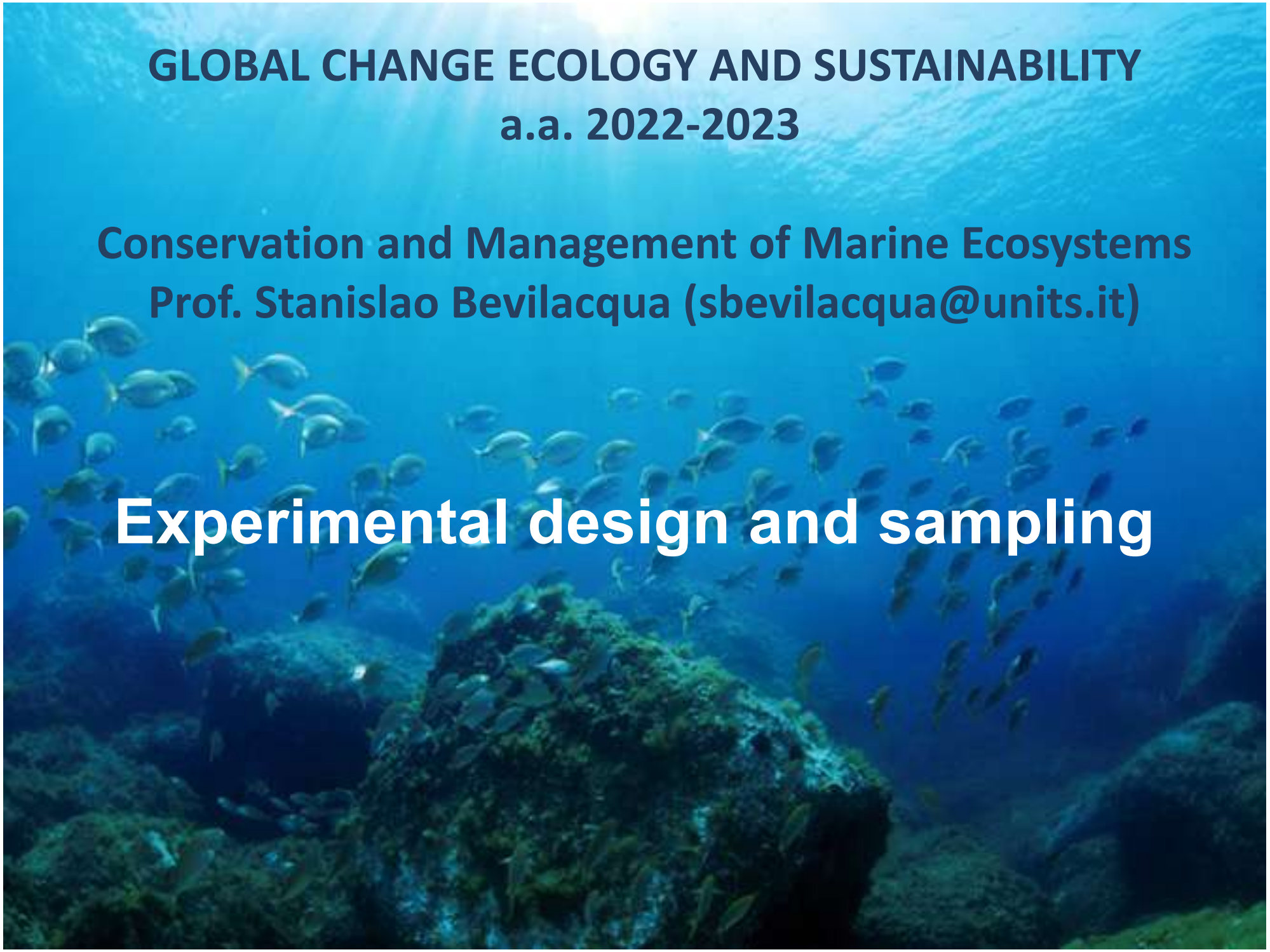
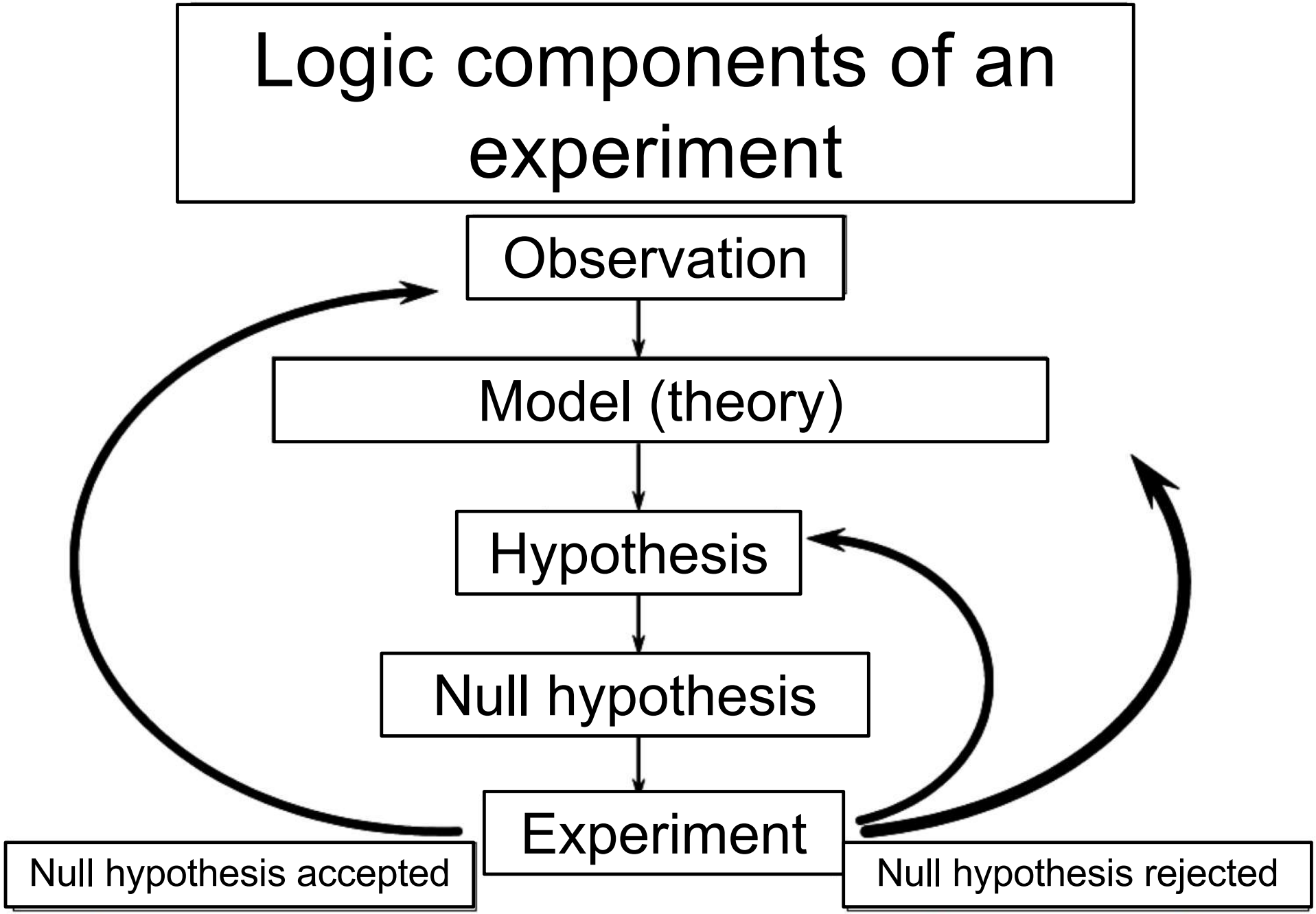


GLOBAL CHANGE ECOLOGY AND SUSTAINABILITY
a.a. 2022-2023

Conservation and Management of Marine Ecosystems
Prof. Stanislao Bevilacqua (sbevilacqua@units.it)

Experimental design and sampling

Experiments



An example

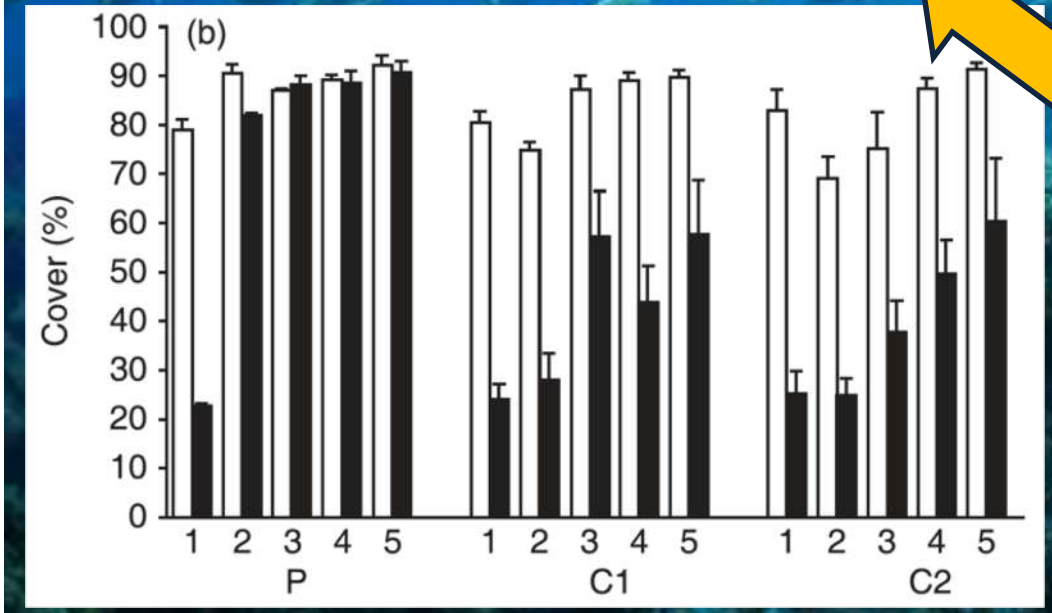
OBSERVATION
Protected assembalges in MPAs have lower temporal variability than unprotected assemblages

THEORY
Reduced human disturbance within MPAs allows to increase the integrity of protected ecosystems and, thus higher resilience

HYPOTHESIS
After disturbance, protected assemblages recover faster than unprotected ones

NULL HYPOTHESIS
After disturbance, unprotected assemblages recover as rapidly as protected ones

EXPERIMENT
Protected and unprotected assemblages are subjected to a disturbance and recovery is monitored



Manipulative and mensurative experiments

Manipulative experiments are those experiments in which the effects of different levels of a predictor variable (factor), which is directly manipulated by the experimenter, are compared. It defines direct cause-effect relationships between factors and response variables.
E.g.: adding sediment to the system, reduce the number of species, etc.

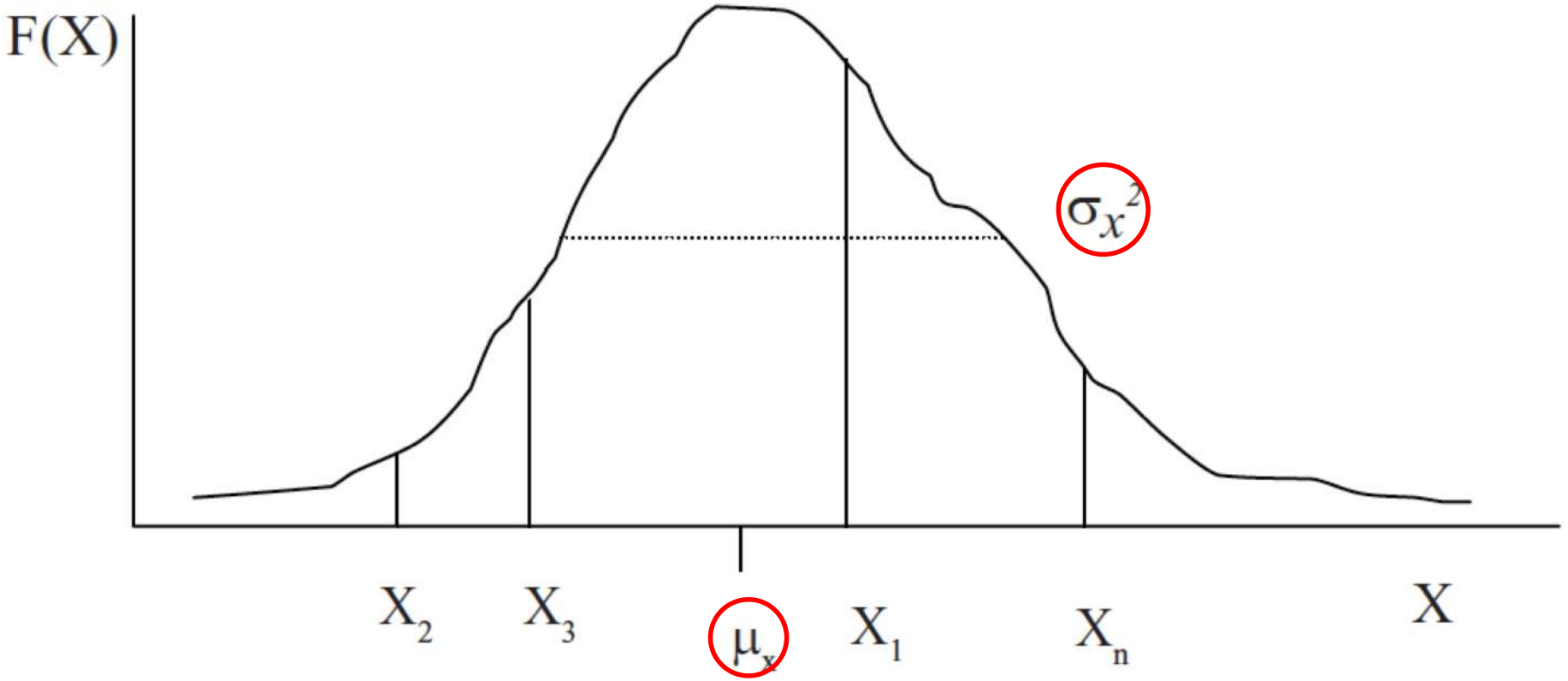
Mensurative experiments are those experiments in which different levels of a factor are not directly manipulated by the experimenter, but comparison is constructed through sampling. It defines relationships between variables without identifiable cause-effect links.
E.g.: assessing populations among different positions along a gradient, or in space, etc.

