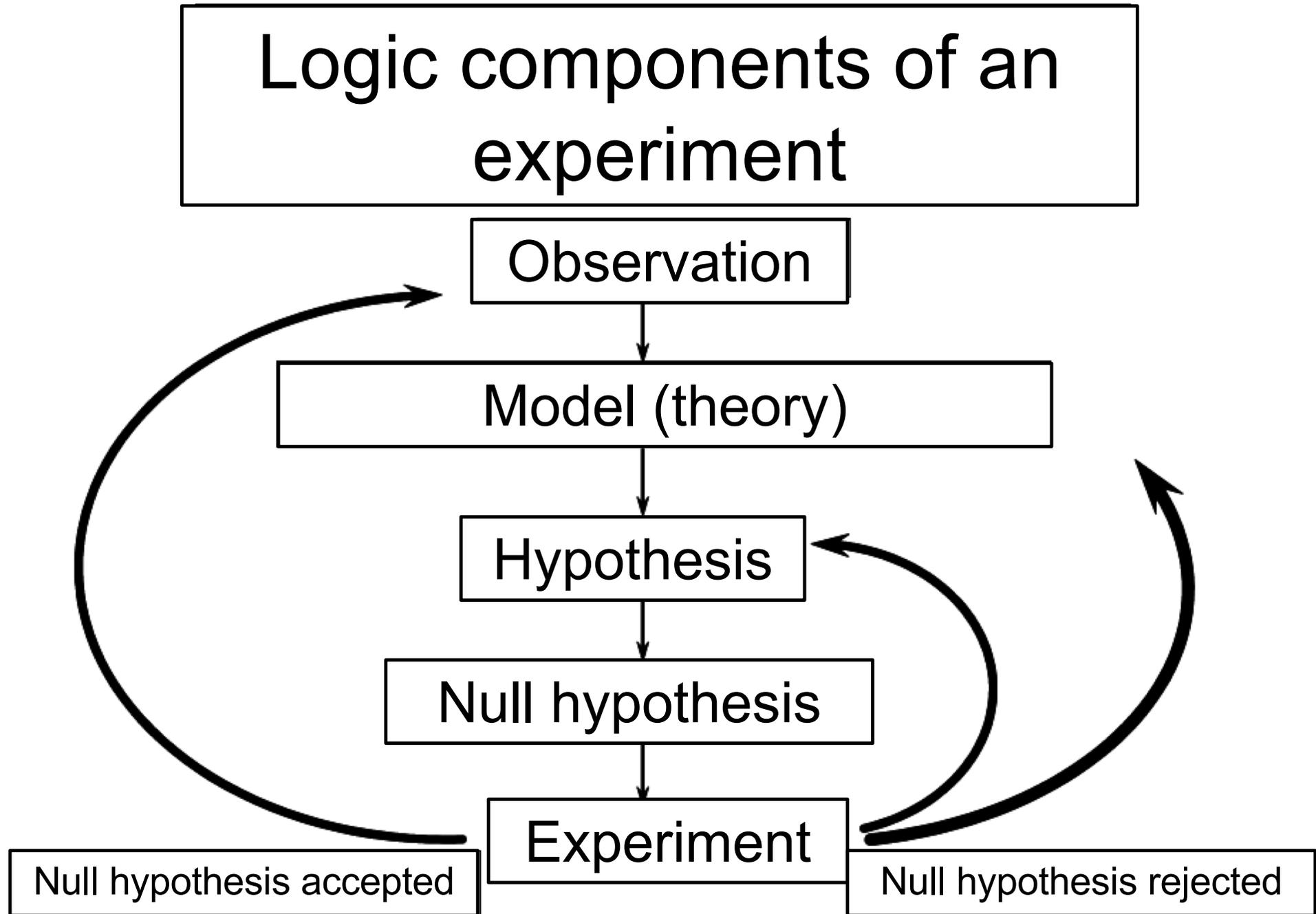
An underwater photograph showing a large school of small, silvery fish swimming in clear blue water above a rocky reef covered in green algae. Sunlight rays are visible filtering down from the surface.

