



## **Marine Biodiversity Observation Network Pole to Pole of the Americas (MBON Pole to Pole)**

### **Sampling protocol for assessment of marine diversity on sandy beaches**

---

#### ***Statement***

Present from Patagonia to the Arctic and more frequented by people than any other type of shoreline, sandy beaches have been commonly neglected in long term ecological and conservation programs, as well as in politicians' and stakeholders' agendas. This is a contrasting situation when considering the intrinsic relationship with human societies and the unique role of sandy shores in providing ecosystem services. A lack of standardized data of this very dynamic environment, with huge variation in geological history, tide range, sediment texture, slope, and exposition to waves, make investigations of large temporal and spatial patterns a big challenge, compromising the forecasting of future ecological scenarios and management activities. For the first time on a Pole to Pole scale, this protocol aims to address simple tools and best practices towards an implementation of standardized tools of investigation at large temporal and spatial scales able to detect relationships between ecological patterns and global climatic changes. We hope to provide common and useful indicators of sandy beach ecosystem functions and environmental changes that can be used by the scientific community and by stakeholders for a sustainable management of coastal zones, such as macrofauna diversity, ghost crab density, macroplastic occurrence and biomass of detritus at the drift line.

---

---

## 1. Aims and expected outcomes

This protocol aims to:

- i. Propose best practices commonly used in sandy beach ecology that can be easily implemented at multiple sites in order to increase our knowledge on marine biodiversity along a large spatial and temporal gradient,
- ii. Propose biodiversity indicators estimated with standardized practices that could be implemented at these scales, and finally,
- iii. Provide essential information to emphasize the necessity to increase management actions in these coastal habitats able to maintain indispensable ecological services of sandy beaches under the pressures of climate change.

With the long-term implementation of the protocol on this spatial scale (MBON Pole to Pole), we expect several further scientific questions to be addressed:

- i. What are biogeographic patterns detected and how do species and communities change from pole to pole?

Do these patterns change over time and what are the drivers of these changes?

What are the changes in functional biodiversity (i.e., detritivores, predators, scavengers, suspension-feeders) related to different beach zones?

- ii. Are the proposed biological indicators a good measure for biodiversity changes on a large spatial scale and how exactly do they reflect these changes?

Can we link these biological indicators, able to reflect shifts in biological trends, with environment indicators of climatic changes?

Do these biological indicators respond to extreme events such as tsunamis, earthquakes, droughts, hurricanes, flooding, extreme temperatures, coastal storms?

- iii. What are the effects of human actions such as beach nourishment on the ecosystem functioning?

What is the effect of climate change on the habitat availability of species from sandy beaches?

What is the effect of climate change on species traits composition of sandy beaches?

## 2. Definitions

### ***Indicator (adapted from SINAC, 2016)***

Characteristic or condition that is relevant, precise and sensitive to changes over time and that can be determined and characterized in an accurate and practical way with reasonable cost.

### ***Monitoring***

Measurement of an indicator applied over time to evaluate the changes in the environment.

### ***Permissible variation***

The minimum biodiversity measure in which each sandy beach can persist both in time and space.

### ***Biodiversity measurements***

Macrofauna composition (species abundance, richness, functional traits). Finding literature and connect species list with this information.

### ***Morphodynamics measurements***

Beach slope, granulometry, sediment temperature, beach orientation, the length of the distinct habitats, beach width and swash width, wave height, wave period

### ***Remote sensing data (Satellites/Database)***

Altimetry, sea level, primary production, wind, storms, cyclones, hurricanes, wave period, wave height, radiation, water temperature.

### ***Locality***

Name of a given location (City, Neighborhood)

### ***Site***

In sandy-beach ecology, a sampling site is a short (no more than a few meters wide), along-shore stretch of beach from which samples are drawn with the express aim of describing features of that stretch of beach only. For example the name of a beach.

