Protocole CIGESMED : Coralligenous based Indicators to Evaluate and Monitor the"good ecological status" of the MEDiterranean coastal waters

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I. Context

CIGESMED is an « ERANET » European research program for management assistance on the coralligenous **habitats**[1], marine mediterranean ecosystems of great natural value. In this project, an ecological approach (indicators development and testing, non invasive sampling by diving, **transects**[2] and « *visual sensus* » techniques, photographic treatments) is coupled with genetic approaches (barcoding, méta-barcoding, **phylogéography**[3], **population genetics**[4]). These approaches will enable to describe the species composition of the red algae, bio-builders of this environment. The genetic approach also consists to provide innovative tools for bio-indication, based on intra-specific genetic diversity. Using Next-Generation Sequencing (N.G.S.) this project will characterise, by meta-barcoding, organism compositions (several animals and algae phyla) for differents coralligenous ecological **profiles**[5].

There are currently severals index developped for the monitoring of marine habitats, used by national monitoring networks. However, the indexes, their use and meanings, often vary from place to place (Borja *et al*., 2009). A review of the human-driven impacts on mediterranean marine habitats, conducted in 2010, concluded that there was a lack of knowledge on the spatial distribution of habitats, especially in the eastern Mediterranean. Data standardization is the *sine qua non* condition for a proper assessment of human impacts on marine habitats (Claudet *et al.,* 2010). Knowledge gaps pointed out are even more obvious when it comes to coralligenous habitats, which are so unknown.

In this context, CIGESMED project must involve managers and scientists working on the coralligenous, in order to develop a network of **observers**[6] using standardized and inter-calibrated methods.

All CIGESMED's work packages are concerned. WP2; WP3, WP4, WP5, WP6 are the most concerned.

[1] An area distinguished by geographic, abiotic and biotic features (definition of EEC Directive 92/43).
[2] Trajectory followed by the diver while he surveys (occurrence, abundance, biomass…), which can be done systematically or randomly. In our case these records will be made from photo-quadrat of the coralligenous bio-concretions.
[3] The study of processes that explain the distribution of genealogical lineages within the same species (may go as far as speciation processes).
[4] Study of the distribution and changes in the frequency of gene versions (alleles) in populations of the same species. Population genetics allows for example to answer questions about the connectivity of two populations of the same species.
[5] Within CIGESMED framework, a profile is a situation, mixing the exposition (i.e. slope + orientation + rugosity) and the covering type.
[6] Responsible person for the acquisition of the data *in situ*, following a protocole.

II. General Organization

A. Four protocols "matrix"

Protocols tested in CIGESMED framework will provide information on the functioning of the biogenic component, in terms of organization, connectivity between individuals or populations of matrix and builders species. In order to refine knowledge of the conditions related to different structural arrangements, four biological approaches must be overlaid to characterise the study **sites**[1]:

- Cartography of the major populations (and associated profiles) along two depths on at least 3 sites per **locality**[2] [For Marseilles area the depths are 28 m ± 1m and 45 m ± 1 m, for the others these two isobaths must be adapted depending on the luminosity (important factor owing to the nature of coralligenous algal components) and monitored profiles].

- Sampling of individuals fragments from two species: (i) (*Pseudolithophyllum cabiochiae* Boudouresque & Verlaque, 1978, species currently considered [cf. WoRMS) to be *Lithophyllum stictaeforme* (Areschoug) Hauck, 1877) but the taxonomic status is not steady, and *(ii) Myriapora truncata* (Pallas, 1766)]. 32 samples must be collected at each isobaths studied, on profiles presenting very disctinct features, and on opposite sides of each site. This should enable the research of **cryptic species**[3] and the assessment of populations' connectivity and genetic structuration, at different scales (local, regional and pan-Mediterranean).

  NB : Since it's very difficult to distinguish the two taxons, we will name the complex as Lithophyllum stictaeforme/cabiochiae. Lithophyllum cabiochae is considered to be endemic of the Mediterranean Sea, and L. stictaeforme would be from the Atlantic. Indistinguishable while diving, but we observed differences during the samples conditionning. Thus the study will possibly focus on the two taxons (and may highlight the existence of hybrids).

- Sampling by scraping, on the two identified profiles, on four small areas (0.01 m^2). This must be done on each side of each site at both depths (that is 32 samples per island, so 96 samples in total) and should be as complete as possible. It will enable to determine the global specific diversity (taxa number) and the relative differences of biodiversity levels observed between the phyla considered, thanks to a visual assessment (sorting, photograph) completed by meta-barcoding methods.

[1] Within CIGESMED, it can be an island, a small island, a shoal, from the coastal fringe or coralligenous fringe, as long as it has a maximum of different orientations and at least two that are diametrically opposed.
[2] Geographic region where sampling sites of one partner are grouped (e.g. Marseilles locality).
[3] Species defined as such because isolated in term of reproduction and / or whose genetic line has a significant genetic differentiation, indicating an ancient divergence between one and the other, but that is not distinguishable from a morphological point of view.

- Analysis of communities by photo-quadrat sampling along transects on the main profiles. These photo-quadrats will enable to use additional **metric**[1] in terms of relative covering by the species/taxa. Some transects will enable to inter-calibrate methods and materials used by various steakholders (Harvey *et al*. 2004).

The number of samples takes in account the need of statistical **robustness**[2] but is limited in order to preserve the environment. Interventions should be conducted with utmost care.

