Long-term Ecological Research Program
Coastal Habitats of Espírito Santo

Monitoring protocol for coastal reefs

Benthic Cover

Larval Recruitment

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Universidade Federal do Espirito Santo

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LTER HCES

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Version 1.0

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Aim

The aim of this protocol is to elucidate the methodology and rationale applied in the Long-Term Research Program for Coastal Habitats in Espírito Santo to monitor marine biodiversity in coastal reefs of the Eastern Brazil Marine Eco-region, in the South Atlantic.

About the LTER HCES

The Long Term Research Program Coastal Habitats of Espírito Santo LTER HCES started in 2017 and is located on the east coast of Brazil (Fig. 1), with a focus on coastal ecosystems, including estuaries, mangroves, coastal reefs, and rhodolith beds. This is an innovative initiative to assess the spatio-temporal patterns of coastal ecosystems in the Eastern Marine Eco-region of Brazil, where there is a decadal trend of warming. The program combines research and conservation, applied at three Conservation Units: Costa das Algas Environmental Protection Area, Santa Cruz Wildlife Refuge and the Municipal Integral Protection Area of the Piraquê-Açu and Piraquê-Mirim rivers. The projects in LTER HCES aim to investigate the interactions between abiotic, climatic and ecological dynamics in the benthic communities and ichthyofauna assemblies within several coastal ecosystems.

The PELD-HCES has a research team coordinated by Universidade Federal do Espírito Santo in partnership with Universidade de São Paulo, Universidade Federal do Sul da Bahia, Universidade Federal da Bahia, Universidade Federal do Paraná, Universidade Federal Fluminense, Universidade do Sul de Santa Catarina, Universidade Federal de Santa Catarina, and University of Oregon. The program is funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo a Pesquisa no Estado do Espírito Santo (FAPES).

About the coastal reef monitoring

This monitoring protocol for coastal reef biodiversity was developed to obtain information about the spatio-temporal variability in reef biotic cover along the intertidal zone and larval recruitment rates of benthic fauna associated with macroalgae banks. The monitoring is carried out monthly at a site at Costa das Algas (Praia de Gramuté, Latitude 19 ° 57 '58.68 "S,

Longitude -40 ° 8' 2.4" O; https://deims.org/6bc83779-d2a7-48ed-a180-0e1e1758a9dd) and complemented with other measures and monitoring protocols throughout this region (4-30 km scale). This protocol was developed based on scientific methodologies specific to the monitored species (see References) and the sample design was adapted to the aims of the program. The data generated in LTER HCES allows the calculation of environmental indexes (e.g. abundance, richness and diversity of species) and provides subsidies for monitoring a list of essential biodiversity and ocean variables (EBVs and EOVs). The environmental conditions in the region is monitored by satellite remote sensing and in situ loggers. The LTER HCES database is made available open-access through the Ocean Biodiversity Information System (OBIS) and the Global Biodiversity Information Facility (GBIF) repositories (http://ipt.iobis.org/wsaobis/ and http://ipt.iobis.org/mbon/).

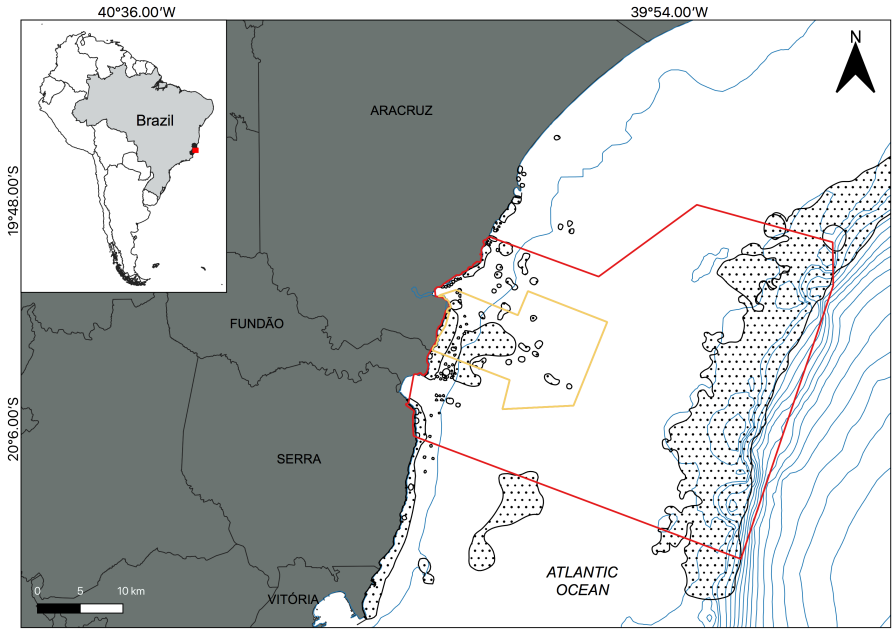

Figure 1. LTER HCES study area in the Eastern Coast of Brazil, within Costa das Algas Marine Protected Area (red) and Santa Cruz Wildlife Refuge (yellow). Note: reef areas and depth isobaths are highlighted (dotted and blue lines)

Session 1 - Benthic Cover

This monitoring protocol is based on photo-transects to obtain ecological information about the reef benthic community.

1. Where to monitor?

Coastal reefs exposed at low tide. Concentrate photo-sampling in the low mid-tide area.

2. When to monitor?

Frequency: monthly.

Temporal window: spring tide, full or new moons.

Hours: daytime low tide (morning), preferably minimum > 0.2 m.

3. How to monitor?

Materials:

- Waterproof camera with good resolution (e.g. GoPro);
- PVC square (quadrat, 50cm x 50cm);
- Flexible measuring tape (minimum length 20 m).