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

**Conservation and Management of Marine Ecosystems**  
**Prof. Stanislao Bevilacqua ([sbevilacqua@units.it](mailto:sbevilacqua@units.it))**

**Experimental design and sampling**

# Experiments



# An example

## OBSERVATION

Protected assemblages 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

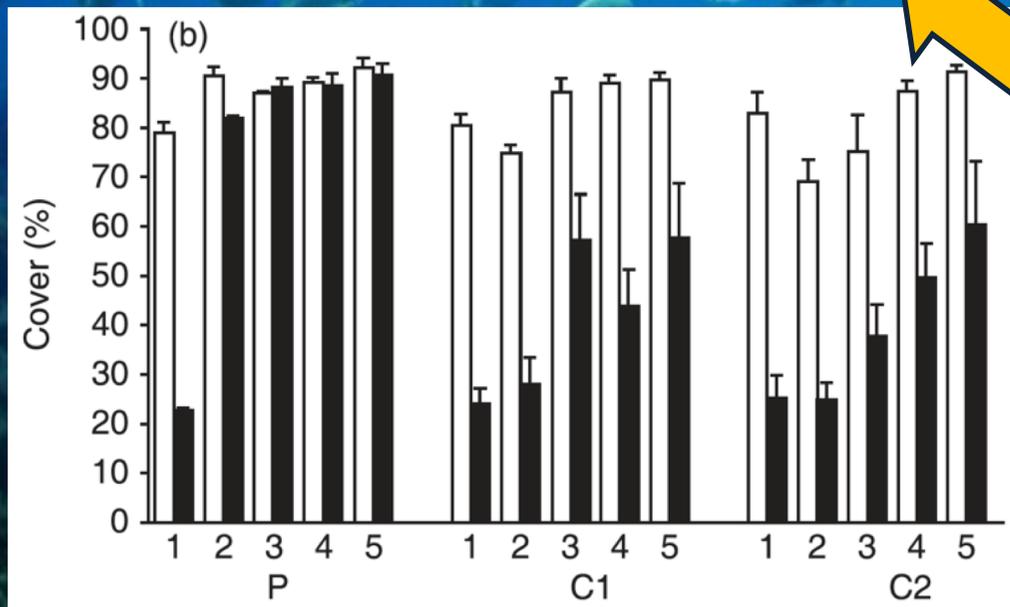


## EXPERIMENT

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

## HYPOTHESIS

After disturbance, protected assemblages recover faster than unprotected ones



## NULL HYPOTHESIS

After disturbance, unprotected assemblages recover as rapidly as protected ones