B. Choice of sites

Whether for tests, exercises or sampling for the first *corpus*, sites must be easily accessible. One quite unknown site requires 1 or 2 exploration dives. The whole protocol requires 8 or 10 dives per site for a complete mapping, and 5 or 6 dives for mapping focus on collected profiles. Adding supplementary location may integrate sites with difficult access if the organizations and divers have enough time, resources and authorisations.

In order to cover a maximum of environmental conditions, site selection should be on areas suffering different pressures. Knowing that some samples must be collected at the same season (study of species diversity), and to avoid weather problems, it is preferable to conduct all operations on one site before starting operations on another site. The first operation is to achieve the site cartography and to select profiles to be sampled.

C. Additional sites specifications

Sites specification on abiotic factors and their spatiotemporal variability (temperature, current patterns, sedimentation, light) and biotic factors (especially food inputs, population and remarkable individuals measures) will complete as far as possible this characterization. And this one must be as shared as possible with all the regions despite the different ecotypes. According to the same outline, the surrounding anthropogenic pressures will also be described in terms of desciptive categorical values.

This characterization (population measures) must be done to best avoid saisonal species ecological successions that could disturb the corvering evaluation, therefore rather from October to March (autumn should be preferred). Over the same period, it's planned to try to assess the trophic inputs and the biomass if a relevant technique enables it. Assessment of the biomass of suspension feeders communities associated with coralligenous can be inspired by True's work (1970). A complementary method must be proposed for that and could be inspired by the techniques of studying the biomass in phytosociology (Bråthen *et al.*, 2004; Bråthen *et al.*, 2007).

[1] Parameter describing a phenomenon or a variable, biotic or abiotic.
[2] An indicator is considered to be robust if it retains a constant value during the repetition of the same event in identical conditions.

Example of criteria for site selection (Marseilles region):

- Sites in common with other projects (e.g. IndexCor)
- Presence of a recording thermometer
- Distributed in and out of creeks
- Maximum exposures represented (i.e. orientation + slope)
- Site with many orientations in a limited area
- if possible, nears of ARMS (measuring device for recruitment) of Devotes project
- Different exposures to pressures (mechanical and chemical)
- Well-defined natural variability (turbidity, current, food intake)
- Diving feasibility (positioning of different exposures at 28 m and 43 m (+ / - 1 m)

Characterization considered for these sites:
- Temperature variability (TMedNet)
- Light variability (4 well-defined **facies**[1]): to define
- Fine particles, food, nutrients contribution: traps positioning for 1 month x 4 seasons x 2 depths was suggested: to define
- Current variability: to define. Current measurement on and near the **substrate**[2] should be preferred. Models developed by Ifremer can probably be exploited to understand the glocal context of currents - including connectivity patterns - even if they often have a resolution too low to explain the spatial variations of considered habitats. Experiments with instruments measuring the Doppler Effect could also be tested.

⚠ Protocols need to be adapted in function of presence/absence and density/frequency of coralligenous bio-concretions. Indeed, it's possible that in some regions of the Mediterranean Sea, coralligenous bio-constructions do not allow the use of our protocols in their full version (e.g. bio-constructions size too small). It will therefore be absolutely essential to specify the changes made or indicate their sizes in order to standardize the data

In order to complete the dataset efficiently, the selected sites must be easily identifiable, and include notable points easy to find at the depths mapped and sampled. These points will be used as starting points for the cartography and for the identification of the sampling areas. It's preferable to choose sites on which other experiments are ongoing. These can also be used to characterize the sites. For example, around Marseilles, the main CIGESMED sites are located in areas where the temperature is monitored (Tmednet program: http://www.pytheas.univ-amu.fr/?Acquisition-haute-frequence-de). These loggers are used as notable points and / or starting points for the map making. Thermometers areas are in most cases shared with IndexCor sites (see part III.F in the protocol), or with gorgonians monitoring (population genetics, phylogeography, resistance, acclimatation...: http://www.imbe.fr/anr-adacni.html) and some of them are equipped with « ARMS » and « ASUS » from the Devotes program (DEVelopment Of innovative Tools for

[1] An aspect exhibited by a biocenosis when the local predominance of certain factors causes the prevalence of either one or a very small number of species, essentially animal ones.
[2] Natural support (usually the bottom) on which organisms develop.

understanding marine biodiversity and assessing good Environmental Status: http://www.imbe.fr/arms-structures-autonomes-de-suivi.html) which aims to understand better the recruitment dynamics using meta-genomics tools (http://www.devotes-project.eu).

D. Test of methods and pre-existent indexes (inter-calibration)

Site characterization will be completed by transects of different sizes (length, shape, depth, boundaries) detailed later on in the protocol. These transects can be done randomly (unmarqued) or permanently (marked), and can correspond to random profiles or selected profiles. Different **operators**[1] will identify a number of metrics from these sets of photos, in order to characterize the sites and / or their ecological conditions. Two types of data will complete these site characterizations: tests of existing methods including the observer changing on permanent transects (inter-calibration on a study site), and the investigation of each metric's variability provided by the pre-existent methods according to the considered profiles. These comparative approaches will be conducted during fieldwork sessions held in common with other countries involved in CIGESMED, as well as in other regions if volunteer researchers and managers want to get involved (e.g. Spain, Italy, Croatia, Malta, North Africa…).

E. Training sites and gear

Cartography and inter-calibrations of the methods and indexes previously designed must be tested first at shallow depth (about 15 m): one exercise site will therefore be designated and marked for this purpose, in each participating region, on a coralligenous shallow place, with diversify facies and easily reachable (under a rock overhang, or in caves' entrances). These sites will be important to initiate new observers, and so that they could assess the efficiency of these methods and indexes. In particular, they will enable observers to fix their mistakes before taking on monitorings in more sensitive areas in terms of safety and **accessibility**[2].