Factors are predictor variables of measured response variables. They have at least two levels, each represented by a set of observations (sample) related to the factor and subjected to the same experimental conditions

<u>Hypothesis</u>
Species richness increases with depth

<u>Hypothesis</u>
Larval survival decreases at increasing temperature

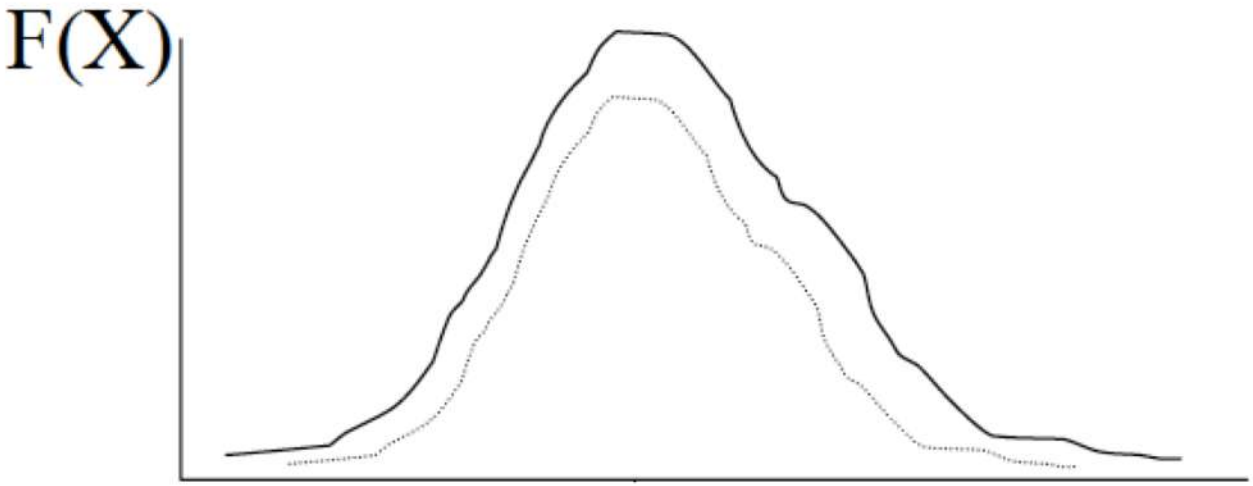
Variables and sampling



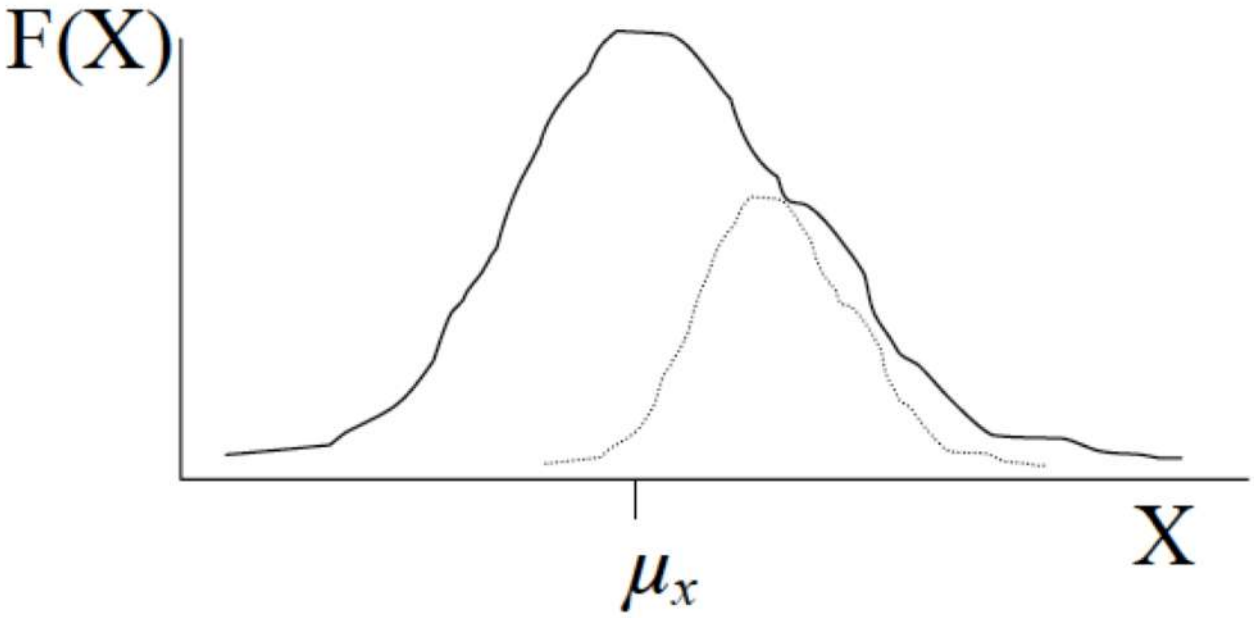
Parameters are mathematical constants that identify the frequency distribution of a variable (i.e. an attribute or quantity that can have different values). Mean and variance are two parameters. The first defines the position of the distribution, the second the dispersion of the values around the mean value.

The sample is a set of random values from the frequency distribution of possible values of a variable.

Variables and sampling



The parameters of a frequency distribution are estimated through sampling. Estimates are useful only if they are representative.

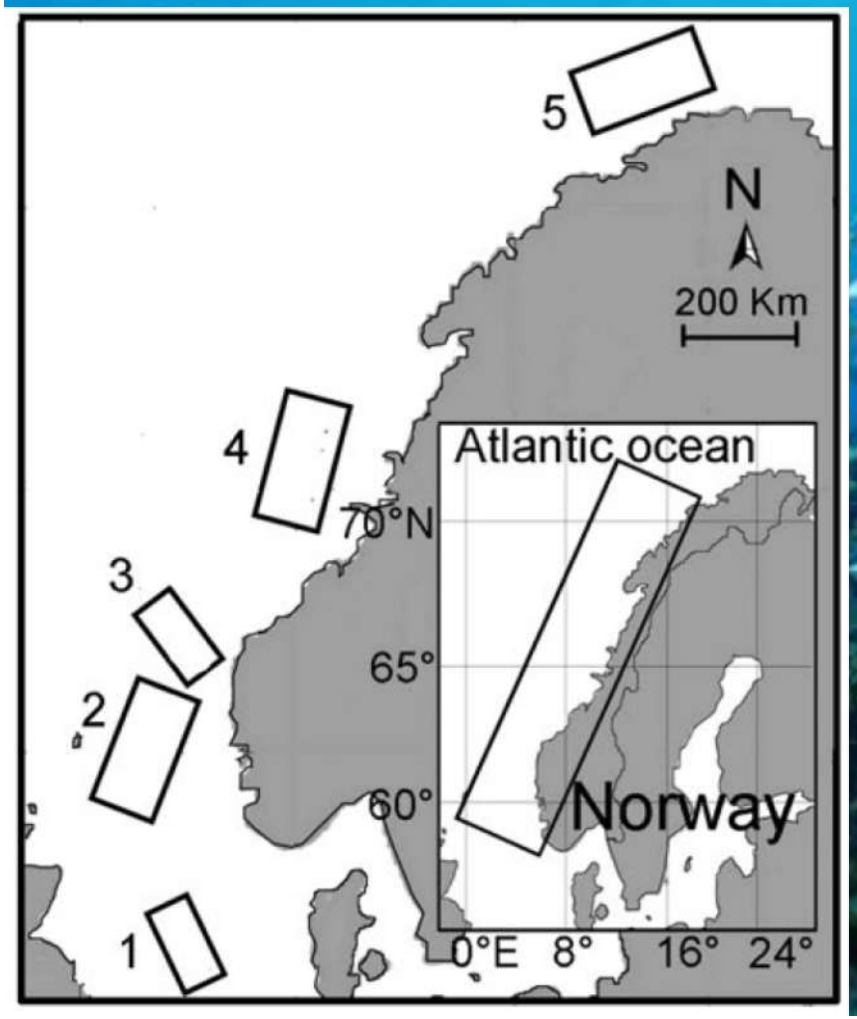


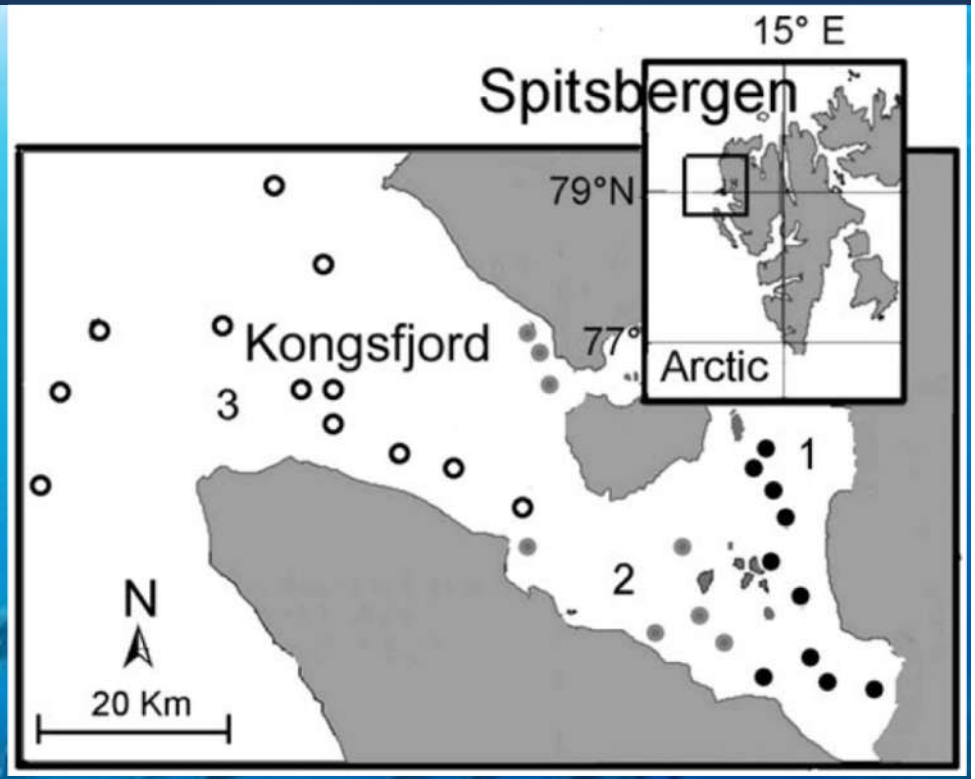
Therefore, in order to be representative, sampling must capture the whole range of possible values. For a sample to be representative, all possible observations must have an equal probability of being considered.

Representativeness is secured with the randomness of the observations, as well as a suitable number of replicates, the random positioning of sampling units

Fixed and random factors

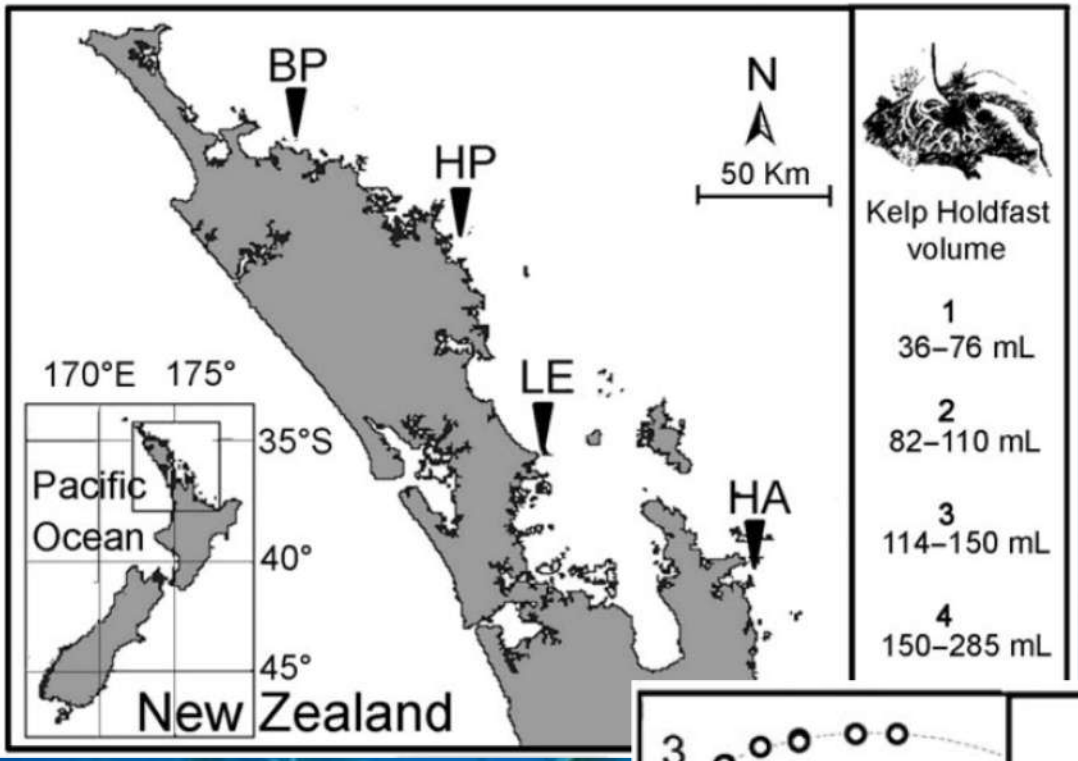
A factor is *fixed* when the levels to be included in the experiment are defined by the hypothesis under consideration and are identified by the experimenter. In the case of a fixed factor all levels relevant to the hypothesis are included in the experiment.