### ***Marine Protected Area***

Filled with “yes” or “no”. Should be filled with “yes” for sites included in marine protected areas

### ***Latitude***

Site latitude using decimal degrees

### ***Longitude***

Site longitude using decimal degrees

### ***Date***

Date of sampling filled using “Year” - “Month” - “Day”. For example: 2019-15-03

### ***Hour starts***

Time of sampling start. For example: 09:00

***Hour end***

Time of sampling end. For example: 13:30

***Rainfall***

None/Light/Medium/Heavy

***Exposure***

Exposition condition: sheltered beach or exposed beach

***Embayment***

Inside a bay formation or an open beach

***Beach Type***

Dissipative, Intermediate or Reflective beach

***Grain size***

Particle size according to Wentworth scale  $= -\log_2 \text{diameter (mm)}$ . The mean particle diameter is defined by Folk (1974).

***Sediment source***

Sediment origin: Biogenic, Volcanic, Oceanic, Clastic

***Tidal Regime***

Macrotidal (beaches with dominate tidal ranges higher than 4m), Mesotidal (beaches with dominate tidal ranges between 2m-4m) or Microtidal regime (beaches with dominate tidal ranges less than 2m).

***Strata***

Zonations in the sampling site divided in: "Supralittoral", "Mesolittoral" or "Swash".

**Supralittoral:** strata between dune crest and the upper limit of tides or swash for microtidal sandy beaches. **Mesolittoral:** strata between the upper and the lower limit of tides (not applicable for microtidal sandy beaches). **Swash:** strata from the point of bore collapse to the upper limit of swash on beach face.

***Supralittoral length/dry zone length***

Distance (meters) between dune crest and the upper limit of tides or swash for microtidal sandy beaches.

***Mesolittoral length/wet zone length***

Distance (meters) between the upper and the lower limit of tides (not applicable for microtidal sandy beaches).

***Swash length/wet zone length***

Distance (meters) from the point of bore collapse to the upper limit of swash on beach face. Wet zone of microtidal sandy beaches.

***Beach slope***

Slope ( $\beta$ ) is defined as the ratio between rise distance and run distance (measured with the same units), the tangent of a given angle (in degrees). The suggestion is to apply the Emery method (Emery, 1961) using a minimum of five points between the dune crest and the lower limit of swash. Include in the field form the mean value of measures.

**Wave height**

Wave height at breaking ( $H_b$ ). The wave height is the vertical difference between a wave crest and a wave trough, in meters. Will be measured in situ or oceanographic buoys (wave sensor).

**Wave period**

The time interval between two consecutive wave crests. Time required for successive crests to pass a fixed point. The measurement of 11 successive crests in a fixed point defined *a priori*, measured in seconds.

**Swash period**

The average time between swashes (upwash-backwash time).

**Beach Width**

Distance (meters) between dune crest and the lower limit of swash on beach face.

**Zone**

Filled with: “Supralittoral” or “Intertidal (including swash zone)”.

**Corer depth**

Depth of the corer penetration into the sand (minimum of 15 cm).

**Organic matter content**

Percentage of organic matter (%) in the sediment.

---

---

### 3. Where and when

Since we are proposing this protocol on a continental scale with high variability the terminology, variations measured and units should be standardized and clearly defined. Where and when sampling should be conducted is described below.

**Where?**

A *Location* in your region is an area of maximum 100 km radius and consists of maximum three sites that should be five km apart. Within that location, at each particular site, an area for sampling should be selected.

**Preferable beach state/characteristics**

Sites must be an oceanic, exposed, dissipative beach, avoiding embayed/closed beaches (> 200 m of beach length/minimum long-shore distance). Sampling should be in the middle of the beaches, at least 200 m away from any freshwater output. Avoid developing this protocol in estuarine/ wave protected sites.

The protocol is intended to cover as much area as possible; please select your sites considering optimal geographic spread.

**Warning:** Dissipative beaches are not present everywhere. Therefore, please make sure that the beach you select is exposed and oceanic, and as dissipative as possible. In any case, before the

monitoring process starts, the researchers interested in applying the MBON Pole to Pole protocol can rely on the network for evaluating selected beaches.

#### *Field sampling design and the number of zones*

The protocol keeps some basic and minimum practices to have comparable sites from a pole-to-pole perspective. All strata and littoral zones are considered in the sampling strategy because of the dynamics and instability of the sand. Because wide variations between the sandy beaches in the American continent exist, a minimum protocol for narrow and wide beaches is proposed.