The training of the first observers will help to define and refine "minimum training" documents, as well as knowledge and skills tests, all along the program application. These tools should allow to improve the level of expertise and the efficiency of the observers network, and to attribute a level of confidence to the identifications (the terms "**data qualification**[3] " are used). This training material will be used as part of the "Work package 5" of CIGESMED, which is about citizen science (http://www.cigesmed.eu/-Work-Package-5-Citizen-science-).

[1] Person who operates the observations and field data (often in digital form) received from the observer).
[2] About a device: ease of use of an observation device (material cost, skills requested, formation, observation and treatment times)..
[3] Metadata or enrichment of this data by an attribute from an ontology. It makes possible to sort the data and / or analyze them with holistic approaches (multivariate) or non-statistical ones (data mining).

F. Particular demands for the whole protocole

The sampling zones (between 30 and 50 meters) present a challenge for SCUBA diving, as they require planning decompression stops, including one with oxygen (at six meters). It's also a zone in which effects of cold, current, narcosis or visibility will have a greater influence on the efficiency and relevance of the divers' observations. The volunteer diver-observers will therefore need a minimum experience level that needs to be better defined.

Part of site's contextualization and genetic approaches are based on sampling of corallinaceae *Lithophyllum (=Pseudolithophyllum) cabiochiae*, bryozoan *Myriapora truncata* and eventually a cnidarian (to be confirmed soon). These species have currently no protection status, but the sampling sites might, in time, benefit from some degree of protection (biological, but also historical or archaeological). In this case, it will be essential to formally request all authorizations and to inform relevant organizations in advance (see the authorization form in the appendix). While, in the CIGESMED context, some organizations have permanent authorizations for sampling (these are granted by the maritime prefecture for unprotected species). It is still preferable to inform the scientific council (if any) of protected places of the planned protocols, and in any case to then formally request authorization from the maritime prefecture.

It is necessary, in order to ensure future inter-calibrations (exchanges of material, methodology, staff), to select principal sites based on their accessibility (in terms of cost, security, duration of stay). Planning sites that are accessible in different sea and wind conditions could be helpful (N.B.: coralligenous suspension feeders are often found in strong current, and thus in exposed zones). This aspect will be developed under the auspices of CIGESMED's WP5, launched in may 2014. It will also factor in such factors as the volunteers' level of motivation, their background in biology and ecology, their technical competence (e.g., observations, snapshots), their communication skills, their diligence, and so on.

G. Safety

In term of safety, we recommend observers operating in countries without legislation on professional diving to maximize the security as it's done in France; for example, in terms of medical facilities nearby (with relevant hyperbaric services). For the French staff, national regulation is the minimum to apply in any circumstance, anywhere.

In France, all workers involved in hyperbaric operations are subjected to the decree n° 2011-45 of the Ministry of Labour, Employment and Health from January 11, 2011. The implementing order of October 30, 2012, defines the conditions for entrance, stay, exit and the organization of work for these interventions as part of B "techniques, sciences and other interventions".

It stipulates:

- Diving depths according to divers'levels: 0 m to 12 m, 0 m to 30 m, 0 m to 50 m and beyond (Art 4461-28)
- Decompression procedures (tables MT92 and computer authorized by the last decree) and the maximum time of decompression stops authorized according to the distance with the nearest re-compression chamber. If this one is situated at more than 2 h, no decompression stop is required. If the decompression stop lasts less than 15 min, accessibility to the re-compression chamber must not exceed 2 h. If the decompression stop last more than 15 min, accessibility to the re-compression chamber must not exceed 1 h. (Art. 17 for the implementaing decree, for the category B).
- Composition of dive teams (beyond 12 m, divers must work with a supervisor on the surface) (Art 14, Art 27).
- SCUBA diving and emergency equipment

This led us to define:

- Air diving (the only gaz authorized by the CNRS at the moment). Mixed-gaz diving, e.g. nitrox or trimix, if organization, material and divers are certified.
- Depth of 28 m for the transect (leaving a 2 m margin beyond the 30 m zone): the aim is, especially when far from the re-compression chamber, to minimize, or even avoid decompression stops. Reminder: in profressional diving decompression stops are not authorized if no re-compression chamber is accessible in less than 2 h. But a safety decompression stop isn't exempted. This is not feasible where coralligenous is deeper (Corsica, a part of Var (France), Eastern Mediterranean, and all places with clear water). Coralligenous habitats are circalittoral zones, which implies that thay naturally set beyond the lower limit of *Posidonia* meadows, that is about 30-35m. In this case, study areas situated beyond 30 m will be superficial concretions, **circalittoral**[1] enclaves in the **infralittoral**[2], whose particularity will be taken in account in the analysis.
- Diving time should be shorter than 15 min as much as possible (no decompression stop)
- Work in pairs
- Personal material:
    - Diver's standard equipment: wetsuit, flippers, mask…
    - Scuba tank of 12 to 20 litres at 230 bar, according to individual consumption

[1] The region of the sublittoral zone, which extends from the lower limit of the infralittoral to the maximum depth at which photosynthesis is still possible.
[2] In the mediterranean area, the infralittoral zone is the part constantly submerged whose upper fringe corresponds to the chart datum. Its lower limit is one that is compatible with life of photophilous algae and marine phanerogams

- Individual parachute of decompression stop, in order to be distinguishable on surface, especially in case of rising in open water or lost of partner
- A net to store material (decametre, slate, sampling bags…)
- Lights
- Notepad with a pencil and a spare pencil

NB: Desirable evolution in France is the transition to SCUBA diving with Nitrox 40/60. This would enable longer dives: at same depth of 21 m, 35 min diving without decompression stop.