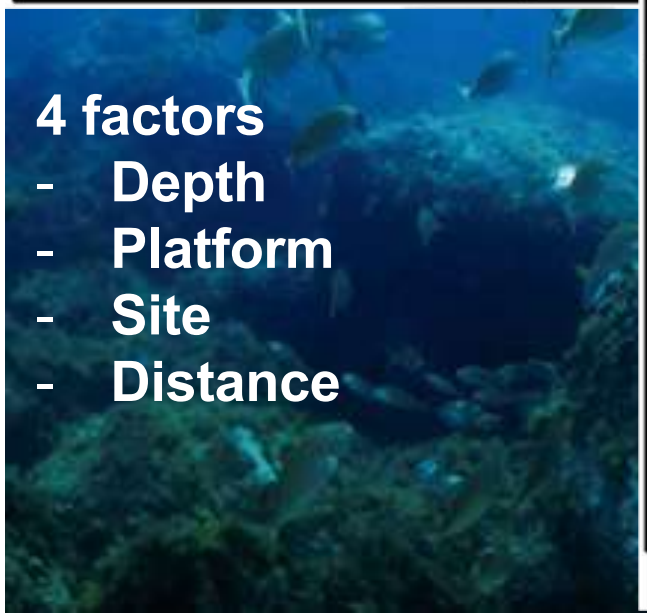


A factor is *random* if the levels included in the experiment are a subset of all possible levels of the factor, and the selection is random. In the case of a random factor only a sample of possible levels is represented in the experiment.

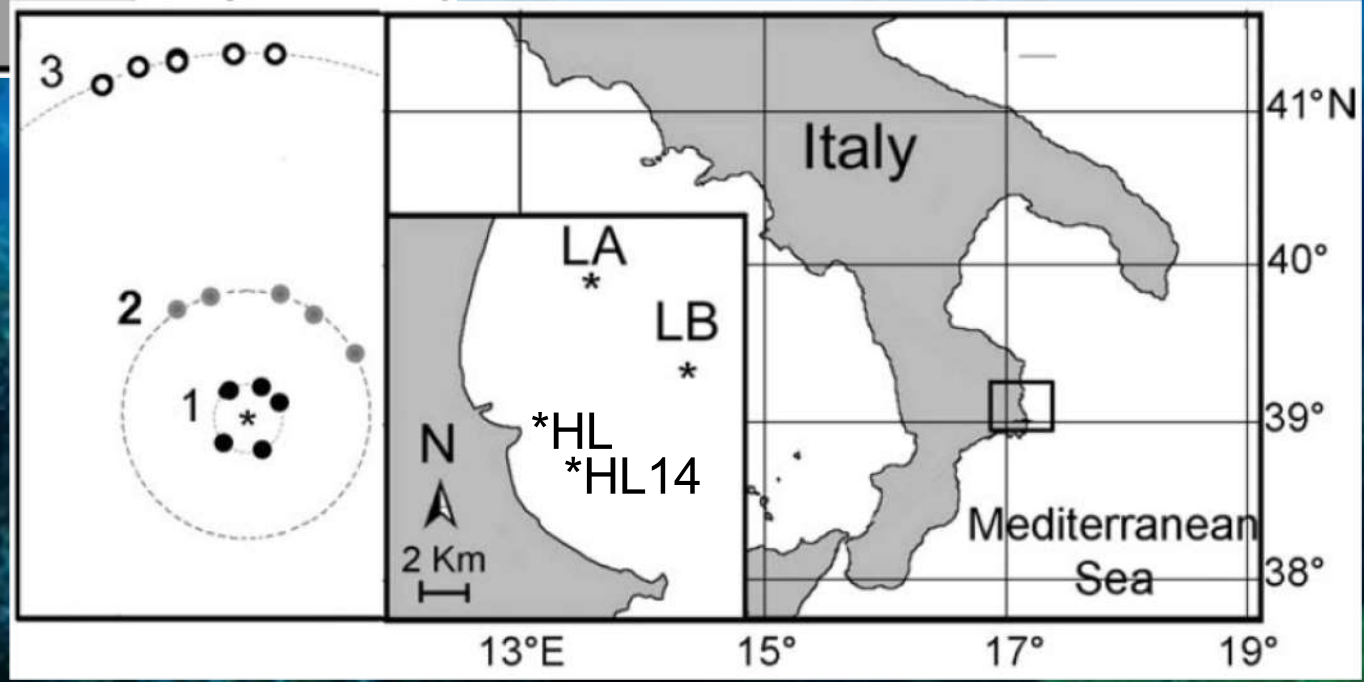
Multifactorial designs



2 factors
- Location
- Holdfast volume



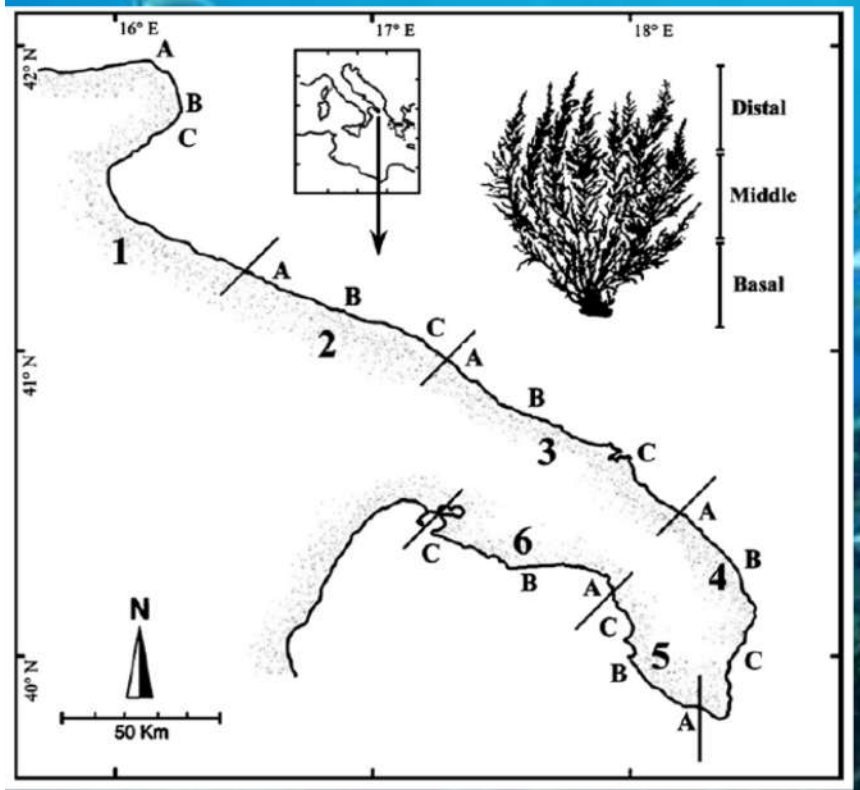
4 factors
- Depth
- Platform
- Site
- Distance



Experimental design

HYPOTHESIS

- **Number of factors**
- **Relationships among factors**
- **Factor levels**
- **Number of levels for each factor**
- **Location of experimental units in space and time**
- **Number of replicates**



Sector 1 2. … 6

Location A B C A B C … A B C

Site 1 2 3 1 2 3. … 1 2 3

Heigth BMD BMD … BMD

n = 10 thalli

Crossed and nested factors

Two factors, A and B, are *orthogonal* (or crossed) when all levels of A are represented in each level of B and viceversa. A given factor B is *nested* in A (the 'nesting' factor) if each level of B is represented in each level of A, but not the opposite.

Depth: 5 m, 15 m, 30 m
Location: 1, 2, 3

For each level of factor Depth there are samples from each location, and for each level of factor Location there are samples from the three different depth.

Location :	1	2	3
Site:	1,2,3	1,2,3	1,2,3

For each level of factor Location there are sample from the three sites, but these samples are specific of sites within the location. In other words, there are no samples from each location in each site.

Confounded experiments

Wrong experimental designs cause confounded experiments which are not adequate to test the hypothesis. This occurs when differences among levels of a factor do not isolate the effects of that factor, but include or are influenced by the effects of other variables.

Season:	Winter	Spring	Summer	Autumn
Date:	1	2	3	4

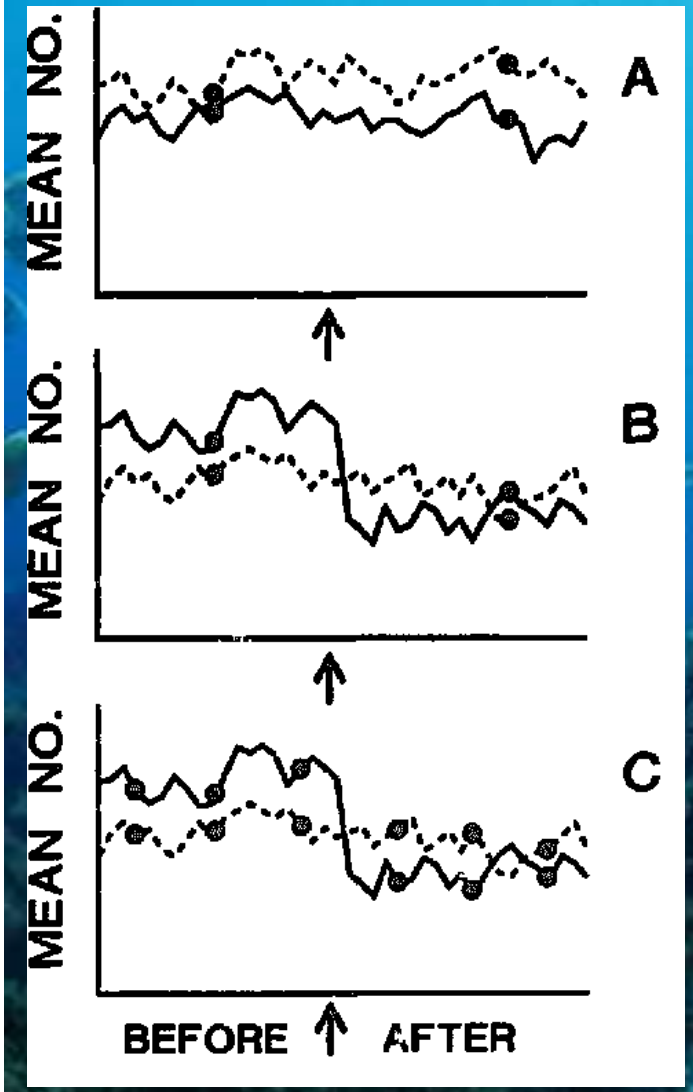
Differences among season in the response variable are determined based on a single date of sampling. This prevent identifying differences among season, since a single date could not be representative of the season. The effect of season is confounded by the effect of varition among dates.

Season:	Winter	Spring	Summer	Autumn
Date:	1,2,3	1,2,3	1,2,3	1,2,3

A correct design must include replicate date of sampling for each season. This is needed to correctly estimate the variability within seasons, avoiding confounding the effects of seasonal changes with those araising from changes among times of sampling.

BACI, beyond-BACI, ACI design

In the assessment of environmental impacts, a putatively impacted site is compared with multiple control sites. Multiple controls are needed to estimate the natural variability in unimpacted conditions. Controls must share all other environmental and biological conditions with the putatively, except for the presence of the source of impact.



Only 1 time of sampling before and after: impact erroneously detected due to a stochastic divergence of the response variable between I and Cs in the second time of sampling

Only 1 time of sampling before and after: impact not detected due to a stochastic overlapping of the response variable between I and Cs in the second time of sampling

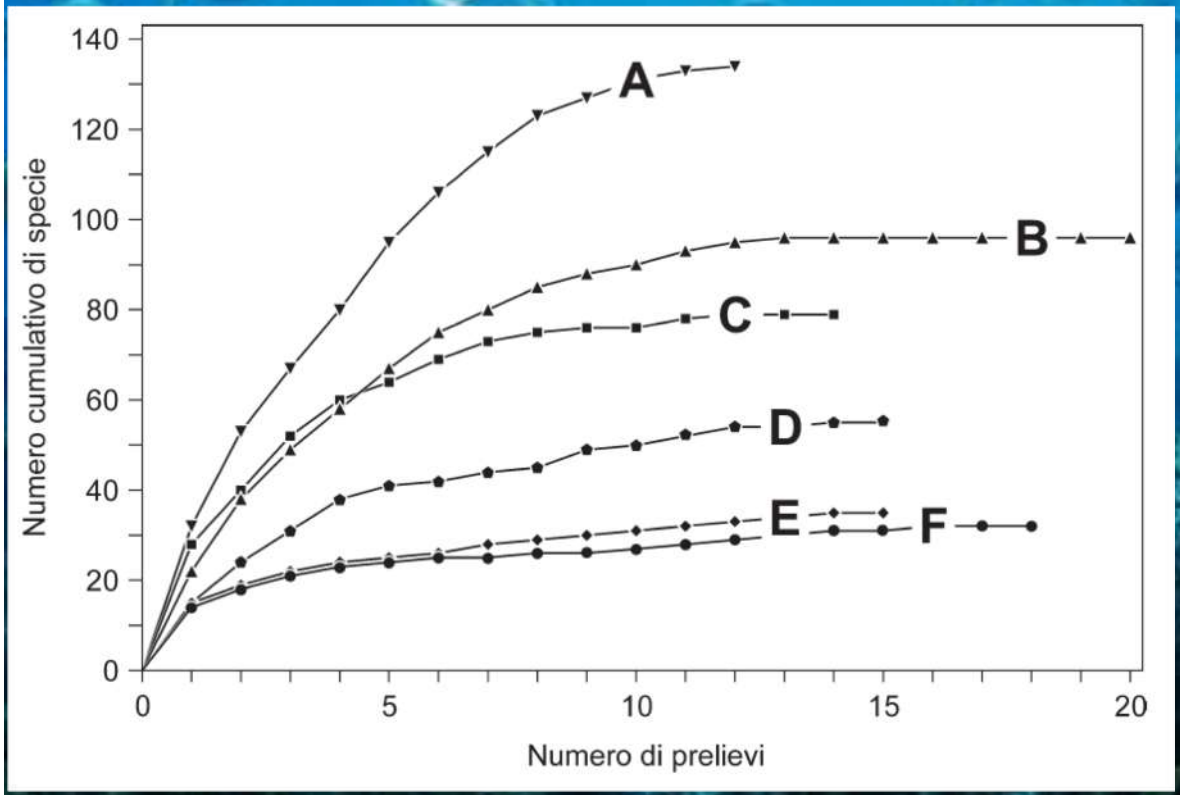
Multiple times of sampling before and after: impact correctly detected

The same may occur when comparing a single putative impacted location against a single control location. Multiple times of sampling and multiple controls are required for a correct assessment of impact, before and after. In most cases only after impact assessments are possible – ACI design

Sampling units

The most common shape for sampling a surface is square of rectangle, and cylinder or parallelepiped for volume. However, shape does not really matter.