# 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

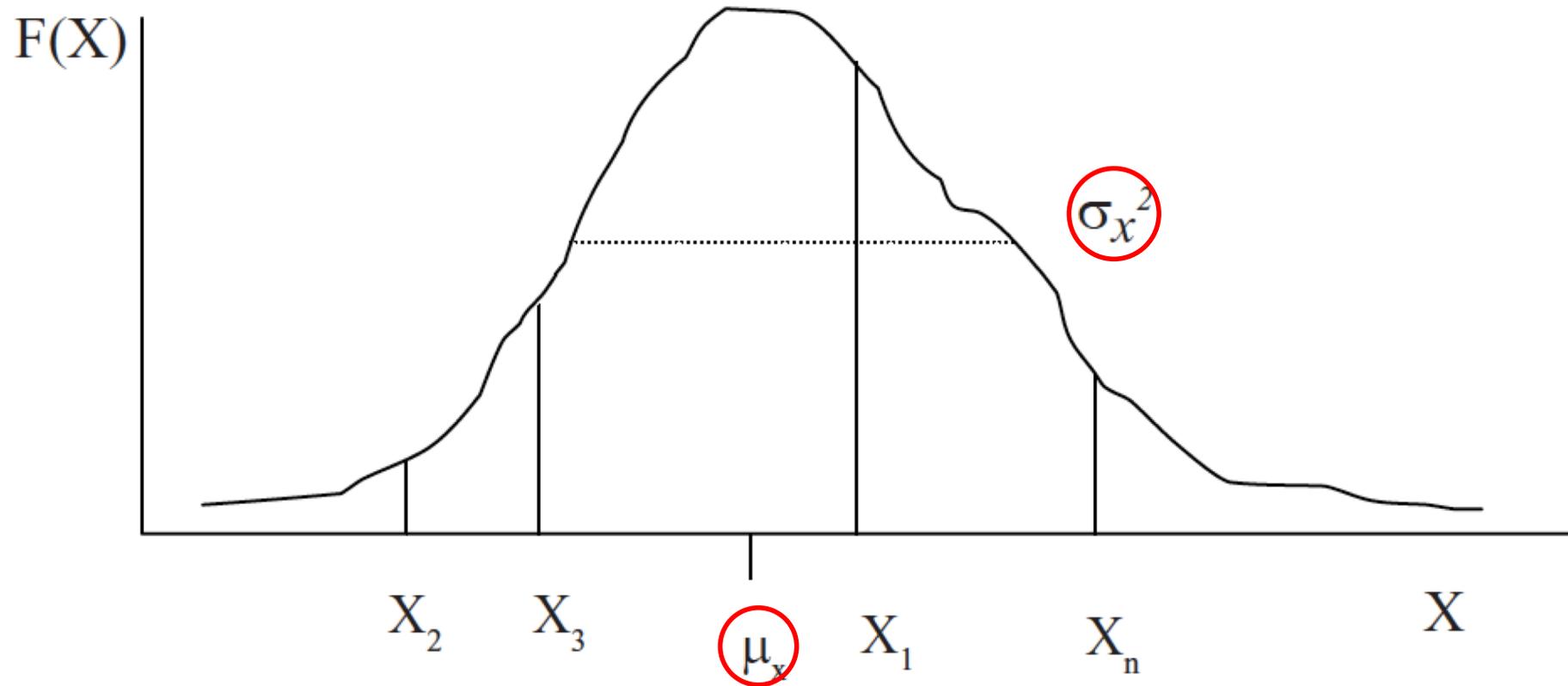
## Hypothesis

Species richness increases with depth

## Hypothesis

Larval survival decreases at increasing temperature

# Variables and sampling

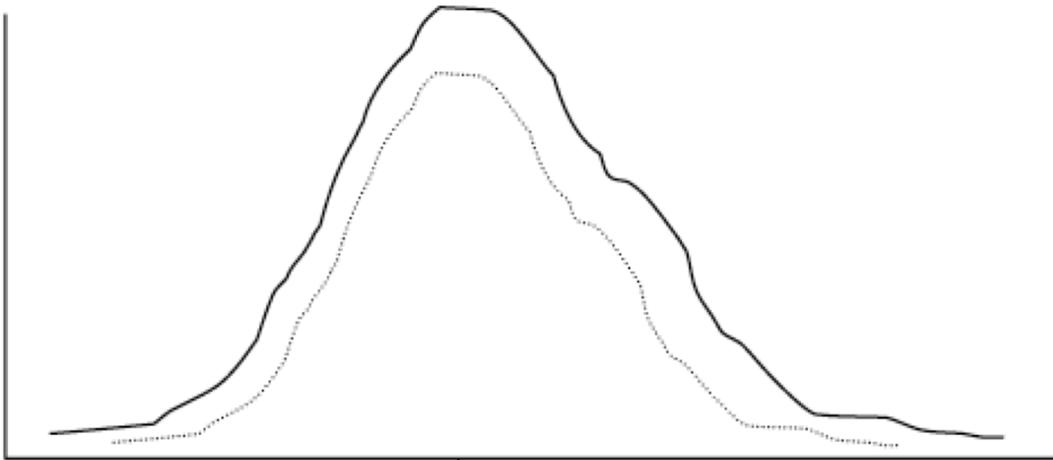


Parameters are mathematical constants that identify the frequency distribution of a variable (i.e. an attribute or quantity that can have different values). Mean and variance are two parameters. The first defines the position of the distribution, the second the dispersion of the values around the mean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