III. Protocols

A. Reminders on the concepts of coralligenous facies

Coralligenous habitats, considered as underwater landscape or as ecological puzzle (UNEP – MAP – RAC/SPA, 2009), having a very complex structure, allow the development of several community types (Laborel, 1961; Laubier, 1966; Laborel, 1987 ; Ballesteros, 2006).

Its great structural and ecological generates multiple biocenotic units (Hong, 1982) and thus many facies.

Description of these facies according to the appearance of different **biocenosis**[1] is influenced by the predominance of one or more notable species (gorgonians, sponges, erect bryozoans…) favoured by the local preponderance of certain physico-chemical and geomorphological factors (current, fine particules input, light and depth, rugosity, slope and orientation).

The only description of these facies based on their bionomic appearance only remains difficult, and their distribution is very relative to surrounding micro-conditions (Virgilio *et al.*, 2006) mainly natural light and currents, that influence and strongly condition the creation and location of various **ecotons**[2] and enclaves.

Establish a precise typology and standardize the description of coralligenous facies encountered in the different regions studied under CIGESMED is primordial. This approach will be intended through the creation of ontology for this type of habitat.

Some facies recognized as coralligenous habitats observed in Marseilles'region, are represented on the Figure 1.

[1] A grouping of living organisms, linked by relationships of interdependence within a biotope with relatively homogeneous major characteristics

[2] Transition between two ecosystems or habitats or areas with different biotic and/or abiotic conditions.

If we focus on **sciaphilous**[1] communities with basal concretions made of coralline algae (*Lithophyllum* and *Mesophyllum*) from the infralittoral and circalittoral zones, a multitude of remarkable facies are cited in the literature:

1) "Pre-coralligenous" facies characterized by the presence of photophilous algae such as: *Udotea petiolata* ((Turra) Borgesen, 1926) and *Halimeda tuna* ((J.Ellis & Solander) J.V.Lamouroux, 1816). This facies has been described by several authors (e.g. Pérès & Picard, 1964; Gili & Ros, 1985; Ros *et al.*, 1985; Gori *et al.,* 2011).
2) Facies characterized by *Eunicella singularis* (Esper, 1791).
3) Facies characterized by *Eunicella cavolinii* (Koch, 1887).
4) Facies characterized by *Peyssonnelia squamaria* ((S.G.Gmelin) Decaisne, 1842).
5) Facies characterized by *Pseudolithophyllum cabiochiae*[2] rims.
6) Facies characterized by *Lithophyllum incrustans* (R.A.Philippi, 1837).
7) Facies characterized by *Mesophyllum alternans* ((Foslie) Cabioch & Mendoza 1998).
8) Facies characterized by *Paramuricea clavata* (Risso, 1826).
9) Facies characterized by *Myriapora truncata* (Pallas, 1766).
10) Facies characterized by *Corallium rubrum* (Linnaeus, 1758).

[1] Refers to an organism, animal or plant, which grows preferentially in the shadows. Sciaphilous is the contrary of photophilous
[2] With the restrictions made above about the knowledge on *Lithophyllum spp*.