Size of sampling units should be selected depending on the size of organisms under study and their spatial distribution. More generally, the size and number of samples should be decided taking into account the system being investigated and sampling constraints. For example, too many samples do not significantly improve estimates of the variable under study, but might increase difficulties related to sampling (e.g., underwater sampling activities).



Common practice on rocky substrata uses square samples of 20-25 cm for macrobenthos (photogrpahic samples, visual census), 15 x 15 cm for scrapings, and normally 10 replicate samples. Sampling units of 1 square m for vagile macrofauna (sea urchins, sea cucumbers, etc.).

Common assessments

Assemblage structure can be quantified through *qualitative* or *quantitative* assessments. The former refers to the identification of species (only presence-absence), without quantifying the abundance of species.

These assessments can also refer to taxa higher than species or morphological groups. When abundance is estimated as ranks, a *semi-quantitative a*ssessment is obtained.

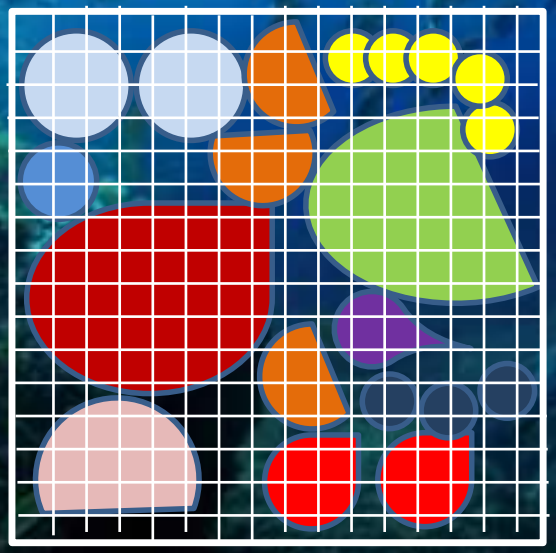
Quantitative assessments, instead, besides the identity of organisms also provide their abundance in terms of:

Biomass

Density

Cover

Frequency



Biomass

Biomass can be expressed as wet weight, dry weight, or ash-free dry weight.

Wet weight is not recommended when organisms in the assemblages are very different, with a wide range of water contents.

The remaining two methods are commonly used. For dry weight, the organisms are placed in ovens at temperatures between 50-100°C to eliminate sea water. The drying period can vary from a few hours to a couple of days, depending on the exposure temperature and the type of organisms (therefore the water content).

Often, especially for many benthic organisms, the problem is not only water content, but also the content of inorganic compound (e.g., carbonate), which does not constitute biomass. For this reason, it is often preferred to quantify the biomass, subtracting the weight of the ashes from the dry weight.

In these cases, the dry mass of organisms is further processed with a furnace where dry mass is exposed to temperatures between 450-900°C to eliminate the organic matter.

Then, the actual biomass is calculated by subtraction of ash weight from the total dry weight, which also includes the mineral parts (shells, exoskeletons, etc.)

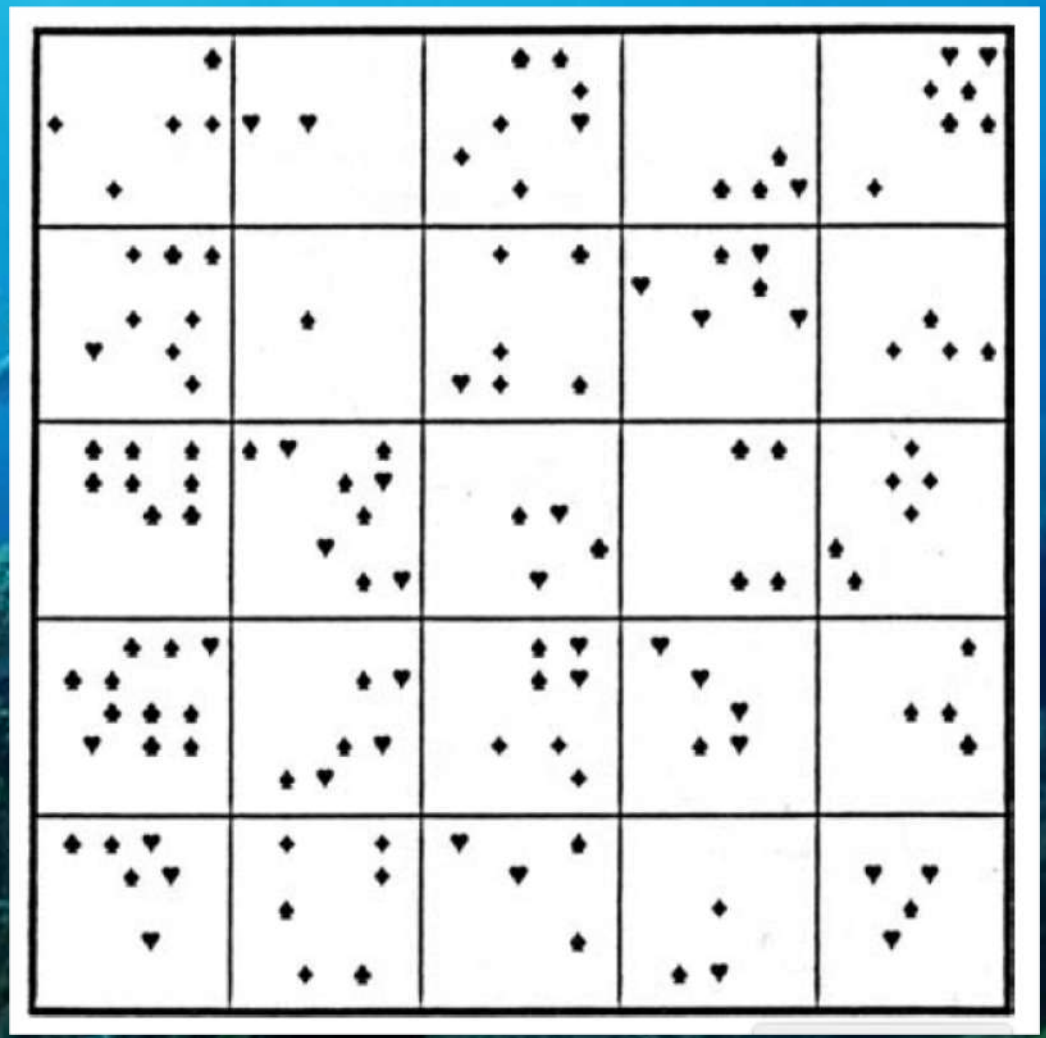
Density, cover, frequency

Abundance of a given species can be obtained by counting all individuals of that species in a sample. The total number of individual can be expressed as density in relation to the sampled surface/volume. This method can be applied to unitary organisms.

% cover on the surface sampling units is recommended when organisms are colonial, or mix among colonial forms and individual organisms.

Frequency consists of counting the number of times a given species occurs in a sample.

The different approaches are selected depending on the type of organisms and the aim of the study



Sampling methods

Direct collection: collecting organisms within the sampling unit through the use of specific tools (grabs, corers) or directly removing organisms (scraping)

Advantages: objective evaluations, accurate taxonomy, voucher collection of organisms

Drawbacks: time-consuming, cost-expensive, need of taxonomic expertise, small sampling units, impact on the community.

Application: biodiversity studies, energetic studies

Video- or photographic sampling: sampling through the use of video-photo tools (e.g., digital cameras)

Advantages: objective evaluations, voucher collection of videos/pictures, fast and not expensive, large areas can be sampled, low or no impact of sampling

Draawbacks: taxonomy can be not accurate, difficulties in identifications, problem with comprehensive quantification of community structure

Application: widely spread, monitoring studies

***Visual census in situ*: estimates of abundance and identification of organisms are carried out directly in the field**

Advantages: low cost, large areas of sampling, data readily available, low or no impact of sampling

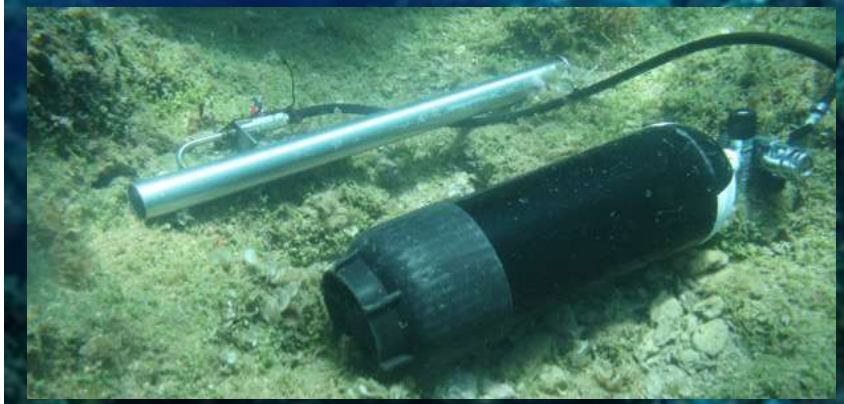
Draawbacks: subjective assessment, taxonomic expertise required, difficulties for underwater sampling

Application: preliminary assessments

Destructive methods

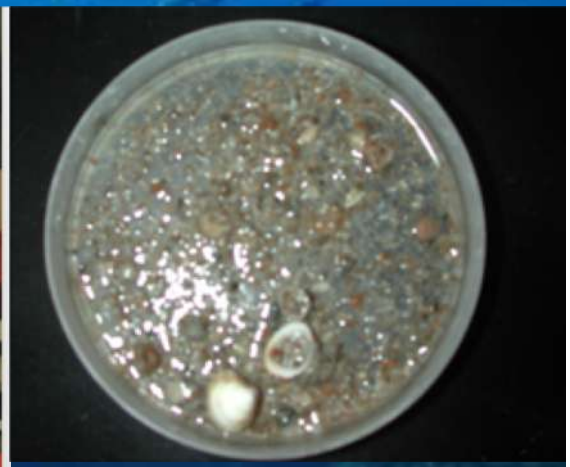
Scraping is a destructive collection method that consists in removing all organisms from the substratum through the use of a hammer and chisel, scrapers, etc. The removal is carried out within a known surface, delimited by a square, often made of aluminium or plastic. The removed material is conveyed inside a mesh bag (generally with a 500 µm - 1 mm mesh for macrofauna).

When the aim is to sample vagile fauna, the airlift is the most frequently used tool. The airlift is a tool that uses the compressed air of the scuba tank to generate suction at the end of a pipe (aluminium, steel or plastic), that creates a flow from one end of the tube to the other which allows organisms to be sucked into a mesh bag. It can often be alternated with scraping, if the aim is to sample all organisms, including the vagile one, or to collect all the fragments removed during the scraping.



Sample processing

Once in the laboratory, samples collected by scraping and/or airlift are processed. The first step is to sort samples, that is separating organisms by large taxa, from non-biological material (sediment, rocks, detritus). In case of sampling that includes vagile fauna, samples are left to rest so that the organisms escape from the interstices. The sample is then fixed in seawater and 10% formalin for organism preservation. Prior to fixation, the use of anesthetics is recommended (magnesium chloride). At the time of sorting, the sample is first sieved (with a 0.5-1 mm mesh) and rinsed abundantly to eliminate formalin residues. Subsequently, the organisms are manually separated from the inorganic material. The operation is carried out under a stereomicroscope. The extracted organisms are first separated by large taxonomic groups, and then identified to the species level and counted. The organisms are preserved in alcohol at (70%) or in formalin at 4%.



Non-destructive methods

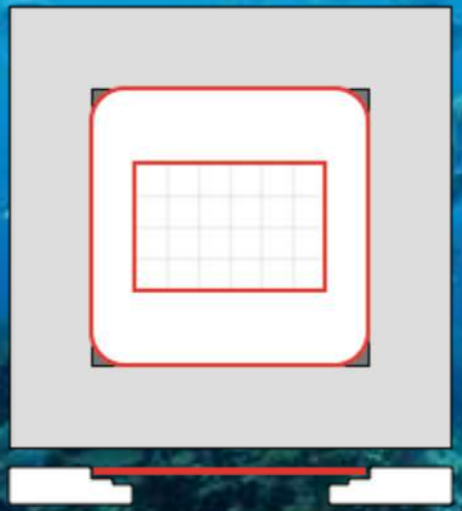
Photographic sampling is a non-destructive method, mostly used on hard bottoms, which consists of taking photos on a standard surface, often delimited by a plastic or metallic frame. Light is provided with strobes, especially in shaded or deep environments.

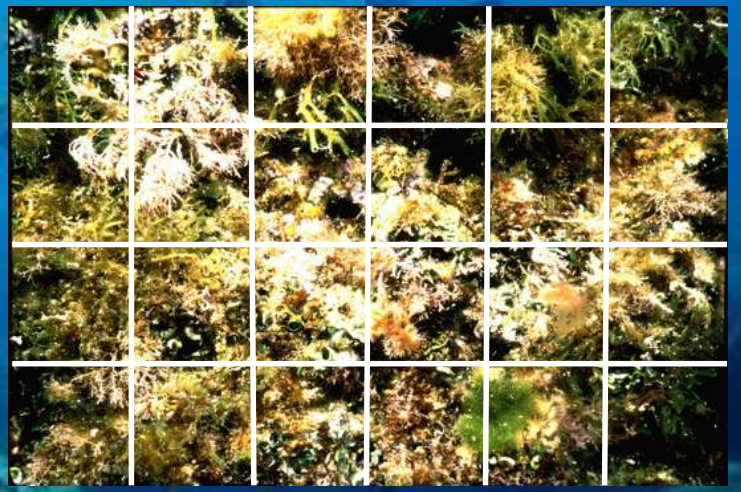
Another non-destructive method consists of direct counting and identifications in situ, during scuba diving or snorkelling. This help identifying organisms when photographic methods will lead to information loss (e.g., when sampling assemblages associated to conspicuous macroalgal canopies.