#### *Daily conditions*

Morning – low spring tide (below 0.2 meters).

#### *Periodicity*

The sampling should occur preferably seasonally (two to four sampling events per year).

**Warning:** Considering logistical and time capabilities, we must define the limit to biannually, the minimum sufficient periodicity to be comparable as part of the MBON Pole to Pole program. The network does not provide financial support for carrying out the field surveys.

Once at the site, take note of the exact geographical location and fill the general data in the field sheet (attached)

## **4. Sampling strategies**

### *4.1. Environmental measurements*

Upon arrival at each site, several environmental measurements should be described in the field data sheet. Information on the variables can be found in the definition list and in the field form. Note that the slope measurement requires a specific methodology, which can be found in the protocol attachments.

### *4.2. Indicators*

#### **4.2.1. Macrofauna**

##### *Macrofauna diversity*

Macrobenthic invertebrate fauna of sandy beaches primarily comprises crustaceans, mollusks and polychaete worms. Intertidal invertebrates play a crucial role in sandy ecosystems, can exhibit responses associated to different levels of disturbance and provides several advantages as indicators sandy beaches health, including relative facilities of sampling and accurate response to environmental conditions over multiple temporal scales.

### *Traits of the macrofauna community*

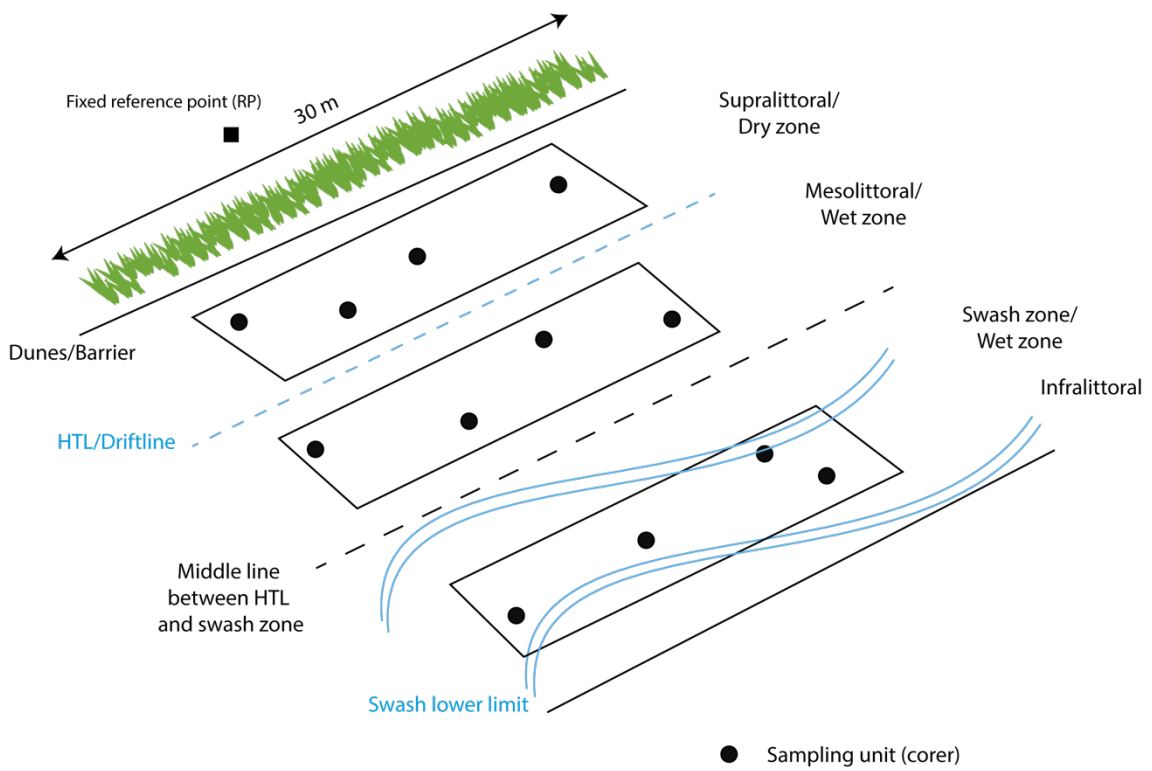
Sandy beaches have a very specialized community including carnivores, herbivores, scavengers and filter feeding species. Because species composition is intrinsically related to environmental conditions, including natural and anthropogenic drivers, the structure of the macrofaunal community is considered a suitable ecological indicator of sandy beaches changes. For example, the decrease in grain size leads to an increase in organic matter composition, lower oxygen availability and consequently a shift from a community dominated by filter feeders to a community dominated by detritivorous species.