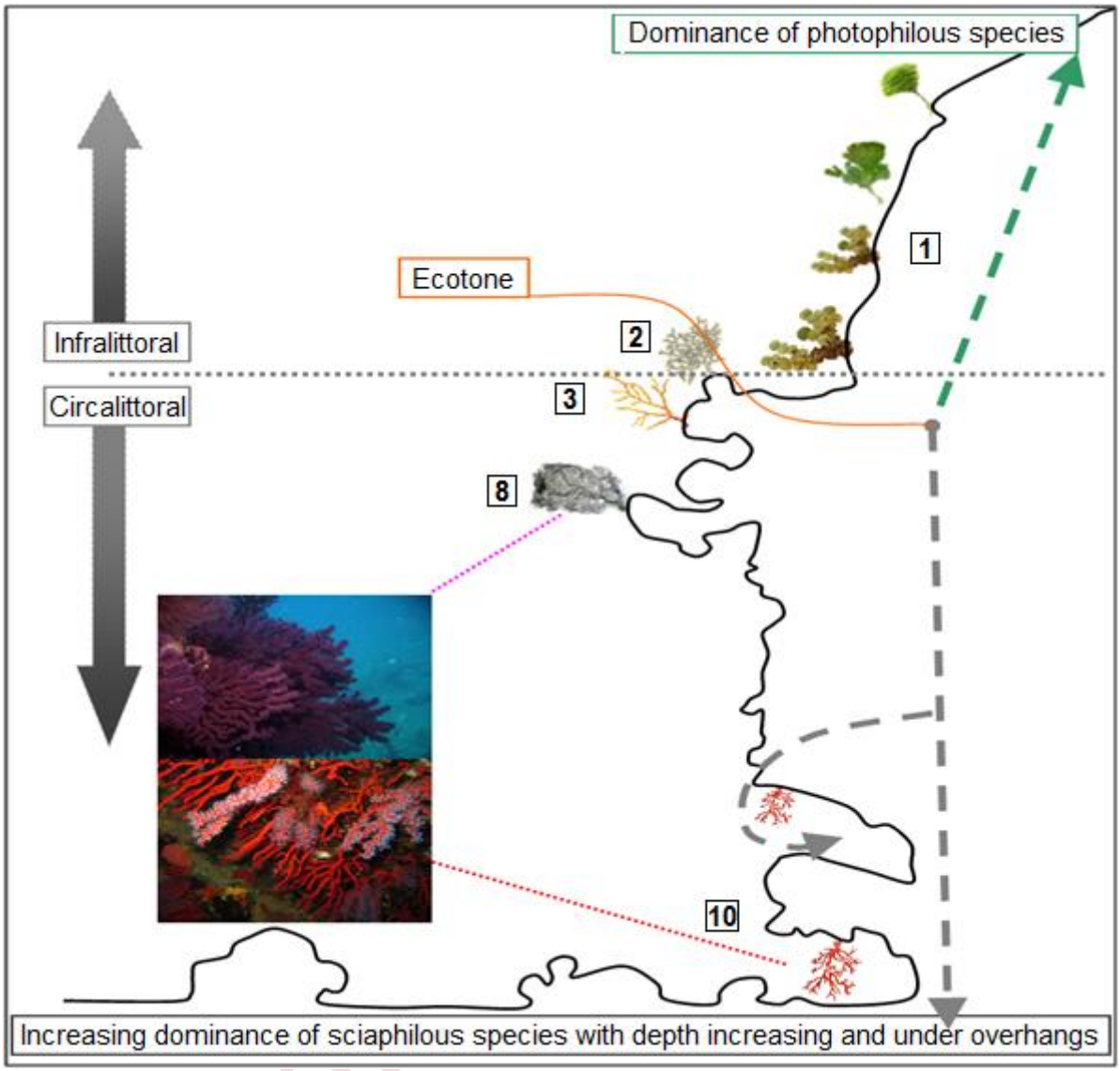


Figure 1: example of remarkable facies of coralligenous habitats observed in Marseilles' region

The SPN (service of natural heritage of the French national Museum of natural history) listed Mediterranean biocenosis in a more completed way, in which we can find these facies of coralligenous environment:

IV.3.1. Coralligenous biocenosis (C)
IV.3.1.a. **Association**[1] of *Cystoseira zosteroides* (Syn. *C. opuntioides*)
IV.3.1.b. Association of *Sargassum spp.*
IV.3.1.c. Association of *Laminaria rodriguezii* on rocks
IV.3.1.d. Association of *Flabellia petiolata* and *Peyssonnelia squamaria*
IV.3.1.e. Association of *Halymenia floresia* and *Halarachnion ligulatum*

[1] Permanent aspect of a biocenosis with vegetal physionomic dominance where the species are linked by an ecological compatibility and a chorological affinity (from UNEP, APM, RAC/SPA, 2006).

IV.3.1.f. Association of *Rodriguezella spp.*
IV.3.1.g. Association of *Lithophyllum spp.* and *Mesophyllum spp.*
IV.3.1.h. Facies of *Eunicella cavolinii*
IV.3.1.i. Facies of *Eunicella singularis* / *Eunicella verrucosa*
IV.3.1.j. Facies of *Leptogorgia sarmentosa*
IV.3.1.k. Facies of *Paramuricea clavata*
IV.3.1.l. Facies of *Parazoanthus axinellae*
IV.3.2. Biocenosis of coralligenous shelf

(http://spn.mnhn.fr/servicepatrimoinenaturel/docs/rapports/SPN%202011%20-%2013%20-%20SPN_2011_-_13_Rapport_TypoMed.pdf)

Within the context of CIGESMED, we will consider mainly biocenosis present in the studied regions and represented in the most typical profiles, on which the sampling should be sufficient (IV.3.1.d., IV.3.1.g., IV.3.1.h., IV.3.1.i., IV.3.1.k., IV.3.1.l. in bold in the list above). Moreover, other profiles sheltering remarkable species will be taken in account independently (*Corallium rubrum, Leptosamia spp*.) See example on Figure 2.