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

# Variables and sampling

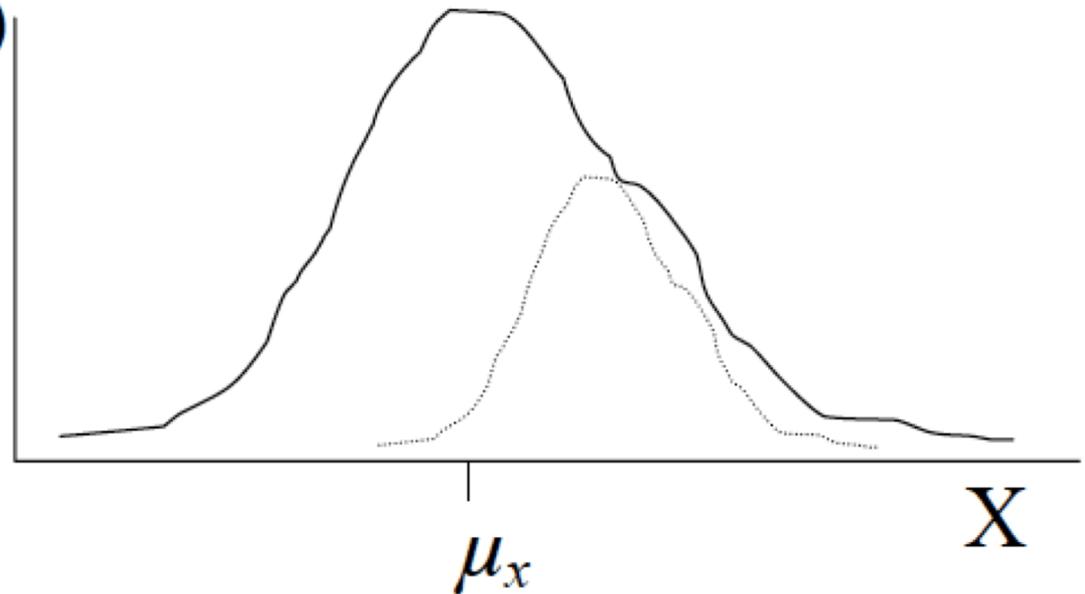
$F(X)$