### *Randomized sampling design*

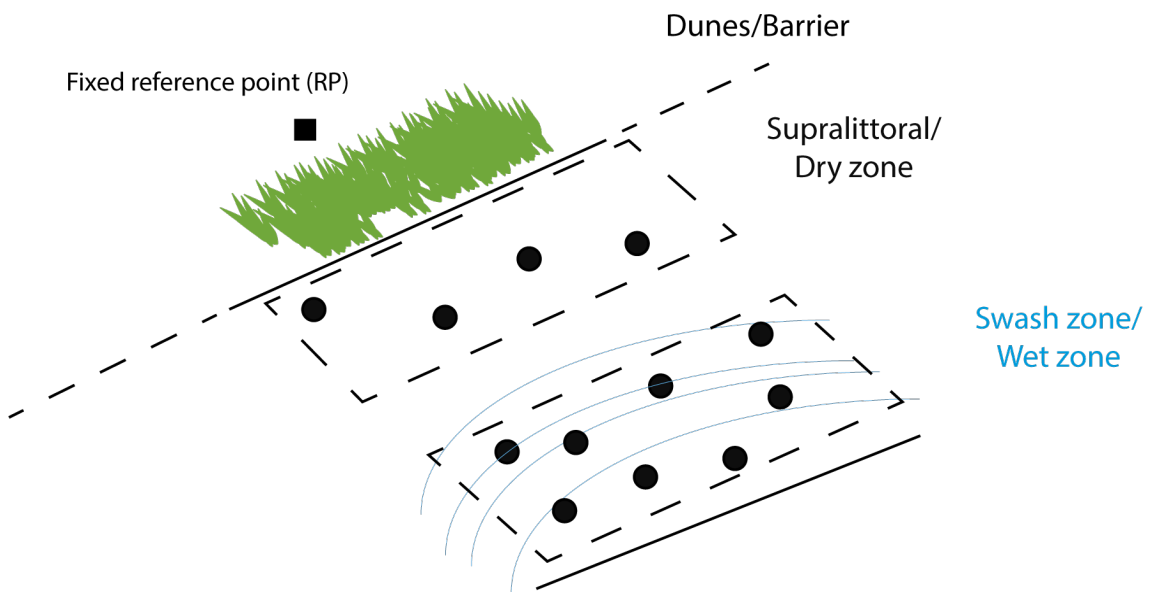
Select three sampling zones within the beach face (inside a 30 meters long-shore length, named as site). The position of the zones will be defined following littoral zones. The first zone is delimited by the upper limit of the high tide level (HTL), usually marked by the drift line, until the dune vegetation (or a wall for urbanized beaches), and named supralittoral. The second one, lower to the HTL until the upper limit of the swash zone, is named mesolittoral. The third one in the swash zone is named as “swash”. The second and third zones are defined as a wet section of the beach (Figure 1A). At each zone, a minimum of four sampling units should be taken randomly ( $n = 12$ ). For the microtidal or narrow beaches, only two zones will be sampled, one above the upper HTL, named as supralittoral, and the second one in the swash zone (Figure 1B).

