

Sampling protocol for assessment of marine diversity on rocky shores

Prepared by the Marine Biodiversity Observation Network Pole to Pole of the Americas
MBON Pole to Pole

(Modified from the SARCE South American Research Group on Coastal Ecosystems for
sampling on rocky shores protocol)



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INTRODUCTION

Rocky shores are among the most studied marine habitats, and their communities are often used as models to describe biodiversity dynamics worldwide. There is a need to understand and monitor environmental changes at global scales and the impact of climate change and other global stressors to marine biodiversity, this protocol provides a standardized methodology and sampling design for marine diversity at rocky shores in the intertidal zone. This protocol was produced by a group of experts (SARCE, 2012) and is updated by the Marine Biodiversity Observation Network Pole to Pole of the Americas (MBON Pole to Pole) in 2019 in order to create a standard method for intertidal biodiversity assessment valid across the American continent.

Ecological variables measured by this protocol:

- Abundance
- Cover
- Presence/Absence

MATERIALS

- One 30 m metric transect tape.
- One Hand-held GPS unit.
- Two 0.5m x 0.5 m (0.25 m²) PVC quadrat, one gridded – see construction instructions below and one not gridded.
- One Waterproof camera.
- One Clinometer or Telephone with clinometer app.
- 10 m stainless steel chain (1 cm link) or any other length can be used, as long as 10 m are measured.
- Field spreadsheet.
- Paper/pencil.
- Manual Cell Counter (Optional – for easy count of small abundant organisms).

DESIGN AND NUMBER OF QUADRATS PER SITE AND STRATA

Sampling is organized following a stratified design, which includes LOCATIONS, SITES, and STRATA (Fig. 1).

Note that **LOCATIONS** are **separated by 30 – 100's of kilometers** (Fig. 1)

For each **LOCATION** it might be possible that you end up sampling different **SITES** (maximum three) depending on how much you decide to do. Those **SITES** will be separated by units of kilometers (maximum 5 km, Fig. 1, Fig. 2). Please note below different combinations of protocols are given depending on your time availability. Each site will be divided in **THREE STRATA (tidal levels)** parallel to the coastline using the almost universal biologically-based characteristics of rocky shores of **high-, mid- and low zones**. In each stratum **10 QUADRATS** will be sampled as specified in the protocol below (Fig. 5).

Number of QUADRATS per SITE and STRATUM: See Table 1 for details of each site.

In the **10 QUADRATS within each stratum (tidal level)**, count **slow moving animals and the percentage cover of space occupied by invertebrates and algae**. The latter includes those organisms that cover a considerable fraction of the rock surface and that are too many to be counted or cannot be easily separated into individuals; e.g., barnacles, mussels, some colonial invertebrates, including zoanthids and ascidians, algal turfs or macroalgae. Organisms adhering directly to the rock surface and moving across it are considered the **“primary layer”**. If canopy-forming organisms such as macroalgae are present, then these should be moved aside for counts, cover and photographs in order to effectively quantify the primary layer. The composition of the canopy- **the secondary layer-** should also be conducted.

Use additional (n = ~ 10) non-gridded quadrats to register the presence of species (Species List). Do not make counts, just register the species. Try to search in crevices, cracks, etc.

TIMING

The exact date will depend on the tides, but in a first step, all samplings should be done between October-March in southern hemisphere and April-September in northern hemisphere. Some flexibility is allowed depending on particular circumstances and logistics of a given site/location. Sampling should, whenever practicable, be conducted during spring tide series when the maximum extent of the intertidal zone is accessible.

PROTOCOL OF MONITORING PROGRAMME



Figure 1. Scheme of locations, sites and strata.

- 1) Get to a specific site within a location (Fig. 2). The yellow line in this case, defines a LOCATION in the eastern side of Venezuela. The diameter of that location is about 15 km. Within that location a specific site was selected. **That specific site must be a stretch of rocky shore that goes along at least 50 meters (ideally 100 meters).**
 - Remember that we want to cover as much as we can; please select your SITES trying to get the best geographic spread.
 - If possible, avoid estuarine sites.