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.

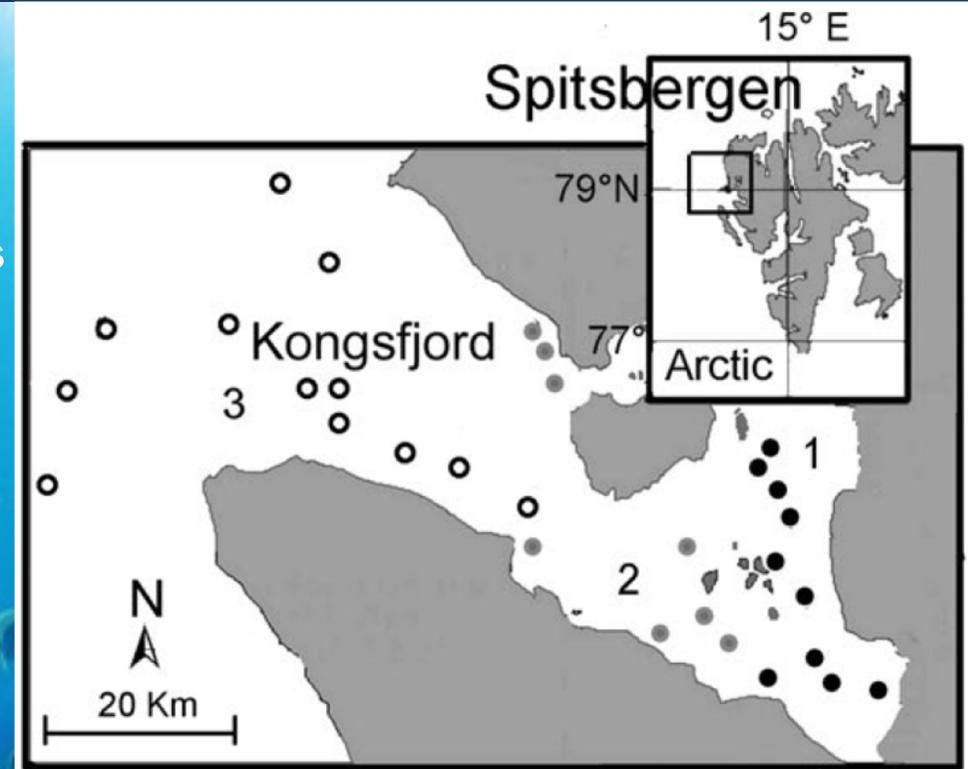
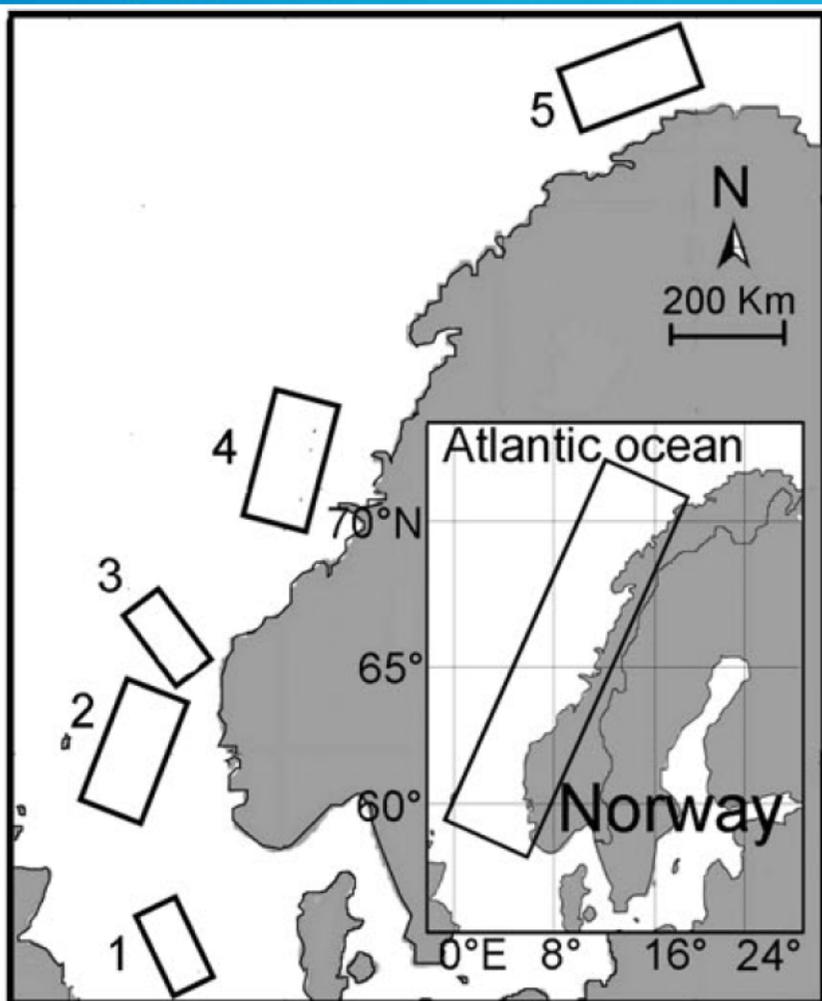
$F(X)$