In the case of microtidal beaches that experience tides ranges  $> 1$  m we suggest that eight sampling units are taken in the swash zone because the second and third zone are considered as one. The samples from the swash zone of those microtidal beaches will be comparable to the wet zone from the wide beaches. The positions of samples are randomly assigned along both zones. Such approaches provide significant advantages in terms of analytical power by avoiding spatial autocorrelation among individual samples. The distances between dune base and HTL, and from dunes to upper and lower limits of swash zone have to be registered. Two samples from each strata can be used to measure total organic matter content by weight difference using subsamples of 5 g of dry sediment previously burned at 500 °C for 1 h.

A



B



Microtidal beaches situation

● Sampling units (corer)

Figure 1. Randomized sampling station (plots) strategy for beaches with well-defined tide range marks (macrotidal or mesotidal regime) (A), and with microtidal or a narrow beaches (B).



### *Sampler*

A corer with a 15 cm diameter reaching up to 25 cm in the sediment will be used. The depth can be marked inside the corer. To perform macroplastics sampling the sediment of each sampling unit must be sieved on a 5 mm sieve and the retained items counted (See macroplastic sampling strategy).

Samples must be subsequently sieved using a mesh size 0.5 mm. When the corer is used close to the drift line, the corer should be closed off with a mesh sieve. Several talitrids live associated with the wracks and can jump quickly out of the corer.

**Warning:** Our sampling strategy with 12 units will make up 0.32 m<sup>2</sup> with 15 cm diameter corers. Consider to increase your sampling effort, e.g. number of transects, levels, or increase your area to 0.57 m<sup>2</sup> with 20 cm diameter corers, or 0.88 m<sup>2</sup> with 25 cm diameter corers, if you want to answer other ecological questions beyond the MBON Pole to Pole aims.

If a research facility is located close to your site, the samples should be transported in plastic bags to guarantee the washing process occurs without losses. If not, there is a possibility to wash samples in the field (with caution, since in general the size of the animals makes this a delicate process). Where the sediment particles are coarser than 0.5 mm and heavier than the animals, decantation or elutriation techniques may be used. In both methods, it is recommended to repeat the processing at least 5 times for each sample and to verify if any animal remained in the sediment after the processing. Overall, depending on grain size, the amount of sediment, and the taxonomical aim, it is necessary to narcotize the animals (e.g., magnesium chloride) to extract them from the sample (*see Eleftheriou, 2013*).

Please try to identify the organisms the best you can, preferably to species level. Samples have to be collected for later identification in laboratory conditions. This working group has various taxonomists, who can help with identifications (*adapted from the SARCE*).

Process only live animal, shells with animal inside, and annelids with prostomium.

**Warning:** There is some criticism about how many details and small animals are lost by washing the samples at the beach, and how to deal with sand (from medium to coarse beaches) retained in the sieve. For sandy beaches with fine sand, the sieving and sediment washing at the beach if made gentle and carefully will save a great amount of time in the lab routines. For medium to coarse sand an elutriator for soft body animals and crustaceans may help, but is still a problem for shells, that should be counted during the sorting.

### *Methods of preservation*

Try to identify the individuals alive when possible and photograph them, then use ethanol as a fixative (useful for molecular studies). Alternatively, buffered 4% formalin can be used. Specific fixatives may also depend on the technique and the taxon. For this protocol, we recommend using magnesium chloride, if available, before fixation for morphological studies or microphotographs (*see Eleftheriou, 2013*). If you have a microscope with camera available, take a picture of each species to add to the database.

### *Data entry*

Register your data from the field data sheets and the information on species identification in the Excel data sheet templated provided. Species names need to be checked using WoRMS. Instructions for checking species list for taxonomic quality control using WoRMS are available on the MBON Pole to Pole site ([https://marinebon.github.io/p2p/methods\\_data\\_science.html](https://marinebon.github.io/p2p/methods_data_science.html)). More information on the descriptors is provided within the example data sheet.

Additional information on species characteristics of the species that you encountered in your sampling sites can be added in a separate sheet, this includes:

- 1) what feeding group (separated in groups: primary producers, herbivores, suspension-feeders, carnivores, detritivores, omnivores, scavengers) does a particular species belong to;
- 2) whether a particular species is invasive, introduced or not;
- 3) include the distance from urban areas to the base of the beach backshore (to measure urbanization effects);
- 4) include information from databases or satellite images: chlorophyll a, phosphate and other inorganic nutrients, distance from river mouth, pollution, sea surface temperature (SST), photo-period and tidal regimes.