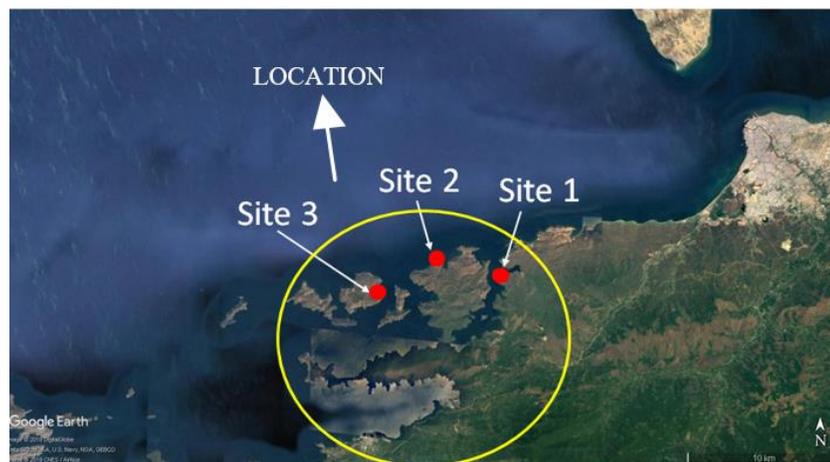


Figure 2. Example of Location and Sites

- 2) Once at the site, take note of the exact geographical coordinates and fill in the **Data Sheet 1**. This information is about the general characteristics of your sampling site and will be important later on to test various hypotheses (Table I).

Table I. - General characteristics of your sampling site.

Descriptor	Definition	Outcome
Location	Name of a given location	It will be unique for this data sheet
Site	Name of a given site	
Urban Area	Located within a radius of 10 kilometers of a human settlement of more than 5000 habitants	Yes/No
MPA	Marine Protected Area	Yes/No
Distance to Rivers	Can be done later using GIS	Distance value in Km
Slope	As measured with a "Clinometer" a scheme of this basic device will be sent shortly.	Degrees. It will be measured in the first 30 quadrats
Sand Burial	Likelihood of a given rocky shore to be affected by sand. To fill this cell you should answer the question: Is there a sand strip in between the rocky shore and the beginning of the land?	Yes/No
Substrate	Composition of substrate	Example: Biogenic (dead coral), sandstone, granitic, etc.
Rugosity	Take 10m stainless steel chain (1cm link) and lay it parallel to the sea line and measure the total length of the chain (contoured length) and the length of a straight line joining the two ends of the rope or transect tape (linear length). In case of short chain, consecutive replicates can be addressed up to 10m). Fig. 5	A number expressing the ratio between the contoured length and linear length

- 3) Divide your site in three STRATA (High, Mid and Low). This is just a guideline as your site might be difficult to divide in three strata. In case of doubt take decisions on a case by case basis taking in consideration that what matters the most is to capture as best as possible the number of species in a given site; which means the biodiversity spanning the width of entire intertidal rocky shore. It is very important to take notes of criteria you used to define the three strata.
- 4) In each **stratum**, estimate **percentage cover of sessile organisms** (e.g. algae, small barnacles and mussels), **density of mobile organisms** (e.g. echinoderms, gastropods) **and** big barnacles on **quadrats** (50 * 50 cm) haphazardly allocated.
- Set a 50 * 50 cm plain frame and take a picture of the quadrat (Photo-quadrat provided by MBON Pole to Pole program).

- The first step of sampling is to make a list of ALL organisms found within the quadrat.
- Then, replace the plain frame by a 50 * 50 cm gridded frame (see Fig. 3) providing 100 intersection points. **Count slow moving animals that you can see to estimate their density** (e.g. echinoderms, gastropods and hermit crabs). Try to identify the organisms to species level. Count organisms if 50% or more of its body lies inside the quadrat.