Step by step:

- Fill out the fieldwork spreadsheet on arrival at the site (date, weather conditions, type of sampling, etc).
- Perform sea surface temperature and salinity measurements.
- Choose the reef area to be monitored (approximately 100 meters in length).
- For each photo-transect, stretch the measuring tape of 20 m parallel to the fringe of the reef and photograph the image of a quadrant every 2 m. Register the transect number with a photo. Keep the camera at a distance that captures the entire area of the quadrat and try to maintain a similar height at all times.

- Repeat the same procedure for 5 transects, separated by approximately 5 m from each other.

4. How to organize the images?

Materials:

- Computer;
- Portable HD;
- Cloud account.

Step by step:

- The images must be organized and stored on the access devices immediately after returning from the field.
- Download the photos to your computer.
- Separate and organize the images by transect, maintaining the original order.
- Make a copy of the files on the HD (or on another computer) and on the Cloud system.

5. How to process the images?

Image processing consists of two steps, species identification, grid counting, and generation of datasets in ecological spreadsheets.

Materials:

- Computer;
- CPCe software;
- Spreadsheet software (e.g. Excel or similar);
- HD.

Step by step (Identification):

- Open the CPCe software and select from the toolbar: File> Open> Raw image file
- Choose the photo frame to be identified.

- Specify the boundaries of the area to be identified. Choose the option "Manually size and position the border" and finish by clicking "ok". Delimit the area of the photo-quadrat that will be identified. To do this, you have to click and hold the left mouse button until the yellow perimeter of the square outlines the limits of the square. That done, click on "Accept border size and position" on the right side of the screen.
- Specify the distribution of points. Another window will open asking for the points distribution pattern for the identification of resources within the previously delimited area. Check the "simple random" option and in the space below specifying the number of points, which are 20. To finish, click on "overlay points". Now the 20 points will appear plotted at random within the area that was delimited in step 4, where the resources will be identified.
- In the right corner of the screen, a column with 20 points will appear, from letter A to T. Each letter represents a point within the quadrat. Just click on the empty space in front of the letter that corresponds to each point and, after identifying which resource the point characterizes, click on the tag that corresponds to this resource. The tags are the various acronyms at the bottom of the program.
- To change the distribution of points within the quadrat, go to the toolbar at: Point overlay> Recalculate point coordinates.
- After identifying all points, the file needs to be saved. To do this, go to: File> Save> Save data to .cpc file 9. Save this file in the project folder.

Step by step (Data spreadsheet):

- To transform the data into spreadsheets in Excel, all the .cpc files of the photoquadrats already sorted must occupy the same folder.
- Open CPCe and go to: Save> Save .cpc file (s) to excel.
- A window will open and you should look for the folder where the photo frames that were processed and will be transformed into an excel table.

- In the same window, after selecting all the .cpc files to compose that table, click on “New excel work” at the bottom of this window and give a name to that transect. To finish, click on “Process files”. ex: Gramute_Nov18
- After completing this process, a window will open and this .xlx file must be saved in the project folder, with the same name that was given in the previous step.

Session 2 - Larval Recruitment

6. Where to sample?

Coastal reefs exposed at low tide. Concentrate the collections on the reef fringes where *Sargassum* is found, usually in the upper low-tide zone.

7. When to sample?

Frequency: monthly.

Temporal window: spring tide, full or new moons.

Hours: daytime low tide (morning).

8. How to sample

Materials:

- Plastic bags (30cmx40cm, 5 units)
- Plastic bag (60cmx80cm, 1 unit).
- Plasticized labels (5 units).
- Thermometer and Refractometer.

Sample label:

PELD HCES Monthly sampling Recruitment - Sargassum October 2018 Gramuté R1

Step by step (Sampling):

- Fill out a spreadsheet on arrival at the site (date, weather conditions, etc.).
- Perform sea surface temperature and salinity measurements.

- Collect the five samples of algae (Sargassum sp.) on the reef fringe or tide pools (remove the entire frond of the stalk to the tip of the rocky substrate, gently remove excess sand in the water, fill half the bag with algae + or - 300g).
- Take the algae to the laboratory in the shortest possible time.

9. How to storage?

The algae samples must be frozen (-5 to -15°C) for at least 24 hours before processing and kept away from contaminants (e.g. formaldehyde).

10. How to process?

Sample processing consists of three steps, washing, weighing, and screening:

Materials:

- Plastic buckets (+ or - 8L);
- Plastic tray;
- 100µm and 500µm metal mesh screens;
- Labeled glass / plastic bottles (+ or - 300ml, 5 units);
- Pissettes with 70% ethanol and freshwater;
- Sorting worksheets;
- Graduated scale (resolution in g).

Step by step (Washing):

- Thaw samples in a bucket of freshwater (ideal, 1 sample at a time).
- Wash the algae fronds under running water or shaking them, keeping the contents (water) reserved in the bucket and allocate fronds for weighing.
- Sieve the water from the bucket and bottom content.
- Allocate sample from sieve to bottle.
- Add 70% ethanol for preservation.

Step by step (Weighting):

- Remove excess water with salad drier.
- Tare the scale with the weight of the tray or bowl.
- Weigh the algae from each sample separately.
- Note the weight of each replicate on the sorting worksheets.

Step by step (Identification and Counting):

- Fraction the sample with 100µm and 500µm sieves.
- Allocate sample fractions to sorting plates (petri).
- Separate each fraction separately in a stereomicroscope.
- Identify, count recruits, and register occurrence in the standard spreadsheet.
- Store part of the recruits in small plastic bottles (5-10ml) in 70% (or 100%) alcohol.

Complementary References

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