#### **4.2.2. Ghost crabs**

Ghost crabs (*Ocypode* spp) species are the most conspicuous invertebrates of open ocean sandy beaches worldwide. They are very active species, visible for visitors and researchers, and construct burrows in a wide zone of the sand, from the backshore to the intertidal zone. Specifically, *Ocypode quadrata* is the single species from sandy beaches of the western coast of the Atlantic Ocean. *Ocypode occidentalis* and *Ocypode gaudichaudii* occur on the coast of the east Pacific sandy beaches. Large populations of these animals are characteristic of well-preserved sandy beaches where they can occupy the full beach profile. Some ghost crab species occur mainly in the supratidal zone and few species are strictly inhabitants of the intertidal zone.

Density of burrows is widely used as a proxy of population density/activity. Also, width of burrows is linearly correlated with the width of the carapace, and that measure is commonly used to investigate population structure. Finally, because of their large size, characteristic behavior, relatively precise and sensitive to changes over time, which can be determined and characterized in an accurate and practical way with very low cost, ghost crabs have been globally used as indicator species to determine anthropogenic disturbance and also to detect changes associated with climatic.

This protocol aims to:

- i) Provide a practical guidance for establishing a monitoring program for ghost crabs populations (*Ocypode* spp) along the Atlantic and Pacific coast of South and North America;
- ii) establish standardized practices that could be implemented at these scales;
- iii) provide an indicator of environmental changes and “coastal health” using ghost crab populations.

### *Where*

Since we are proposing to integrate multiple-evidence studies of biodiversity and environmental change we recommend that *Locations/Sites* should be the same as those selected to apply the macrofauna protocol. If you are only applying the ghost crab protocol please consider an oceanic, exposed, dissipative beaches, avoiding embayed/closed beaches (> 200 m of beach length/minimum along-shore distance). Avoid implementing this protocol in estuarine/ wave protected sites and overly populated areas close to possible sources of pollution.

*Daily conditions:* Morning – low spring tide (below 0.2 m).

*Periodicity:* The sampling should occur preferably at the same time of macrofauna sampling, seasonally (four sampling events per year). When possible, consider increasing your sampling frequency to monthly.

Information on site characteristics will be denoted in the general data field sheet attached. Please see the macrofauna protocol for essential descriptors of sites and their expected outputs. Definitions for the ghost crab protocol are listed below (between parenthesis the header of the spreadsheet).

### *Quadrat*

The number of the quadrat (9 m<sup>2</sup>) sampled at each transect (Figure 2).

### *Burrow Width (Bur\_width)*

The diameter of a burrow, in mm units.

### *Burrow length (Bur\_length)*

The length of a burrow measured with a flexible cable, in cm units.

### *D/V\_distance*

The distance of a burrow to the dune crest/vegetation, in m units.

### *Sampling*

Establish four 3 m wide transects in your monitoring site. Each transect should be perpendicular to the shoreline and stretched from the backshore (crest of the dune/vegetation) to the water line. Every three meters at each transect (9 m<sup>2</sup> *Quadrats*) identify and count only the active ghost crab burrows (you should observe burrow mouths looking for fresh activities and traces like sand marks). Burrows width should be measured using a vernier caliper ( $\pm 0.1$  mm). To avoid the superficial disturbance, measures should be taken inside immediately after the mouth of the burrow. Each burrow length (depth) should be measured using a flexible marked cable into the burrow until resistance. For each burrow the distance between the burrow and the backshore vegetation/dune should be measured.