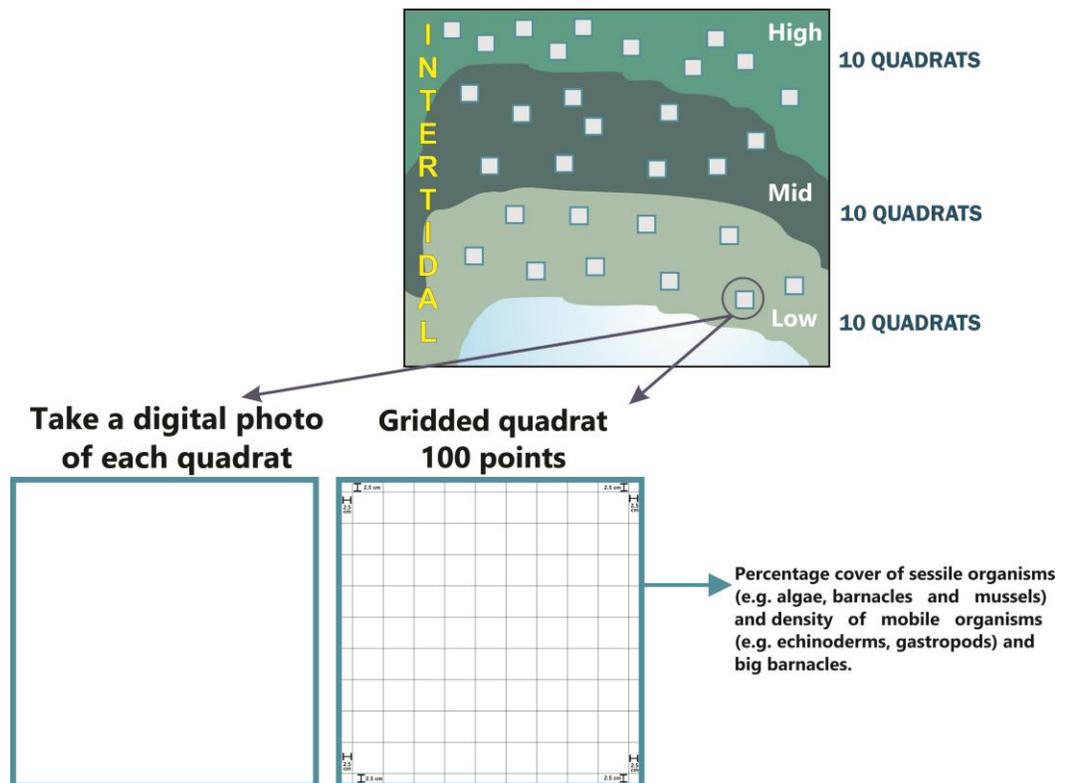
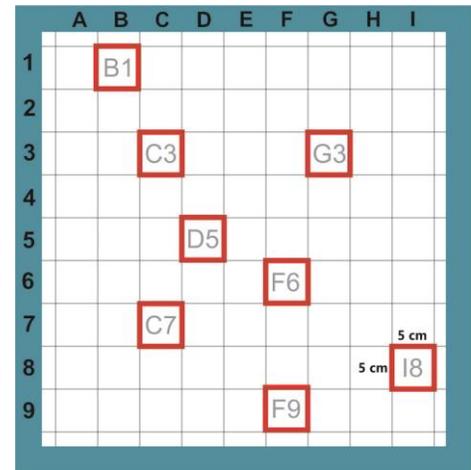


Figure 3.- Protocol for each site

- If there are hundreds/thousands of individuals of a particular species, you may subsample the quadrat by counting individuals in eight randomly pre-selected 5 x 5 cm subquadrats. The randomization needs to be done only once and previous to the sampling campaign; these subquadrats should be marked permanently in the large quadrat (50x50cm) and used for all future counting (see example).



- Estimate the **percent of sessile organisms** (e.g., algae, barnacles and mussels) using the point intercept method (100 points; Fig. 4). Organisms adhering directly to the rock surface and moving across it are considered the “**primary layer**” If canopy-forming organisms such as macroalgae are present, then these should be moved aside for counts, cover and photographs in order to effectively quantify the primary layer. The composition of the canopy- **the secondary layer**- should also be conducted. Try to identify the organisms to species.