Processing of photographic samples

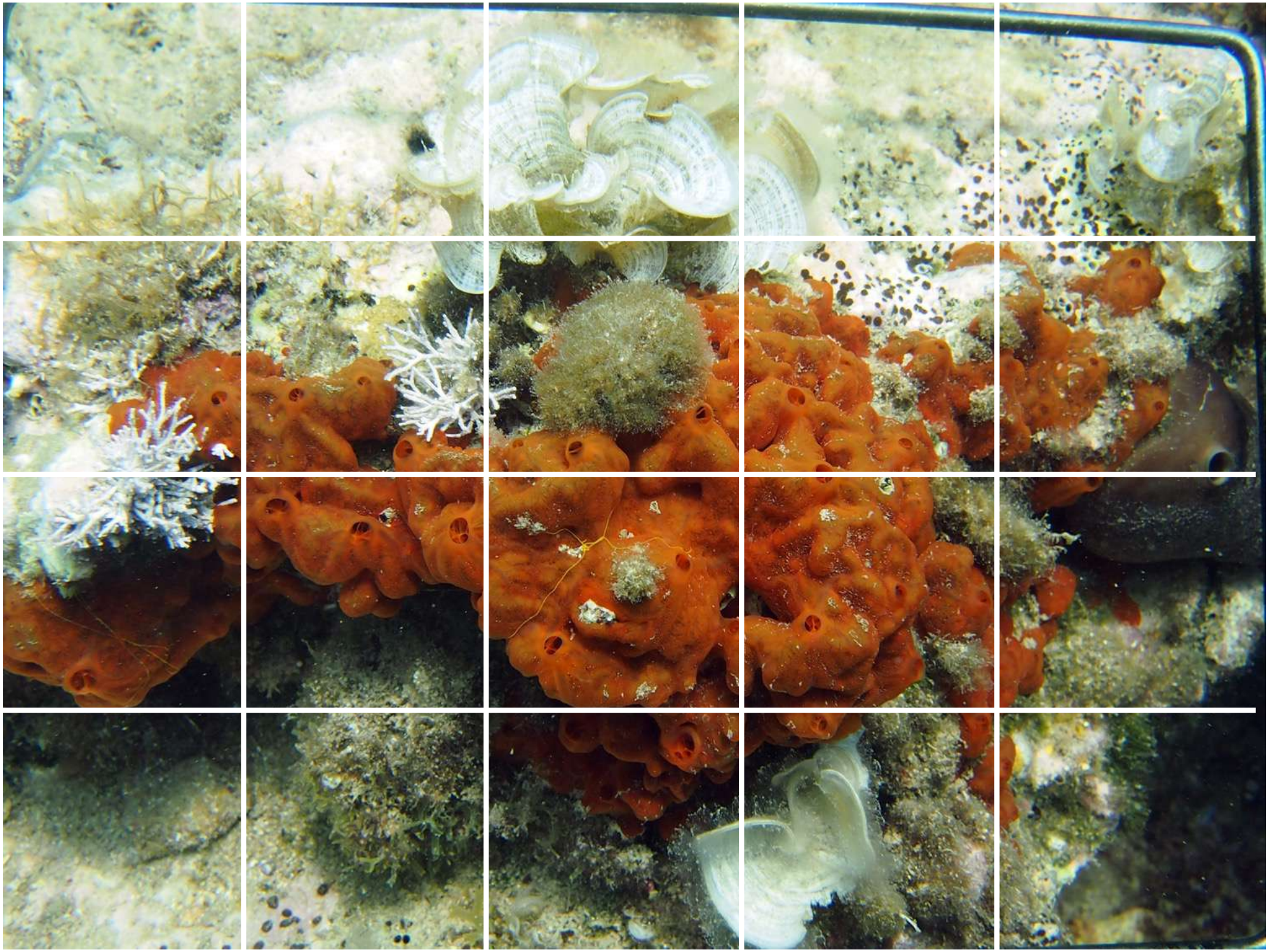
In the past, the photographic samples were developed obtaining slides which were subsequently analysed in the laboratory under magnification. A grid was superimposed on the images to facilitate estimations. Normally the sampled area is equivalent to a rectangle of 16 x 24 cm, divided into 24 sub-squares. Each sub-square is ideally divided into four subunits, each equivalent to 1% coverage. For organisms with lower coverage, a coverage of 0.5% is arbitrarily assigned.

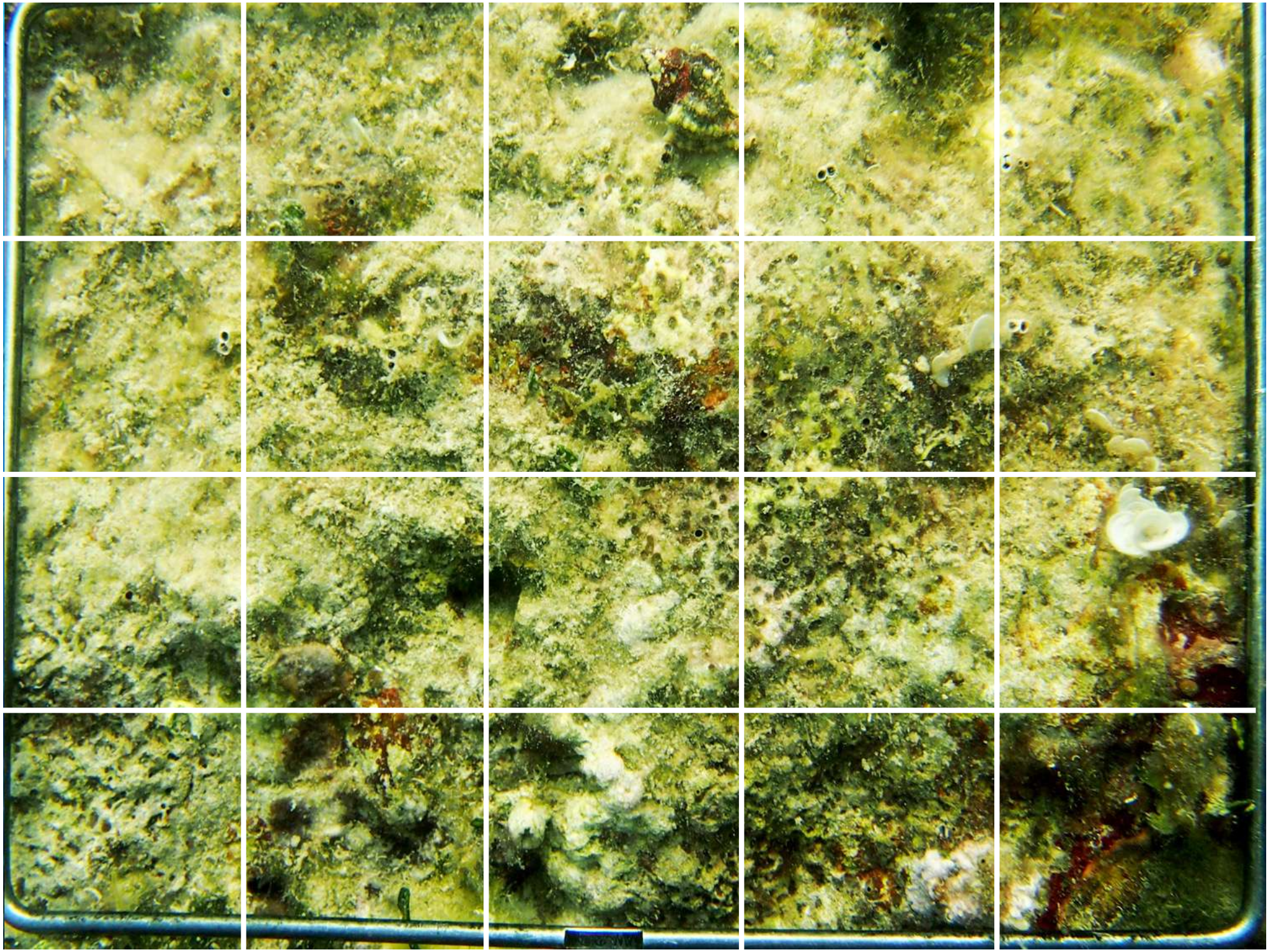
The operator then adds up the subunits covered by each species, obtaining the total coverage for that species (or taxonomic, morphological group, etc.). The % estimates are then brought back to 100% with a simple proportion.