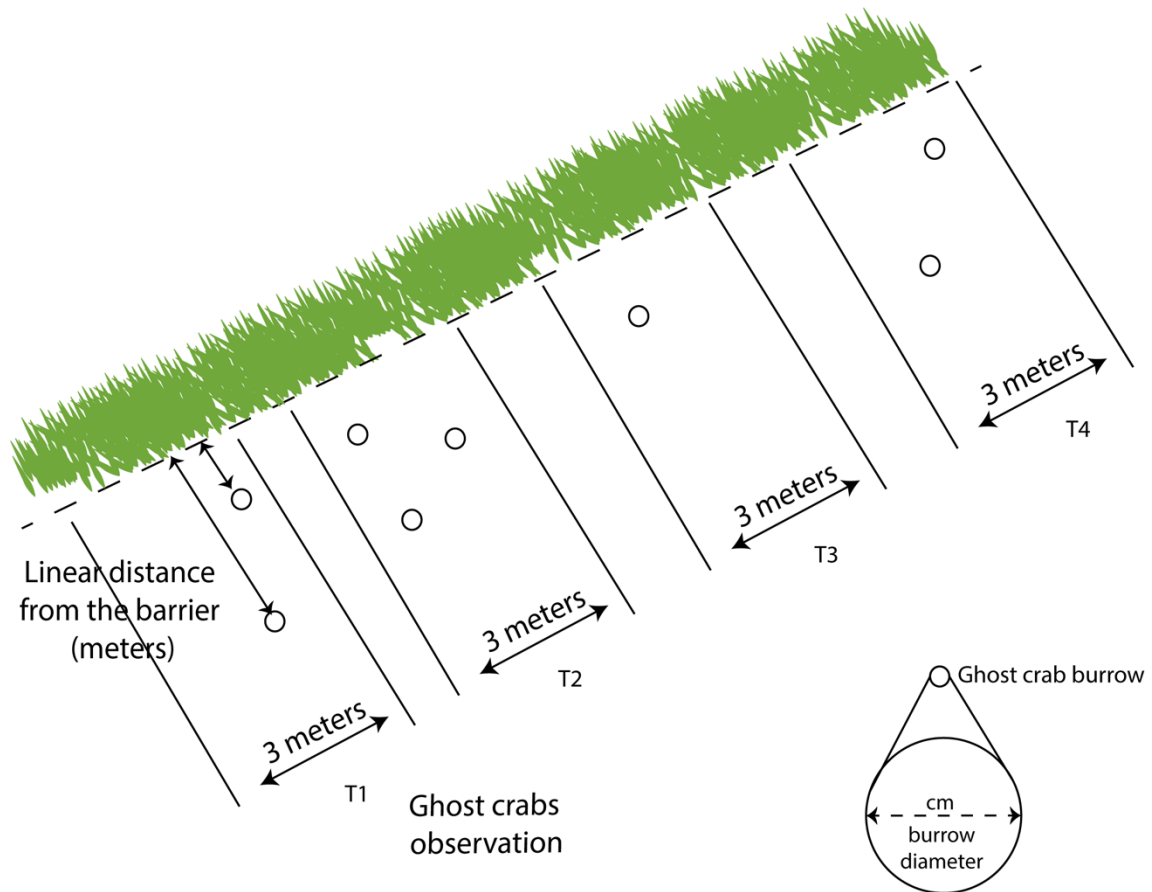


Figure 2. Sampling strategy for ghost crabs and macroplastics. Each transect (T1, T2, T3, and T4) with four meters wide.

#### 4.2.3. Marine debris

##### *Plastics on sandy beaches*

Sandy beach debris is not only an environmental hazard and harmful for human health, but also diminishes its aesthetic value of the landscape and seascape, leading to economic losses for recreational and tourist use of beaches. Particularly, plastic contamination generates an environmental problem of global relevance due to its low cost, great durability and wide range of uses; the increase in its use eventually leads to deposition on sandy beaches where visible litter dominates (Derraik 2002, Sudhakar et al. 2008, López-López et al. 2018). With respect to the physical conditions of the beach, previous studies have shown that abundance of plastic debris could be positively correlated with dissipative characteristics (Lozoya et al. 2016), areas of priority for this protocol.