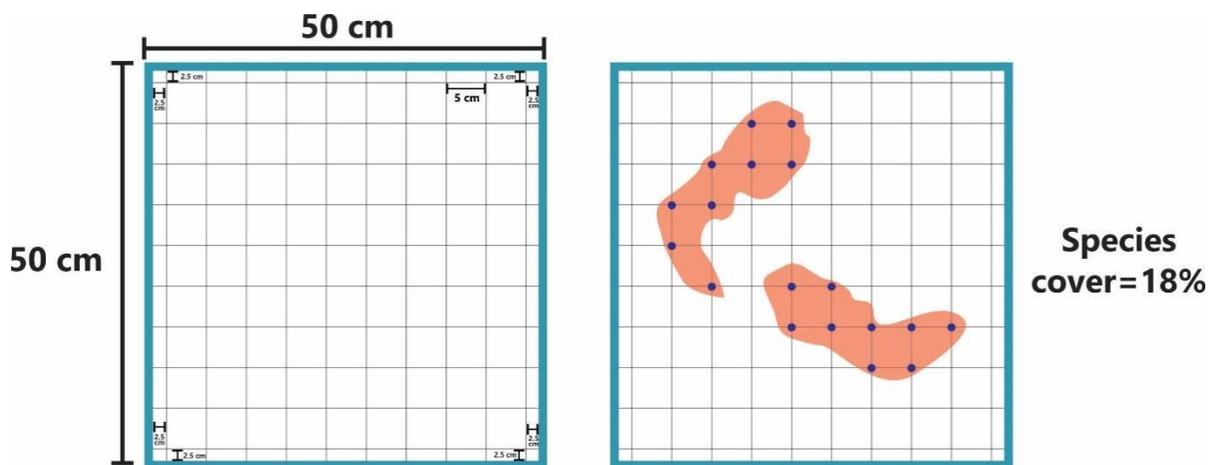


Figure 4. Schematic representation of how to calculate species cover using the point intercept method. There are 100 interceptions and counting the number of interceptions over a given species give an estimation of its cover. In this case the percentage cover is 18%.

- If your site has boulders (preferable look for unfragmented rock sites first), probably you will have situations as shown in the Figure 4. In this case, please include the category “without substrate” as a species and take percent cover as metric. This will help estimate true percent cover in your frame.



Figure 4.- In boulders, sometimes your gridded quadrat could have points without substrate. Please count this points as “without substrate”.

- If identification of species in the field is not possible, you should collect a sample for later identification. There are several taxonomists in the SARCE and MBON Pole to Pole that can help with species identifications. You can also take a look (and contribute, if you want) to our Rocky Shore Guide at Inaturalist:

<https://www.inaturalist.org/guides/9408>

- 5) Move to other SITES within the LOCATION (if you have more than one). These site(s) should be separated by at least 1 km but no more than 5 km (Fig. 4). If there are not two sites in that location, choose at least one extra.
- 6) Repeat steps 2 to 5.