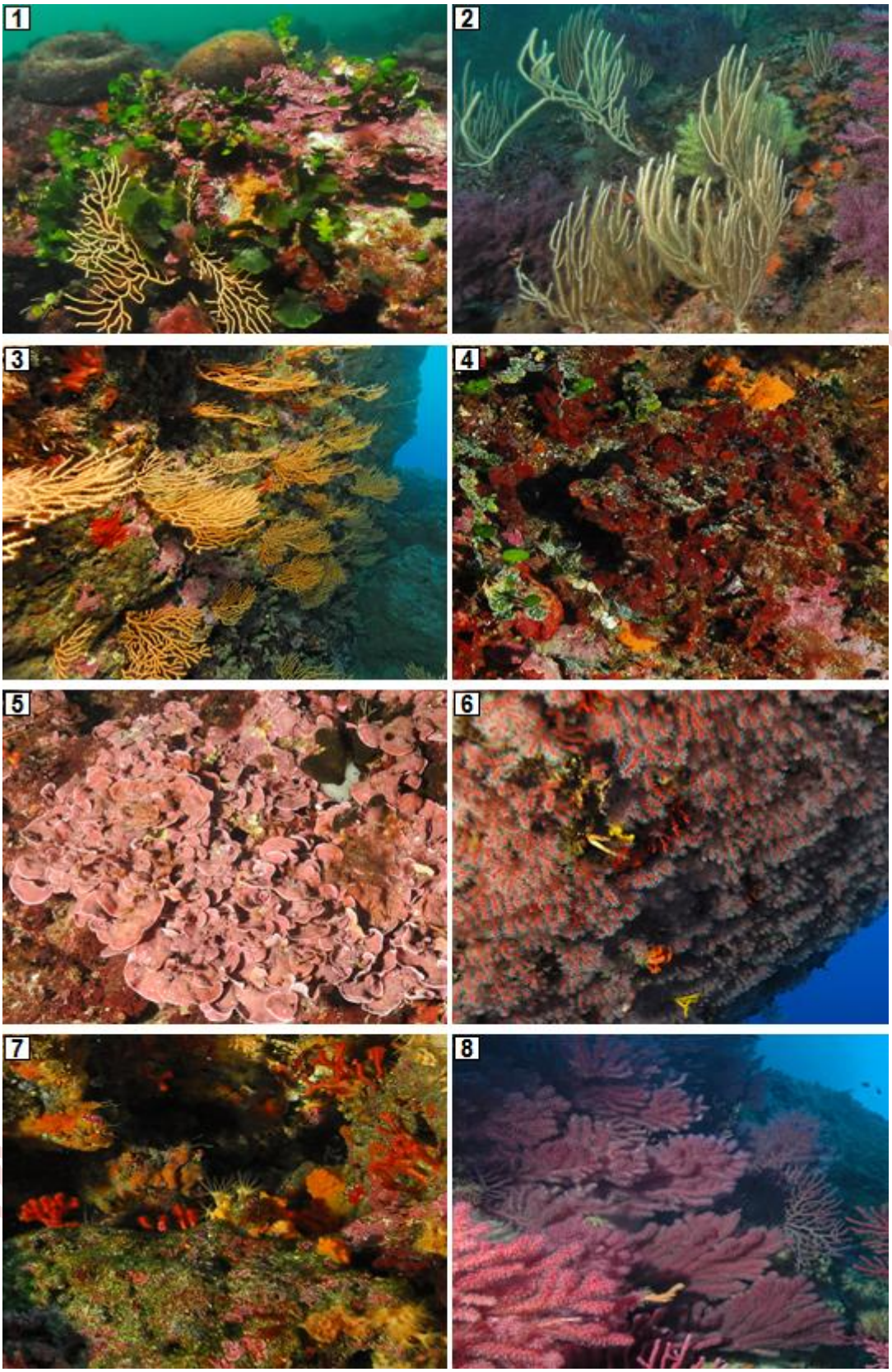
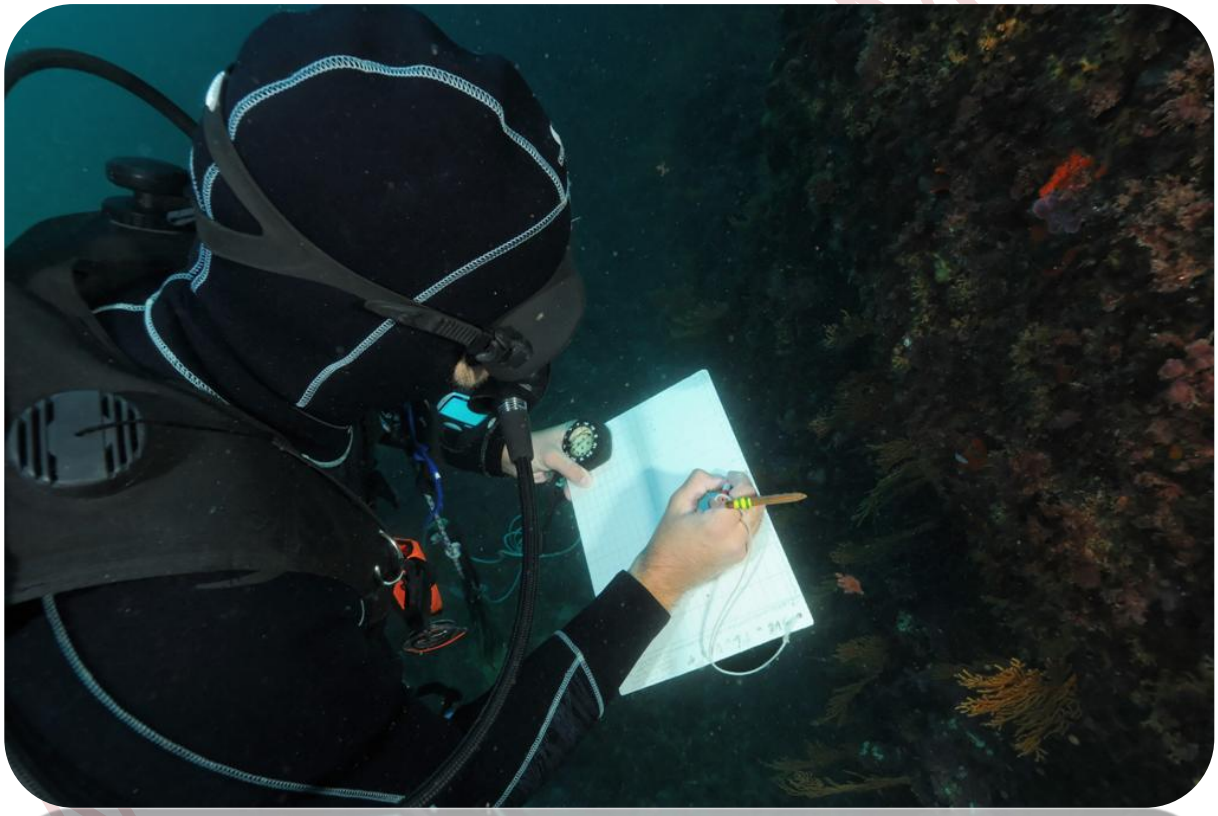