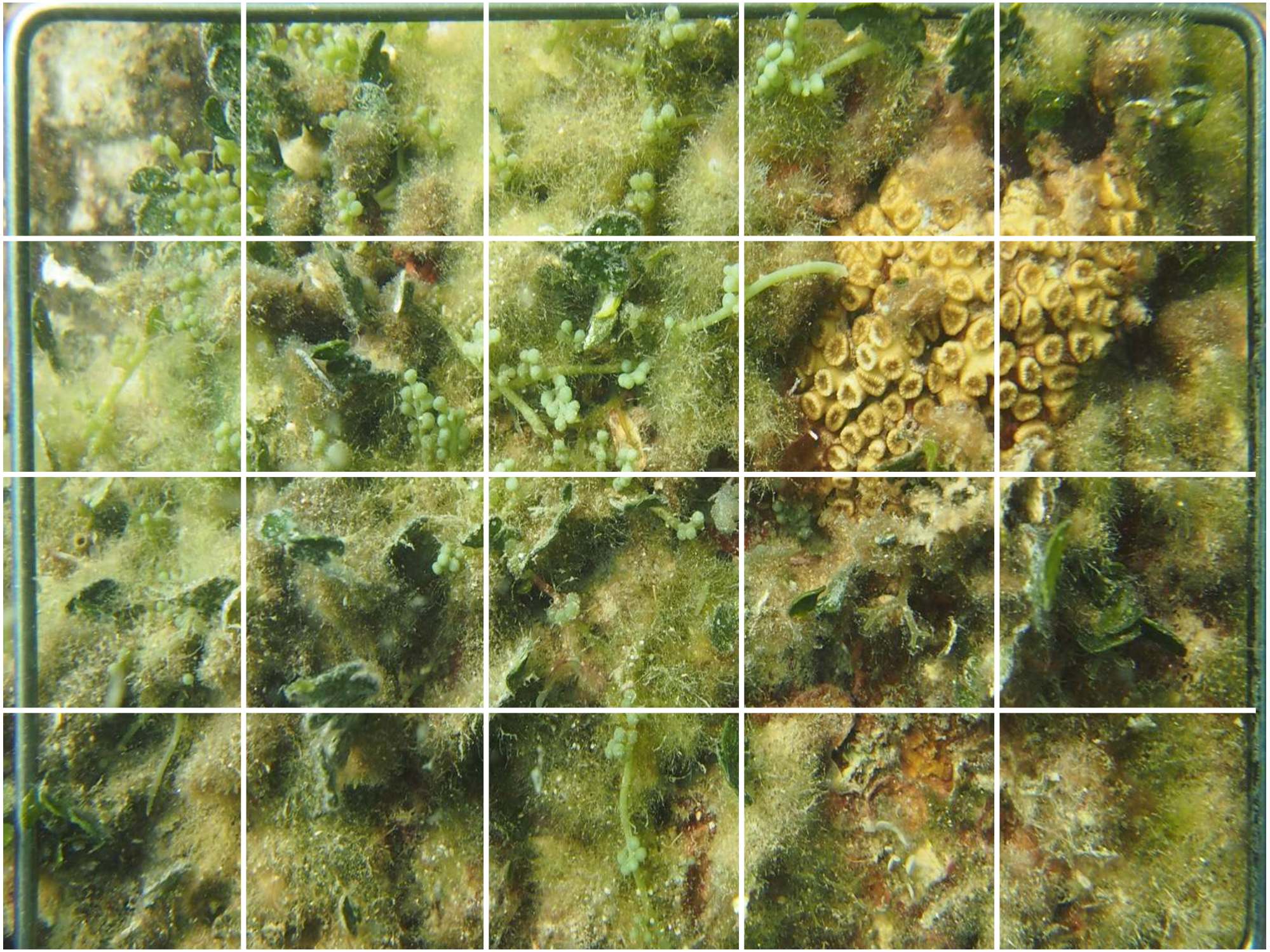


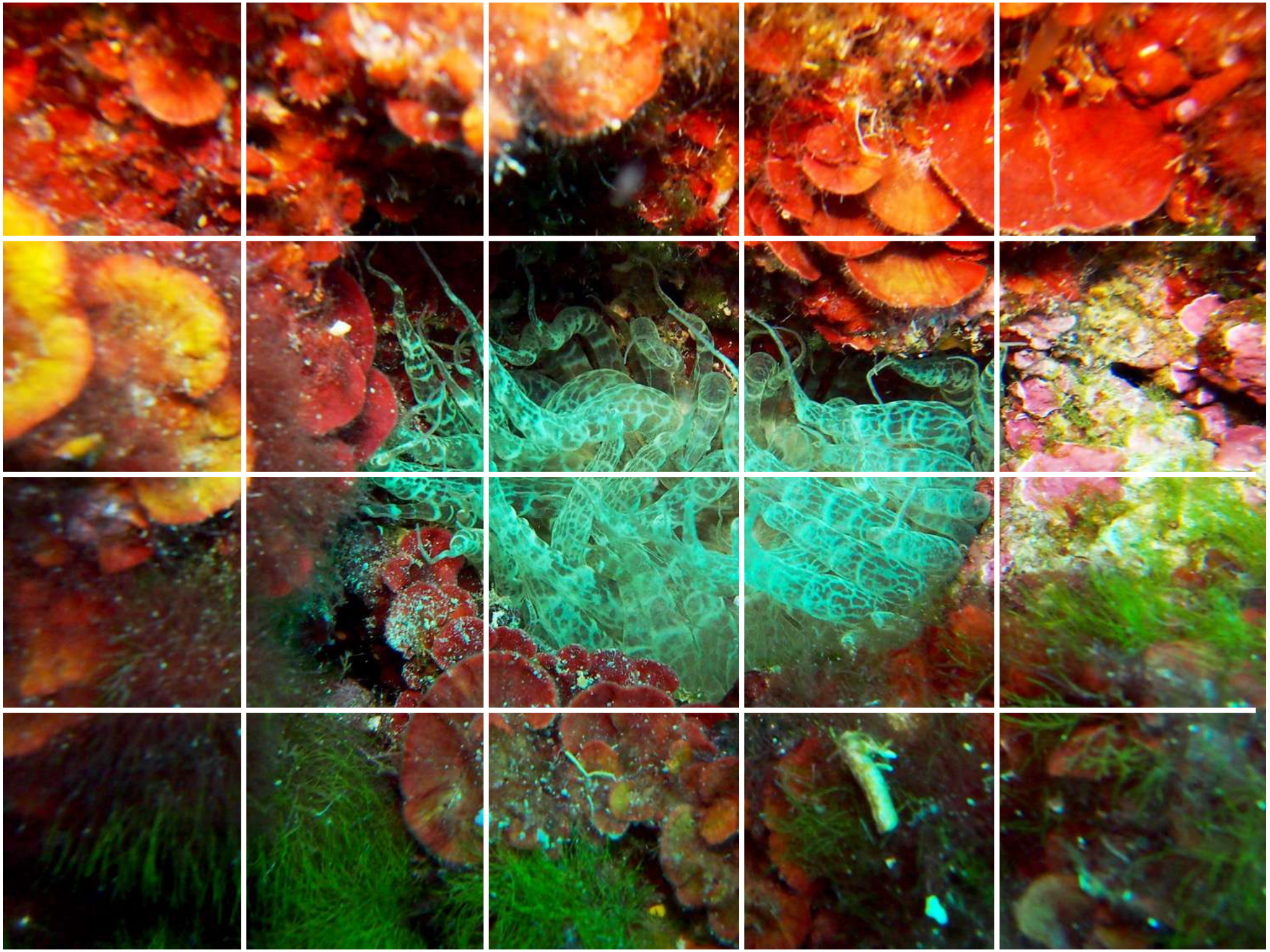


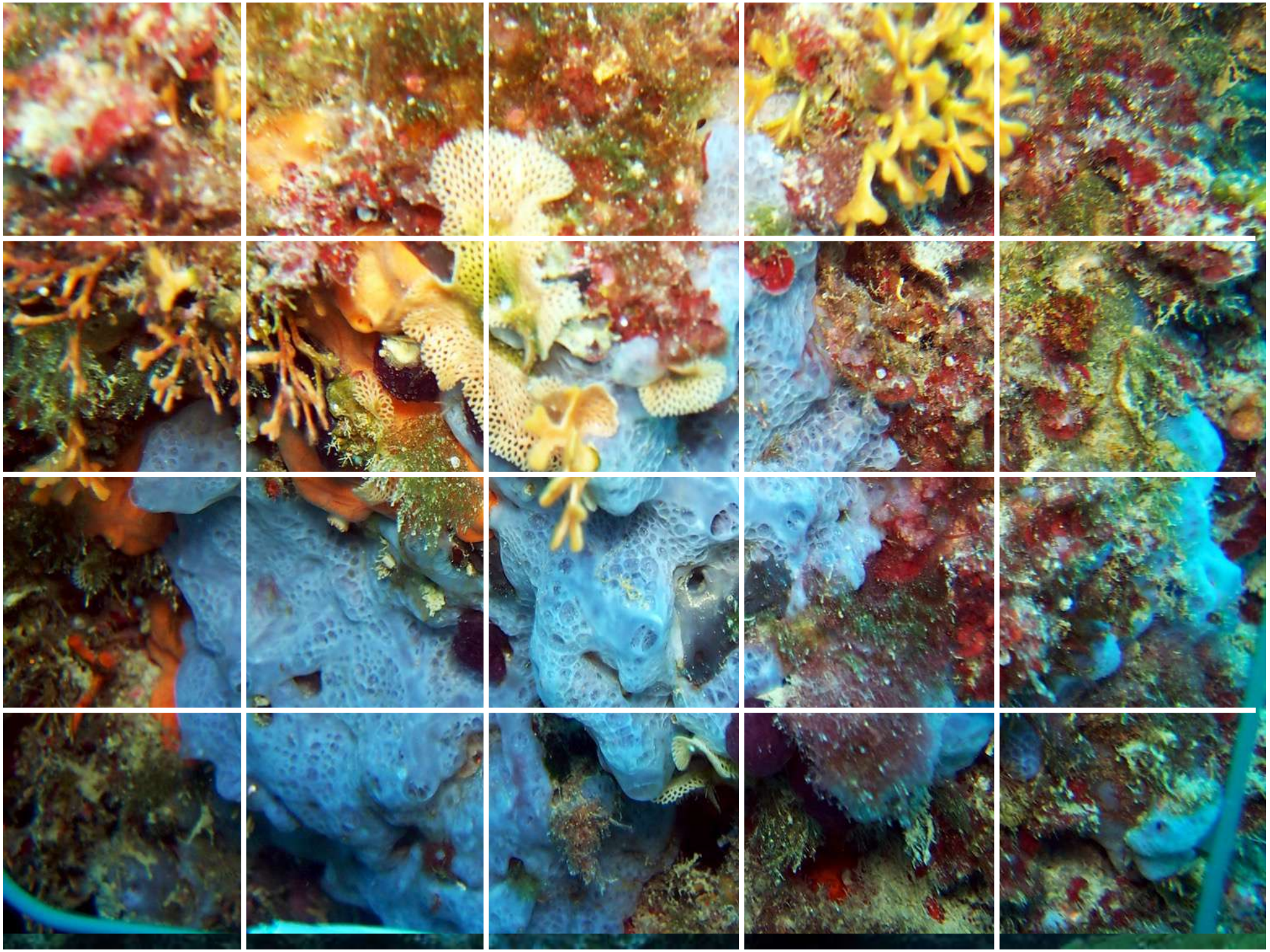
Nowaday, with the spread of digital cameras and imagining, digital picture are analysed with the help of a computer and software, but the role of human operators is still necessary in identifying organisms.

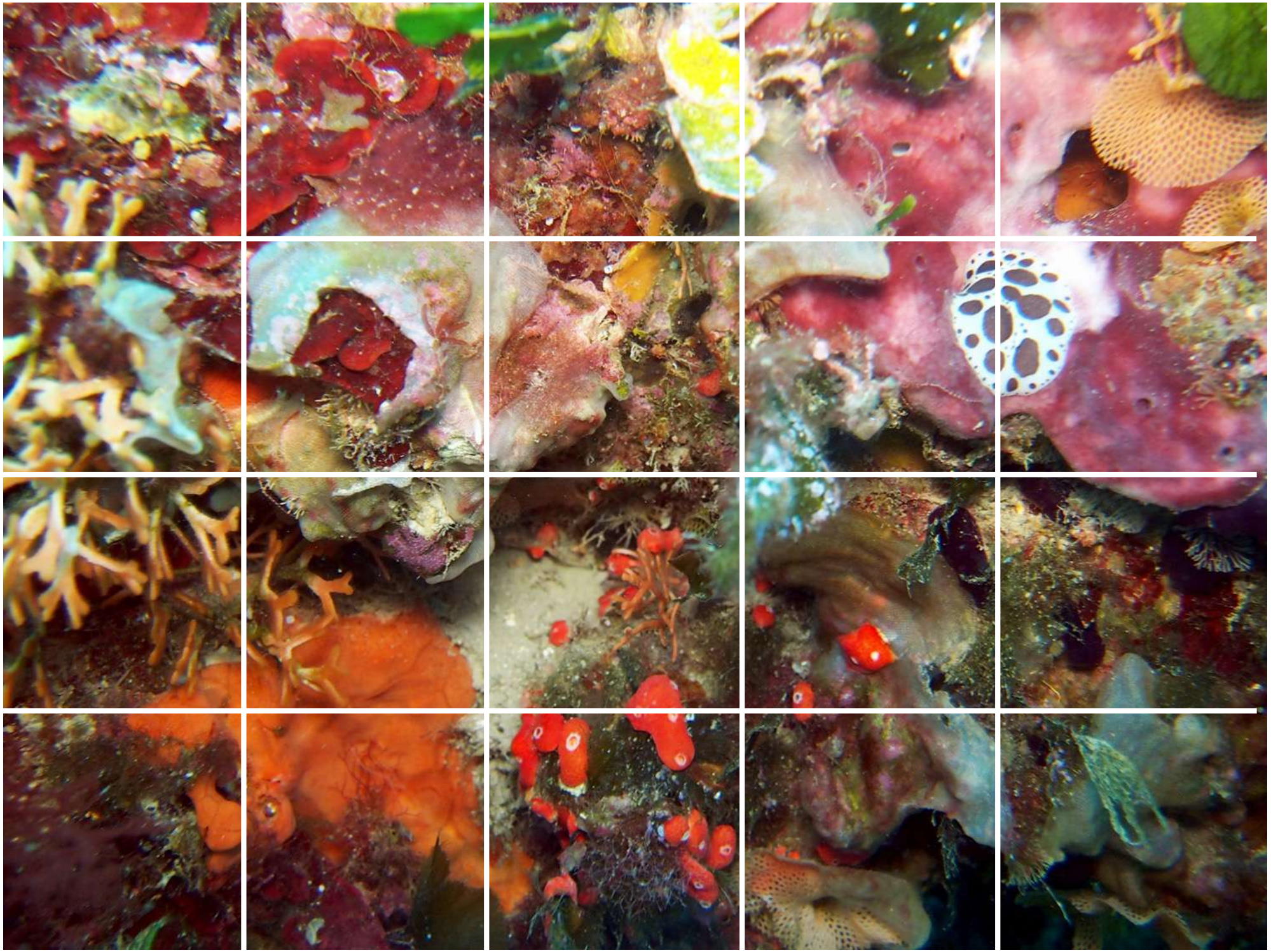


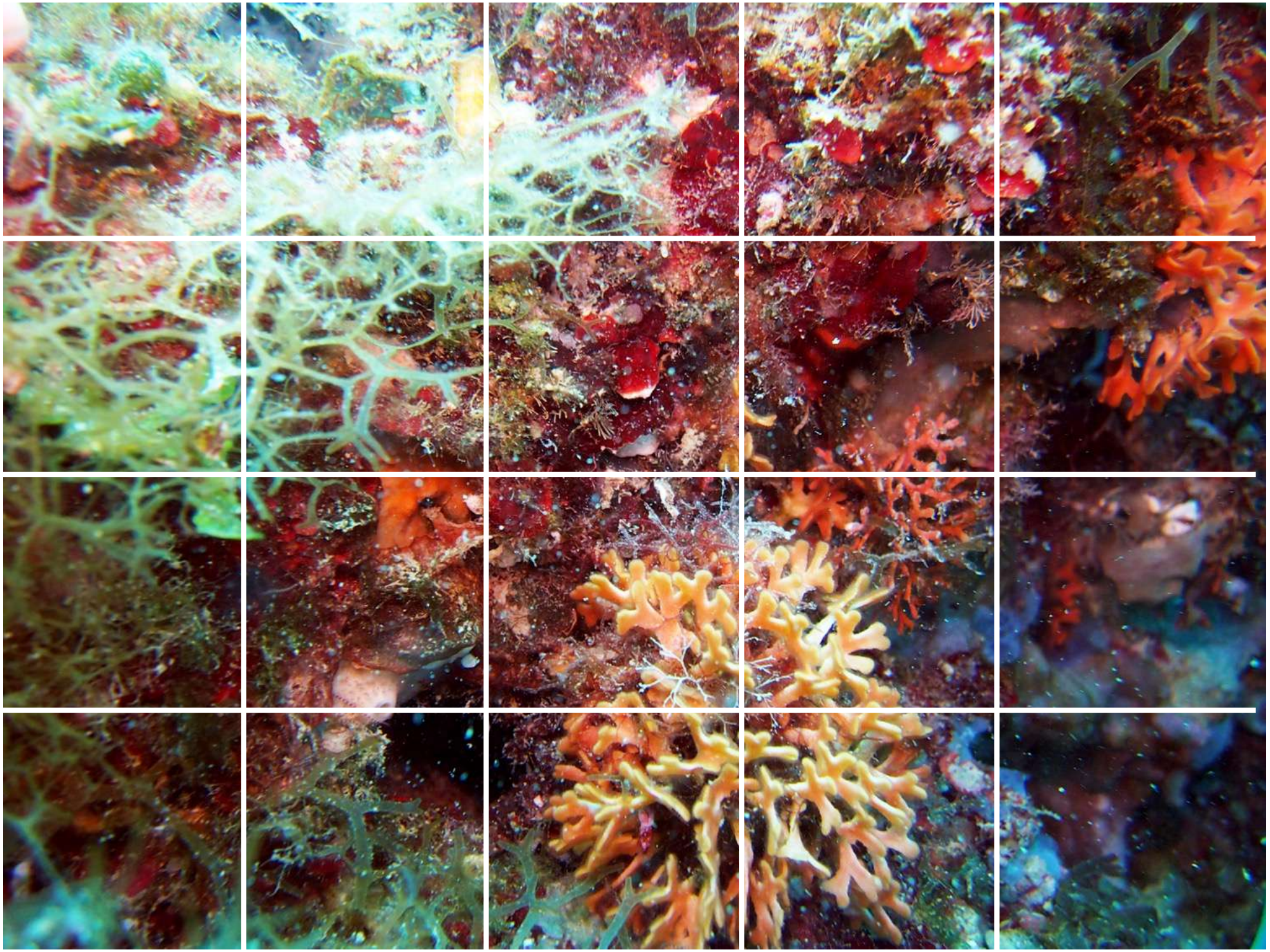












Encrusting corallines (e.g.: *Lithophyllum* spp.)

Articulated corallines(es: *Jania* spp.)

Green filamentous algae (e.g.: *Cladophora* spp.)

Fliamentous algae (e.g.: *Ceramium* spp.)

Chtamalus spp.

Semibalanus balanoides

Perforatus perforatus

Cliona viridis

Rocellaria dubia

Mytilus

Serpulids (e.g., *Pomatocerus*)

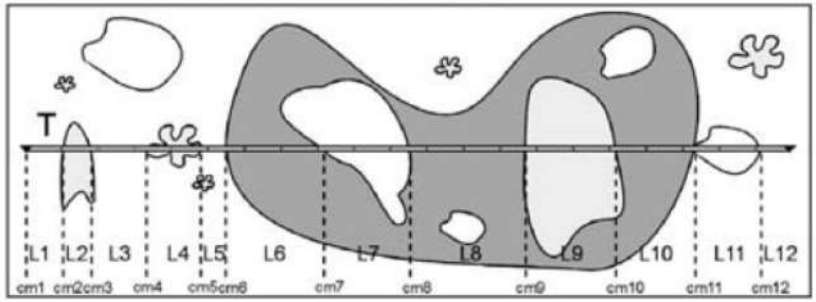
Oysters (e.g., *Ostrea edulis*)

Vermetidi (es. *Dendropoma*)

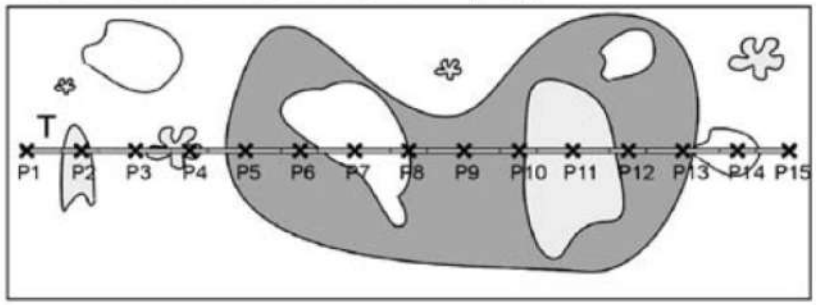
Belt transects

Sampling along transects is done by counting or measuring the organisms along a line track with the help of a metric rope or rib. This is placed for the desired length (generally 10-25 m) and the evaluations are carried out in different ways along the transect.