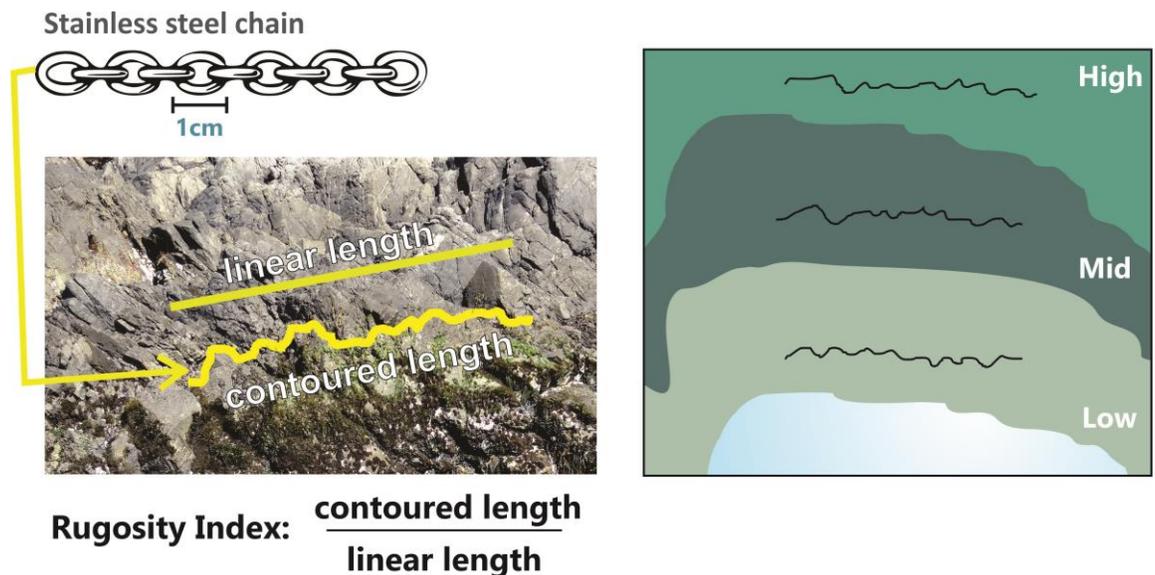


Figure 5.- Rugosity index method. Measurements must be carried out with a 10m steel less chain laying parallel to the sea (Once per strata)

ONCE THE SAMPLING IS DONE

- 7) Upload the data in Excel files using the template available on the MBON Pole to Pole site (https://marinebon.github.io/p2p/methods_data_science.html).
- 8) Filling the spreadsheet:
This is a crucial step in data storage, and all attention must be paid in order to insure data quality and standardization. **Include ALL sites within a LOCATION in one single file.** When transcribing field data to the Excel spreadsheet, the species names should be entered at the proper row (Fig. 6), and only species found at that determinate sampling

event (one site at a given species date) should be added. This implies that only the species found for all three tide levels at the present survey should be included at this spreadsheet. The spreadsheet must be filled with the relative cover associated to each one of the sampled quadrats represented at one of the rows. For each species the number associated to the relative cover of a given species must be entered at the correspondent cell. If one species was present within the quadrat but was not observed at the intersections, i.e. had a relative cover equals to zero, a zero should be added to indicate the presence of the species in that quadrat. Otherwise, all the other cells associated with species not found at that quadrat must be left in blank. The only other case when a zero value could be inserted as 'true' value is for specific target groups or species determined a priori based on specific objectives and included in the species list.

Bare rock at the intersection

In boulders, points without substrate

Species found at this specific sampling event.

Indicate if species found at primary or secondary layer

Species found within quadrat #6 but not under intersection

Survey date (yyyy-mm-dd)	Country	Locality	Site	Stata	Picture number	Replicate / Species	Bare rock	Without substrate	Kermiacea	Chlorophyta	Componente silicea	Dicryogasteria cavernosa	Bryopsis	Ectodictyonella similis	Viverraria mesoatlantica	Galaxaura nigra	Jania	Lithothamnium	Hydractinia solitaria	Pulina gymnospora
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_high_Q01	1	52													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_high_Q02	2	80													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_high_Q03	3	89													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_high_Q04	4	75													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_high_Q05	5	74													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_high_Q06	6	89													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_high_Q07	7	44	1					43							
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_high_Q08	8	76													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_high_Q09	9	68						2							
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_high_Q10	10	93							5						
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_mid_Q01	1	48													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_mid_Q02	2	56													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_mid_Q03	3	45													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_mid_Q04	4	87													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_mid_Q05	5	72													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_mid_Q06	6	20													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_mid_Q07	7	11													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_mid_Q08	8	19													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_mid_Q09	9	11													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_mid_Q10	10	72													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_low_Q01	1	11													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_low_Q02	2	97													
2019-02-21	Brazil	Estado de Noroeste	Francisco de Assis	Noroeste	BR_low_Q03	3	95													

9) Add additional information supporting your observations with regard to species characteristics found in your sampling sites. You should indicate: a) feeding group (separated in seven groups: primary producers, herbivores, filter feeders, carnivores, detritivores, omnivores, scavengers) a particular species belongs to, 2) whether a species is a habitat forming organism, 3) whether a particular species is invasive or not, 4) whether a species is introduced or not (non-native) and 5) whether a species is active at day, night or both

10) The MBON Pole to Pole program will provide server Github repository to upload data files. Detailed instruction on data handling will be provided.

11) NOTE: This collaboration is based on the premise that anyone contributing with data will be offered co- authorship on scholarly products produced under the SARCE and MBON Pole to Pole (peer-reviewed scientific articles, conference papers, presentations, etc.). People retain right to publish data from their own site.