 10% one, through nine passages of 30 minutes each, to pure acetone. Following the acetone bath, samples must be immersed overnight, at 5 °C, in a solution made up of 60 parts of an Epon mixture, and 40 parts of acetone. The Epon mixture must be prepared following this recipe: 50 parts of Epon-812, 47.5 parts of NMA (naphthyl-methyl-anhydride) and 2.5 parts of BDMA (benzyl-dimethyl-amine) put into a shaker for 15 minutes. The mixture can be preserved in a freezer for a long time, provided it is kept carefully hydrated. The infiltration takes place at room temperature, in vacuum, for 4 hours. Then the material must be put into the pure Epon mixture, in vacuum for 1 or 2 hours. Finally, the material is put into containers (usually caps or similar) and put in a stove at 60 °C for 40-72 hours. The solid resin blocks are then partially melted in HCl until the casts of the channels bored by the organisms, fixed in the original position, are evident. The obtained casts can be studied at the stereomicroscope, or at the scanning electron microscope (SEM). Furthermore, the casts can be included in resin and sectioned for observation at the transmission electron microscope (TEM). A less complicated procedure uses two epoxy resin components (Kresten-Nielsen and Maiboe, 2000). Further details on this kind of procedure can be found in Chapter 8 of this Manual.

The three-dimensional reproduction of a macroborer organism can be obtained infiltrating a cold resin (Batson's #17 plastic replica and corrosion kit, Polysciences Inc.) inside the organism, through the bore opening (Burlando *et al*., 1990). An alternative to the resin is the commercial silicon, which is very simple to use but affords a poorer definition of details. When resins harden, the substrate must be eliminated in 10% HCl sequential baths. The material thus obtained can then be examined at the stereomicroscope, or at the scanning electron microscope.

6.8 References

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