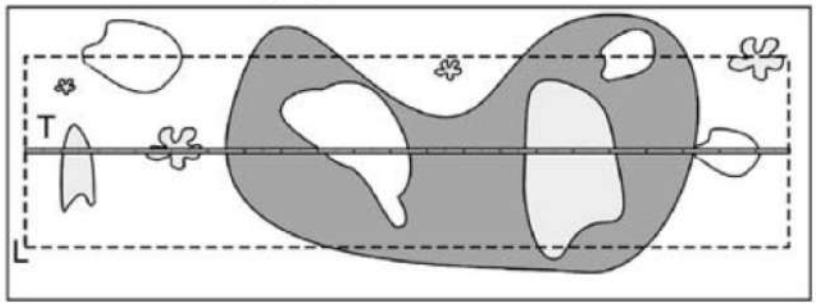
a) LINE INTERCEPT TRANSECT (LIT)



b) POINT INTERCEPT TRANSECT (PIT)



d) BELT TRANSECT (BT)



One method implies measuring the length of the segment cover by a given organism. This procedure is very slow and recommended when organisms are very large, or for preliminary assessments

A second way to proceed is to recording the organisms in fixed point along the transect. This is a relatively fast method, but it does not provide information on the size of organisms (abundance can be estimated as frequency)

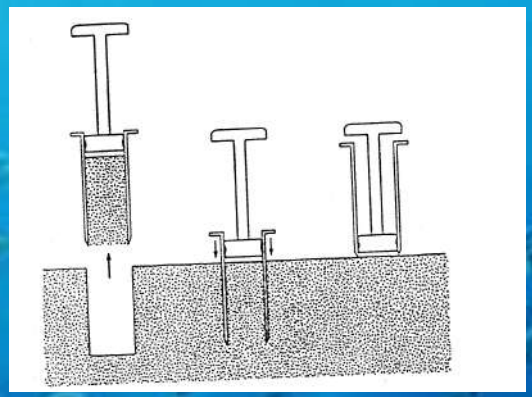
Finally, organisms can be counted within a range of 2 m on both sides of the rope. This method is often used for large colonies (e.g. gorgonians) or for fish

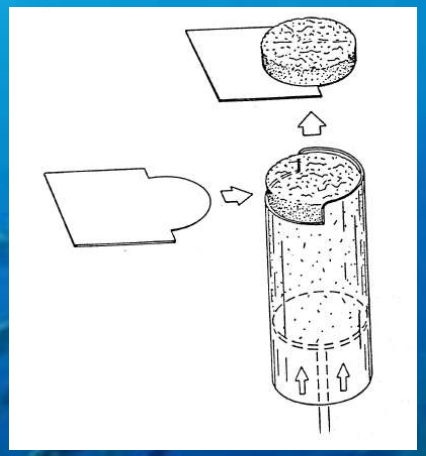
Corers

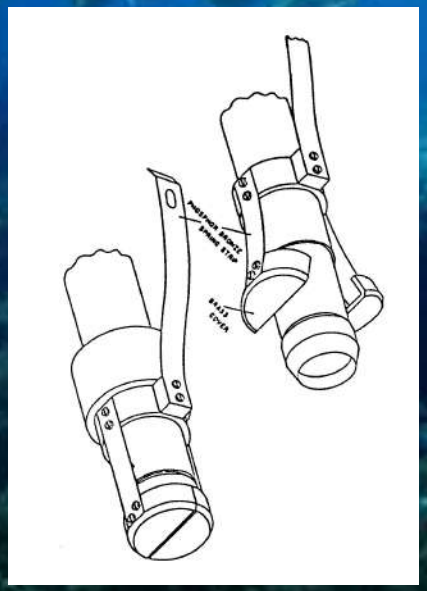
On soft bottoms, corers are the main tools for sampling sediments and the associatd macro- and meiofauna.

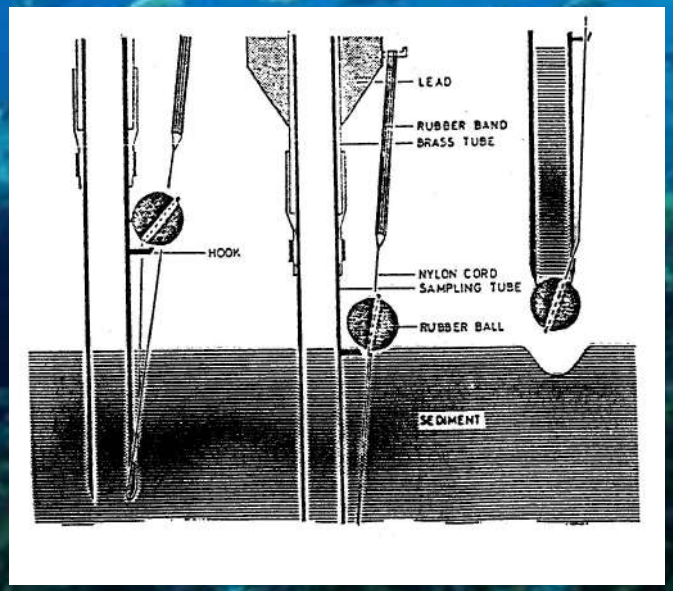
Microorganisms, protozoans, meiofauna and microphytobenthos can be sampled with the help of sterile syringes that are pushed into the sediments, extracted and closed ermetically.

Larger corers can be used when the aim is to sample macrofauna or to sample deeper sediments.



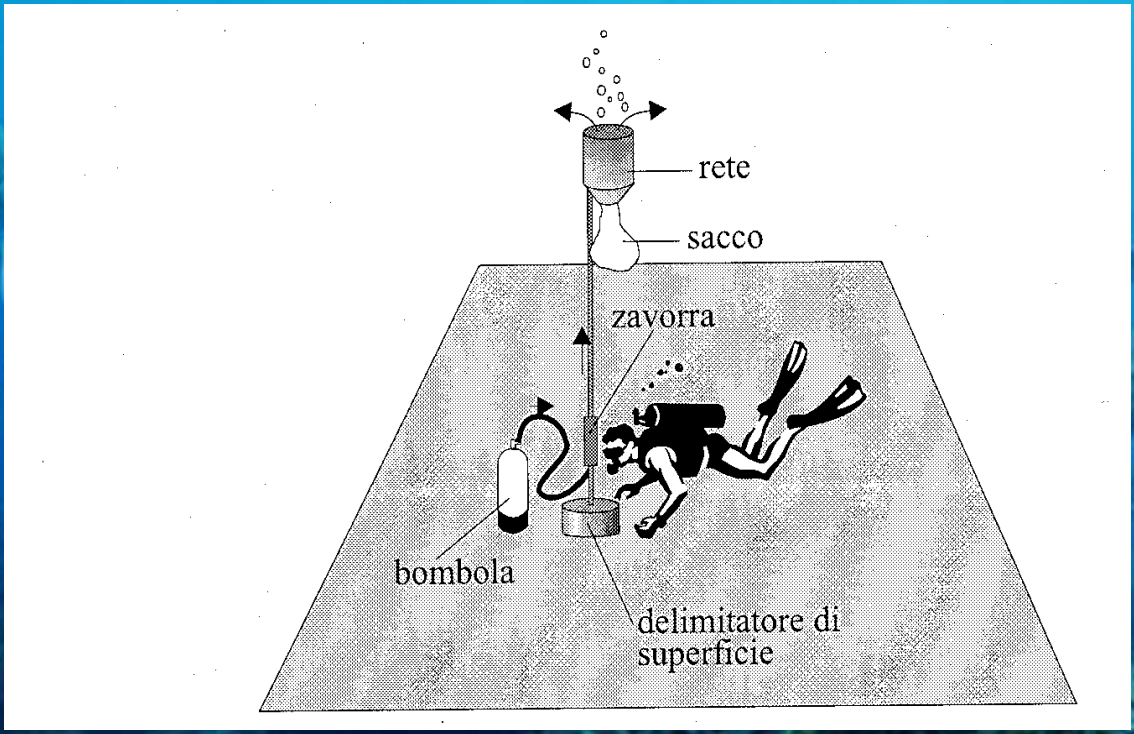


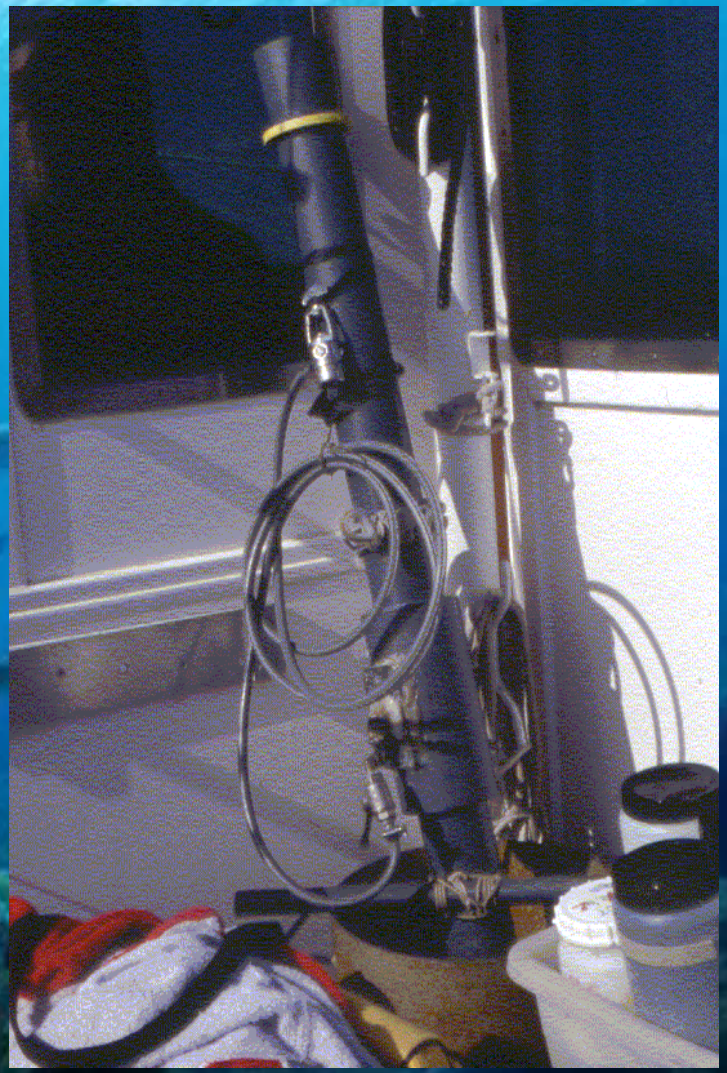




Air lift

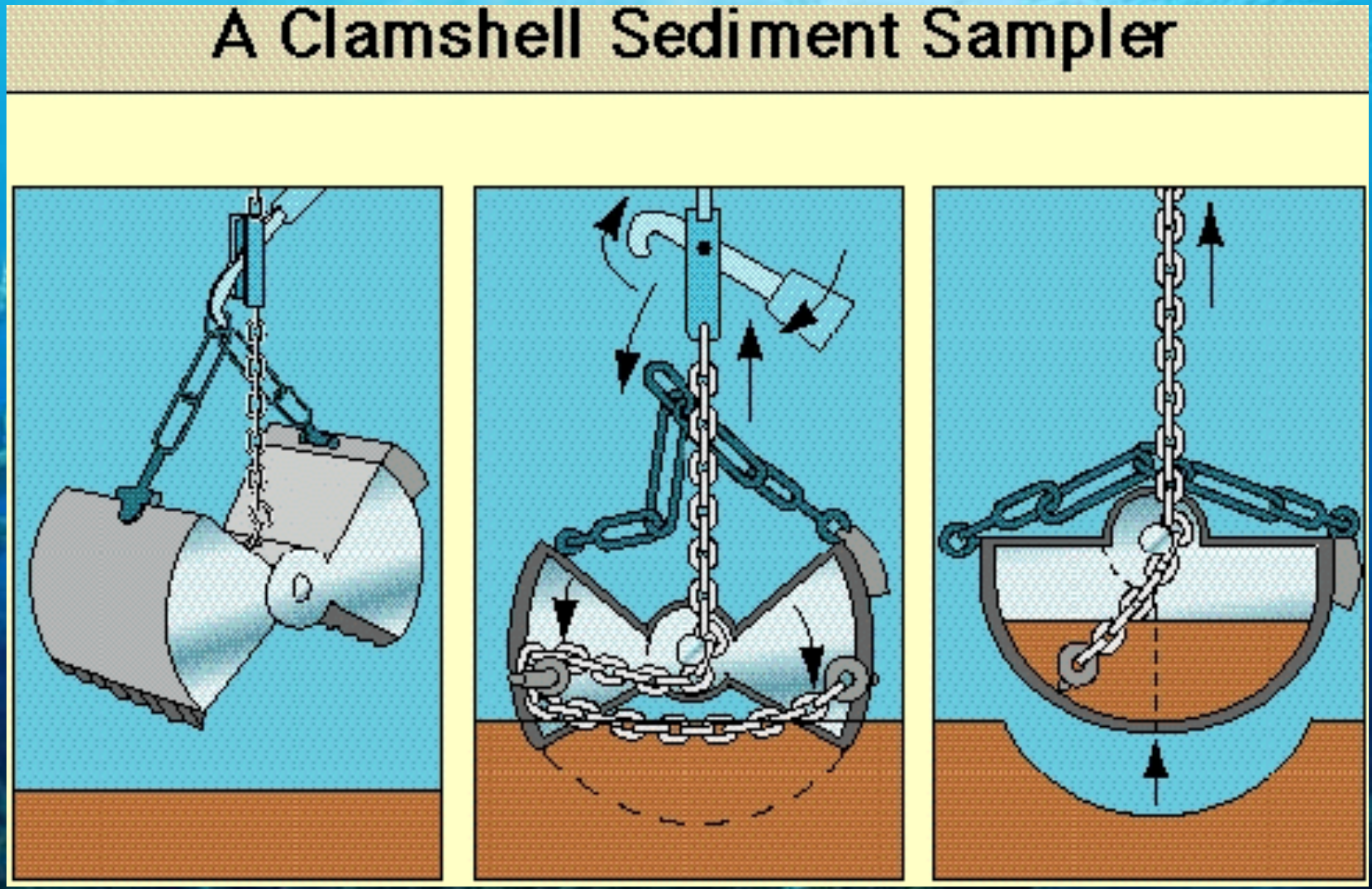
Macrofauna on soft bottoms can be also sampled by using the air lift on a standard surface or volume of sediments