Figure 2: examples of facies of (1) *Udotea peteolata* and *Halimeda tuna*, (2) *Eunicella singularis*, (3) Eunicella cavolinii, (4) *Peyssonnelia squamaria*, (5) *Mesophyllum alternans*, (6) *Corallium rubrum*, (7) *Myriapora truncata* (Pallas, 1766) and (8) *Paramuricea clavata*

MODULE 1

PROTOCOL OF PROFILES AND STANDS CARTOGRAPHY



B. Module 1 : Protocol « Profiles and stands cartography »

1. Principle

Cartographies of marine habitats generally lack data (Claudet, 2010), and uncertainties in the published maps are often poorly documented or even not described (see Cartham results: CARtographie des HAbitats Marins**). This is especially true for the coralligenous environment, which is a complex assemblage at small scale, and for which there is very few precise cartographies. The map realized within CIGESMED will support the knowledge about the spatial distribution of the coralligenous habitats on the sampled sites.

Sites' cartography should be based as much as possible on the concept of facies. This is still difficult to implement in a program of management assistance, because opinions on their definitions still diverge.

Within CIGESMED, it was decided to characterize the sites throught a set of simple and distinct categories of physical and/or biological parameters. That is what we call a profile: it includes information about slope, orientation, rugosity and main stands of population for segments of coralligenous with a length of 5 m.

2. Method

To determine and record profiles, data must be noted on a note-pad, and properly captioned with the name of the observer(s), date, site, transect and depth.

Cartography can be done on different types of sites. Either around small islands and shoals, or along coastline with all orientations represented (Nort, South, East, West and the four intermediaries). For each site, two depths must be sampled: in Marseilles 28 m (+/- 1m) done by CIGESMED's team, and 43 m (about 40 to 50 m in pratice) done by INDEXCOR's team. Sampling of whole sites is impossible, thus samples will be collected along transects (see red lines on Figure 3) cut into **segments**[1] of 5 m long on 1 m high. This height should be centered at the target depth, and a difference of 1 m deep, above or below, is accepted.

To place transects, a notable point must be determined, preferably in a corner, in order to deal with several orientations. This notable point should be described, photographed, and **GPS**[2] coordinates should be recorded as precisely as possible (by a person in surface according to divers'bubbles or floating object). A report on a 3D **DTM**[3]**, WGS84**[4] format, would enable to find it easily later on. From this point, transects can spread in two ways:

- By following all segments in one direction from the notable point,

[1] Mapped length (spatial object) of 5 m to which is assigned a profile.
[2] Global Positioning System.
[3] Digital Terrain Model: map in 3D.
[4] World Geodetic System 1984. global standard geodetic system, used especially by the GPS system.

- By following some of the segments in one direction, return to the notable point, and go in the opposite direction (preferred for divers'safety and precision of the segment's location). If the notable point is located in the middle of the area to be mapped, two **group of diving partners**[1] can work at the same time (Figure 3).

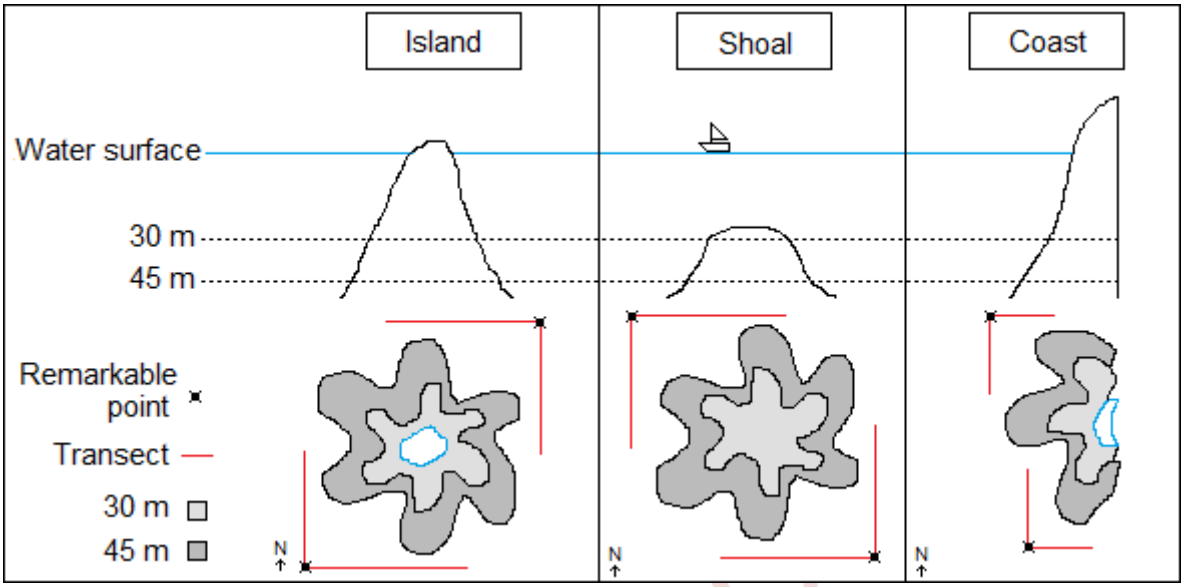


Figure 3: sites' selection and transects' allocation

For all segments, records will be completed by films, which may be studied later on and could complete the observations.