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

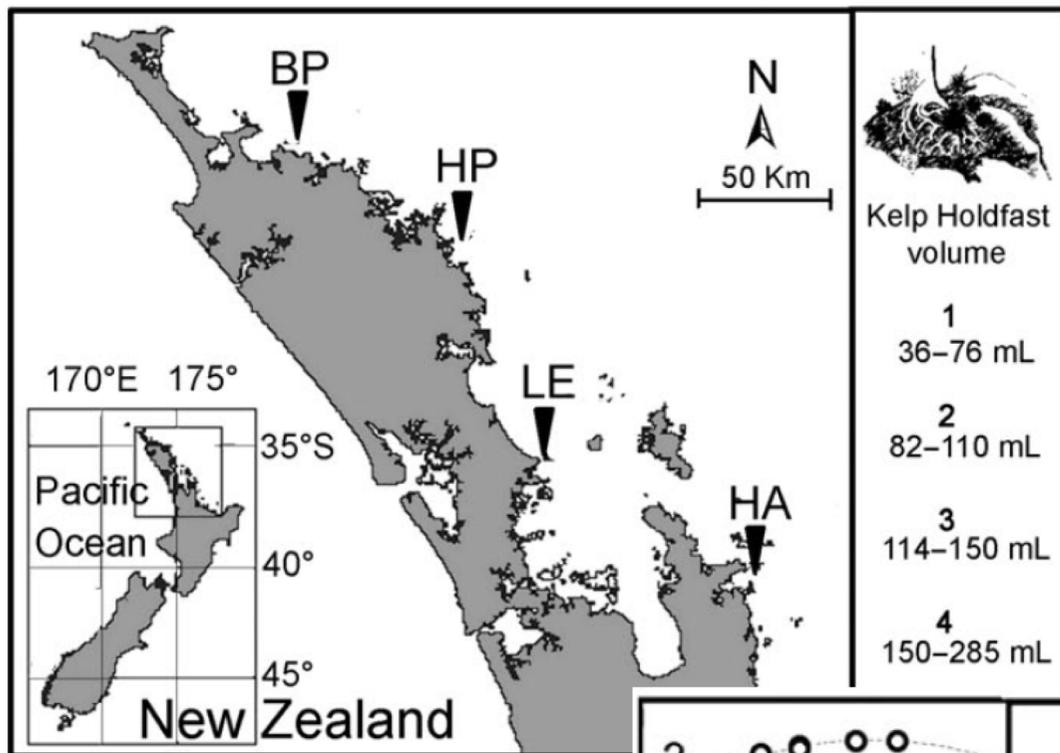
# Fixed and random factors

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

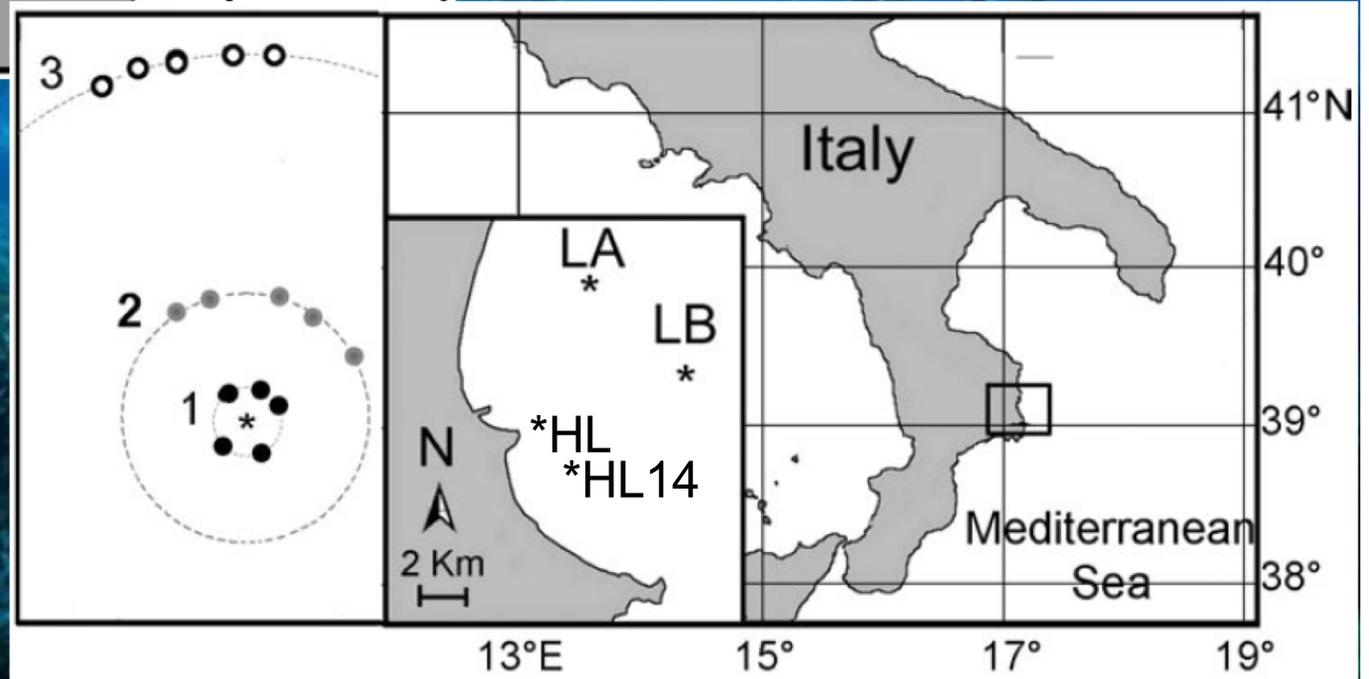


2 factors

- Location
- Holdfast volume

4 factors

- Depth
- Platform
- Site
- Distance

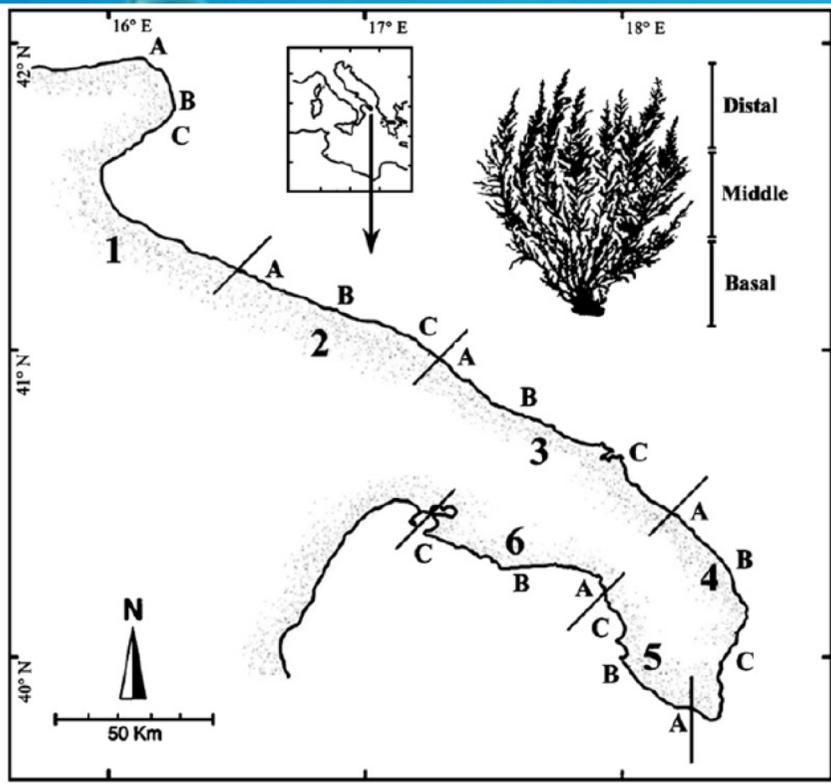


# Experimental design

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
Height	BMD	BMD	...	BMD