Limits:
- Operational depth
- Time and effort

Grabs



Grabs

Washing and mesh

Grabs

Limits :

- disturbance of sediments;

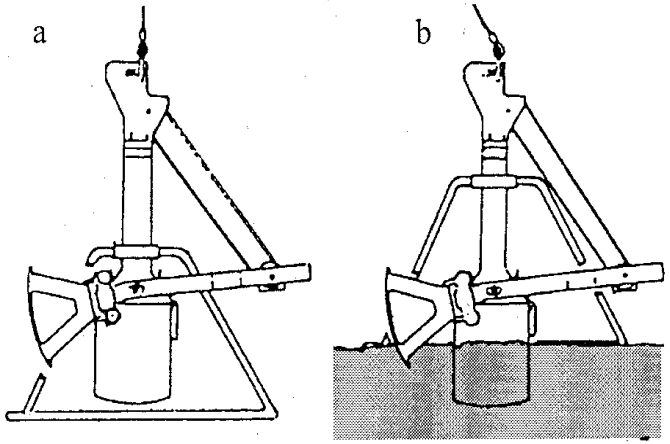
- washing during retriving could lead to loss of sampled material

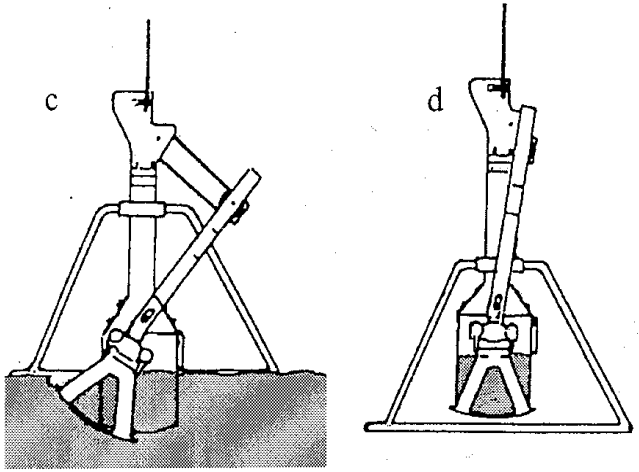
- limited sampling surface;

- difficulties when sampling at high depth.

Box-corer

Box-corer prevents most of problems that occur when using grabs



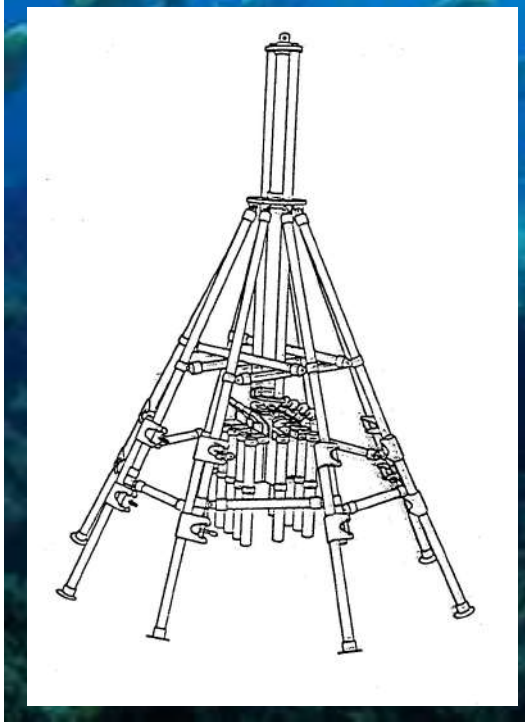


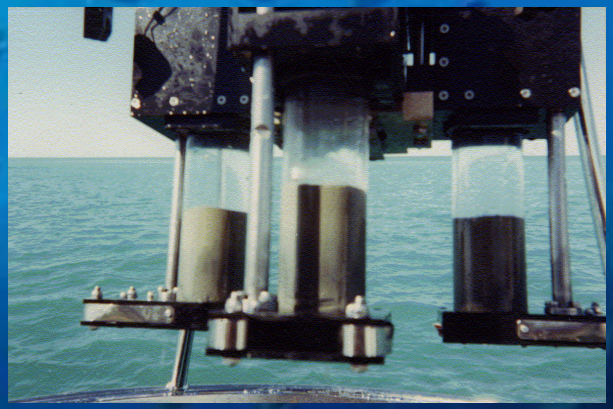
Box-corer

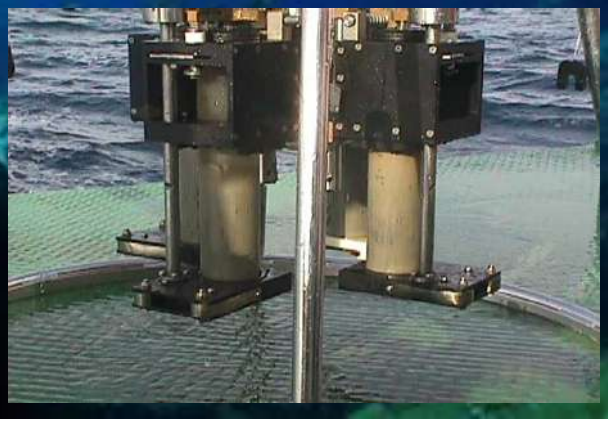
- Limited disturbance of sampled sediments;
- Limited loss of material;
- Allows analysing the vertical distribution of organisms;
- Could cover sampling surface up to a 0.5 m^2;
- Can be used for sampling sediments at high depth

Box-corer

Multiple-Corer represents the best tool for sampling sediments and the associated fauna on soft bottoms.







Dredging

The use of dredges can be used for sampling megafauna on soft bottoms, and allows collecting benthic organisms on the surface of sediments, and also borrowing organisms.

However, it is difficult to standardize sampling, for example to quantify the surface or volume of samples.
Useful for qualitative sampling at high depth or over large areas.

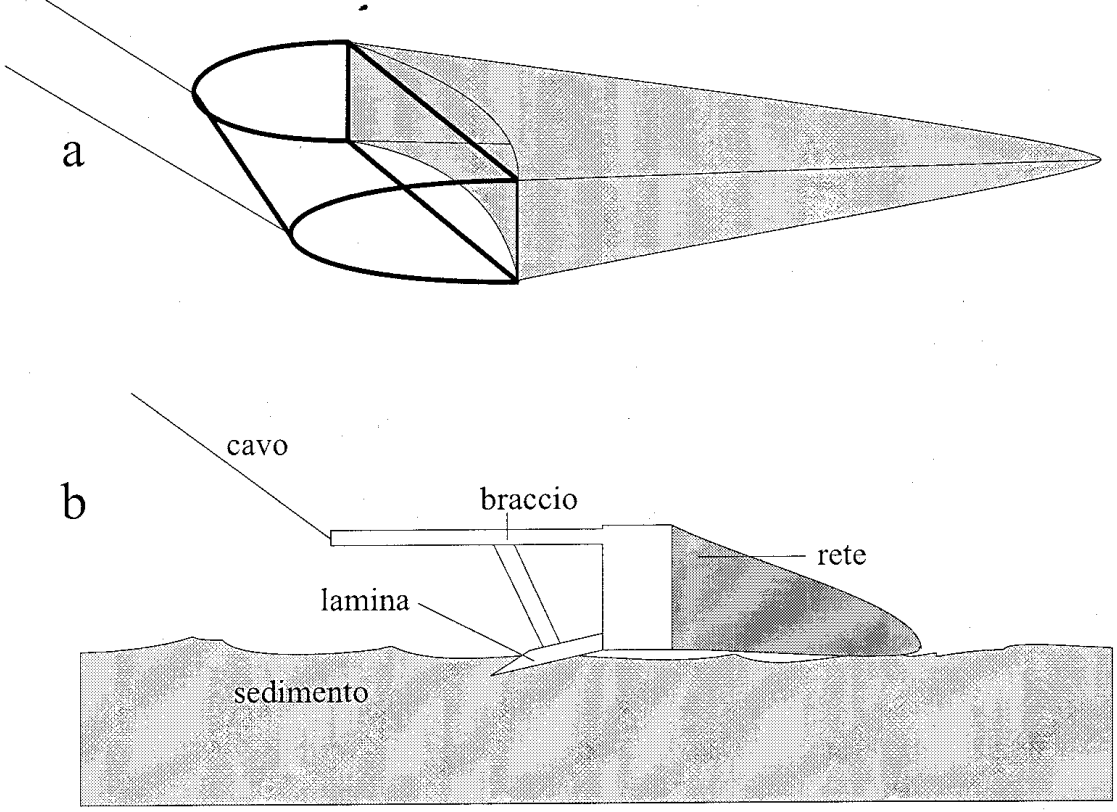


Fig. 10.22 Reti per la raccolta qualitativa di benthos: a) secondo Agassiz; b) secondo Forster.

Data matrices

Once the experiment has been designed and implemented, the data collected through sampling, the next step is to create a data matrix. In the matrix, the data are organized according to the experimental factors and according to the needs of the subsequent analyses. A data matrix is the basis of the statistical processing.

Generally, the matrices that are structured are species x samples matrices, in which for each observation unit (samples) the values found are reported (number of individuals, % coverage, frequency or other) for each species, taxon, etc.

The matrices are the starting point for extrapolating the response variable(s) chosen to carry out the statistical tests of interest, such as, for example, total population coverage, number of species, or diversity indices. In these cases, the approach is univariate, as it considers single response variables.

The multivariate approach, on the other hand, proceeds with analyses that consider several variables simultaneously. For example, analyses testing variations in stand structure as a whole, where all species are considered in the same analysis.

Data matrices

	AC	*Acetabularia acetabulum*	*Agelas oroides*	*Amphiroa* sp	*Anadyomene stellata*	*Aplysina aerophoba*	Attinie	*Ascidia mentula*	*Ascidia* sp. 1	Barnacles	*Balanophyllia europea*	Bacteria	*Botryllus* sp.
CE1A	7	0	0	0	0	0	0	0	0	0	0	0	0
CE1B	21	0	0	0	0	0	0	0	0	0	0	0	0
CE1C	21	0	0	0	0	0	0	0	0	1	0	0	0
CE1D	13	0	0	0	0	0	0	0	0	0	0	0	0
CE1E	28	0	0	0	0	0	0	0	0	0	0	0	0
CE1F	18	0	0	6	0	0	0	0	0	0	0	0	0
CE1G	32	0	0	3	0	0	0	0	0	0	0	0	0
CE1H	16	0	0	2	0	0	0	0	0	0	0	2	0
CE1I	18	0	0	21	0	0	0	0	0	0	0	0	0
CE1L	39	0	0	4	0	0	1	0	0	0	0	0	0
CE2A	0	0	0	9	0	0	2	0	0	0	0	0	0
CE2B	24	0	0	2	2	0	0	0	0	0	0	0	0
CE2C	6	0	0	18	1	0	0	0	0	0	0	0	0
CE2D	29	0	0	0	0	0	0	0	0	0	0	0	0
CE2E	13	0	0	1	0	0	1	0	0	0	0	0	0
CE2F	13	0	0	0	0	0	1	0	0	0	0	0	0
CE2G	12	0	0	0	0	0	0	0	0	0	0	0	0
CE2H	11	0	0	0	0	0	1	0	0	0	0	0	0
CE2I	14	0	0	0	0	0	0	0	0	0	0	0	0
CE2L	24	0	0	0	1	0	1	0	0	0	0	0	0
CE3A	16	0	0	0	0	0	2	0	0	0	0	0	0
CE3B	0	0	0	0	0	0	1	0	0	0	0	0	0
CE3C	13	0	0	0	1	0	2	0	0	0	0	0	0
CE3D	0	0	0	0	0	0	5	0	0	0	0	0	0
CE3E	14	0	0	0	0	0	0	0	0	0	0	0	0
CE3F	17	0	0	0	1	0	0	0	0	0	0	0	0
CE3G	0	0	0	0	0	0	0	0	0	0	0	0	0
CE3H	38	0	0	0	0	0	0	0	0	0	0	0	0
CE3I	5	0	0	0	0	0	1	0	0	0	0	0	0
CE3L	0	0	0	0	0	0	3	0	0	0	0	0	0
CE4A	6	0	0	0	0	0	0	0	0	0	0	0	0
CE4B	0	0	0	0	0	0	0	0	0	0	0	0	0
CE4C	0	0	0	1	1	0	2	0	0	0	0	0	0
CE4D	43	0	0	0	0	0	1	0	0	0	0	0	0
CE4E	27	0	0	0	3	0	0	0	0	0	0	0	0
CE4F	9	0	0	0	0	0	1	0	0	0	0	0	0
CE4G	47	0	0	0	0	0	0	0	0	0	0	0	0
CE4H	22	0	0	5	0	0	1	0	0	0	0	0	0
CE4I	14	0	0	0	0	0	0	0	0	0	0	0	0
CE4L	0	0	0	0	0	0	0	0	0	0	0	0	0
CO1A	0	0	0	0	0	0	0	0	0	1	0	0	0
CO1B	0	0	0	0	0	0	0	0	0	1	0	0	0
CO1C	0	0	0	0	0	0	0	0	0	0	0	0	0
CO1D	0	0	0	0	0	0	1	0	0	0	0	0	0
CO1E	0	0	0	0	4	0	0	0	0	0	0	0	0
CO1F	0	0	0	0	0	0	2	0	0	0	0	0	0