Marine plastic waste is presented in a continuum of sizes and shapes, so these are fundamental measures to include in a monitoring program. Microplastic debris comprises purposely manufactured microparticles to fulfill a function (primary origin) or fragments resulting from wear and tear or fragmentation of larger plastic products (secondary origin) (Galloway et al. 2017, GESAMP 2019). Following size criteria, GESAMP (2019) recommends < 5 mm diameter as the ‘common definition’ of the upper size boundary for microplastic particles for monitoring

purposes, thereby published articles of microplastics distribution on sandy beaches used a 1 mm mesh sieve to retain them (Turra et al. 2014, Hidalgo and Thiel 2013, Lee et al. 2015).

Several groups of coastal researchers have therefore incorporated citizen scientists as active participants to generate large-scale spatial data on the occurrence and abundance of small plastic debris (Hidalgo-Ruz and Thiel 2013), and that helps to collect a temporal records of coastal plastic occurrence and raise awareness about the importance of keeping our beaches in good condition not only for human well-being but also for ecosystem health.

### *Sampling*

Next to each sampling unit of the biological protocol, draw three random 0.5 x 0.5 m quadrant and collect the first 5 cm layer of sand. Sieve the sand through a 5 mm mesh sieve (meso-plastics) and then using a 1 mm sieve (micro-plastics). Although there is no standardized scheme for the morphological characterization of plastic debris in general, GESAMP (2019) suggest five categories:

- i) Fragment: irregular shaped hard particles having an appearance of being broken down from a larger piece of litter;
- ii) Foam: Near-spherical or granular particle, which deforms readily under pressure and can be partly elastic, depending on weathering state;
- iii) Film: Flat, flexible particle with smooth or angular edges;
- iv) Line: Long fibrous material that has a length substantially longer than its width;
- v) Pellet: Hard particle with a spherical, smooth or granular shape.

#### **4.2.4. Driftline detritus**

To determine the species composition and biomass of wrack (detritus) on the driftline all the wrack (detrits) present within a 1 m wide strip of beach centered on each of the transects (same as for the ghost crab) should be collected, separated by species, shaken to remove adhering sand and weighed in a scale. Biomass values for each detritus species should be expressed in terms of kg wet weight of each species per meter of beach shoreline.

## 5. References

- Eleftheriou, A. (Ed.). (2013). *Methods for the study of marine benthos*. John Wiley & Sons.
- McLachlan, A., Defeo, O. (2018). *The ecology of sandy shores*. Academic Press.
- Nielsen, P. (1999). Groundwater dynamics and salinity in coastal barriers. *Journal of Coastal Research*, 15, 732-740.
- Opfer, S., Arthur, C., & Lippiatt, S. (2012). NOAA marine debris shoreline survey field guide.
- (ReBentos) Rosa Filho, J.S., Nascimento Corte, G., Fabricio Maria T, André Colling L, Regina Denadai M, Cruz da Rosa L, Borzone CA, Marques de Almeida TC, Rosental Zalmon I, Omena E, Veloso V, Zacagnini Amaral AC (2015) Monitoramento de longo prazo da macrofauna bentônica entremarés de praias arenosas. In: Turra, A., Denadai, M.R. (eds) Protocolos para o monitoramento de habitats bentônicos costeiros – Rede de Monitoramento de Habitats Bentônicos Costeiros – ReBentos. Instituto Oceanográfico da Universidade de São Paulo, São Paulo, pp 194– 208
- Schlacher, T. A., Schoeman, D. S., Dugan, J., Lastra, M., Jones, A., Scapini, F., McLachlan, A. (2008). Sandy beach ecosystems: key features, sampling issues, management challenges and climate change impacts. *Marine Ecology*, 29, 70-90.
- Sherman D.J. (2005) Reflective Beaches. In: Schwartz M.L. (eds) Encyclopedia of Coastal Science. Encyclopedia of Earth Science Series. Springer, Dordrecht
- SINAC. 2016. Protocolo PRONAMEC: Protocolo para el monitoreo ecológico de las playas arenosas. Proyecto Consolidación de las Áreas Marinas Protegidas. Programa de Naciones Unidas para el Desarrollo (PNUD) y El Fondo para el Medio Ambiente Mundial (GEF), San José, Costa Rica. 39p
- Wentworth, C. K. (1922). A scale of grade and class terms for clastic sediments" *The Journal of Geology*, 30 (5), 377-392.