<i>n</i> = 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

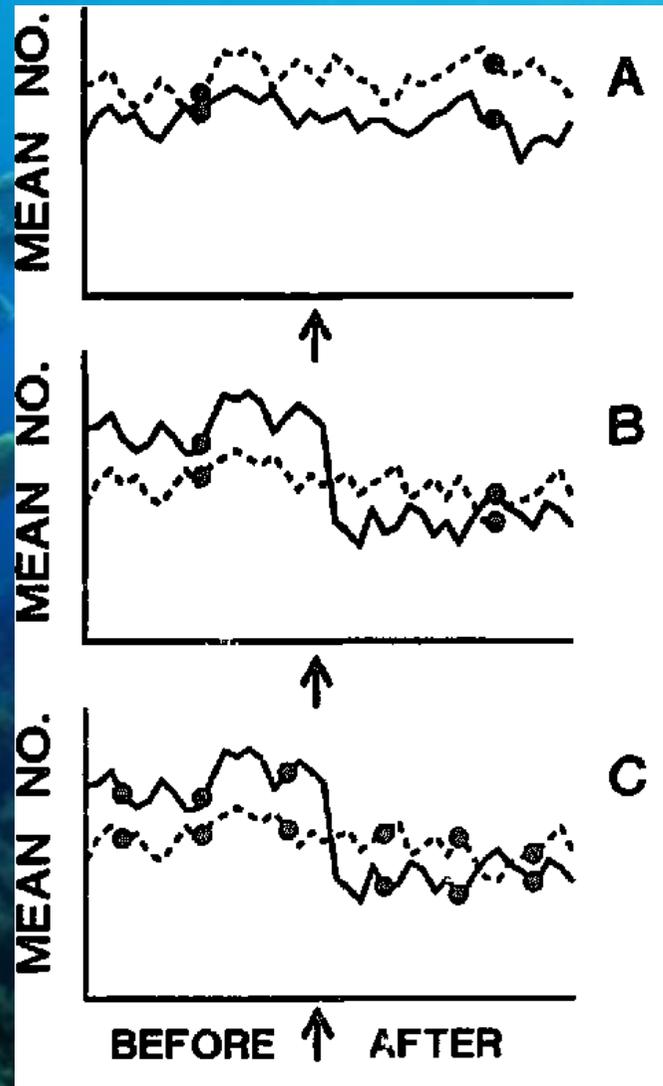
Differences among season in the response variable are determined based on a single date of sampling. This prevent identifying differences among season, since a single date could not be representative of the season. The effect of season is confounded by the effect of varition among dates.

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 arising 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