To define the profile of each the segment, the following typology must be applied:

- Orientation of the wall: North, South, East, West and the four intermediates (Northeast, Northwest, Southeast, Southwest). To determine the orientation, the diver should stand in front of the wall and go towards the wall. If the observed segment has many orientations, take the average one (this happen in case of peak or cavity). Nomenclature on the note-pad: N, NE, E, SE, S, SO, O and NO.

- Wall's inclination: unlike Glasby (2000), who used the inclinations "flat" and "vertical under overhang", CIGESMED's protocol suggests to decline this parameter in 4 values: VIFC = Vertical, Inclined, Flat, Ceiling (Figure 4).

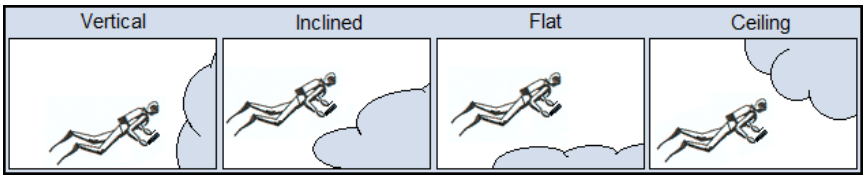


Figure 4: drawing of the different slopes

[1] Group of divers having the same diving parameters (direction, depth, duration, decompression time).

- The « Ceiling » category corresponds to the presence of an overhang above the observer, that is as large as a person at least, and covers most of the segment.
- The « Vertical » category (from 66% to 100% of inclination) corresponds to a strip of substrate (2 m wide around the same isobath) or a series of rims that overlap. If shifting slightly from the wall, this seems that it's at equal distance from the diver, 1 m above and 1 m below, and the wall is entirely visible. Another criteria may be used to characterize the "vertical" category: it's a wall on which a heavy object couldn't settle.
- The « Flat » category (from 0% to 33% of inclination) corresponds to a strip of substrate (2 m wide around the same isobath) or a set of concretions and rocks that seem to be at about the same level and very exposed to light. Another criteria can be used to characterize the "flat" category: an object placed on this substrate cannot roll beacause the inclination is very low. This profile is particularly subject to fine inputs and mechanical impacts.
- The « Inclined » category (from 33% to 66% of inclination) is an intermediary category between « Vertical » and « Flat ». The accuracy of the inclination's estimation depends on the diver's assessment. The "inclined" category is preferred when doubts remain. This category has the most diversity to light exposition, fine inpu and currents.

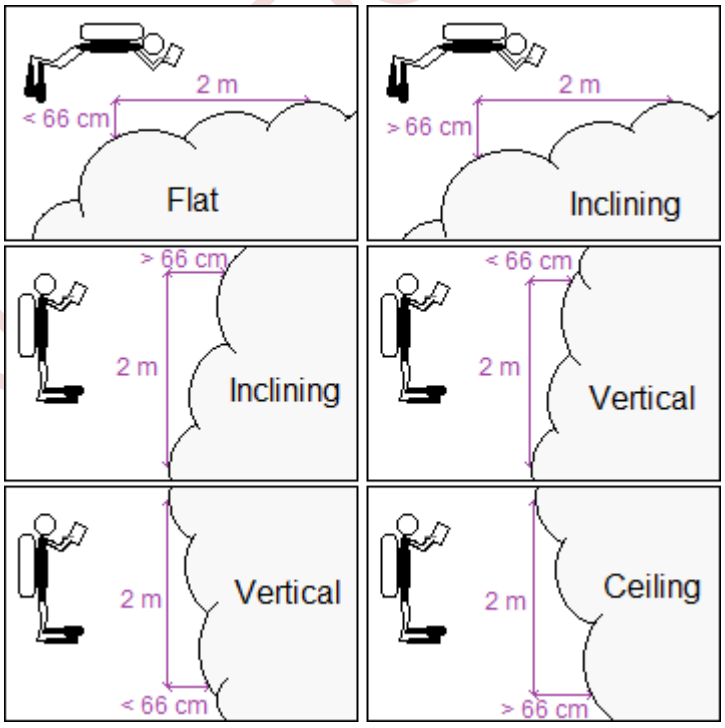


Figure 5: Slope categories

When the segment is not uniform in terms of inclination, the majority parameter only should be recorded, or the darker-shaded profile if there is equivalence (i.e. darker-shaded profile > Ceiling > Vertical > Inclined > Flat > less shaded profile), assuming that this parameter will influence the composition of the stands sampled in terms of cover (same logic will be applied for the rugosity).

Finally, when the segment considered is in the shade (that is when an overhang prevents the light to arrive with an incidence of more than 10 % relative to the vertical (percentage to be discussed)), the category of inclination is « Ceilling ». Quite simply, it means that when the sun is at its peak, the diver seems to be in the shade.

- Rugosity: as in the project RECOR (Deter & Holon, 2012), this parameter must be recorded and easily evaluated by the diver (whatever his level of knowledge). The size of crevices, holes and faults observed on the entire segment will be described as follow:

    - « T » (Tiny): segments whose holes do not allow the passage of a fist (considering the fist is about 10 cm large).
    - « S » (Small): segments whose crevices allow the passage of a fist at most.
    - « M » (Medium): a head can enter in one of the segment's hole at least (the hole must be about 30 cm large).
    - « L » (Large): segments whose crevices can contain at least, the upper body (the crevices must be at least 1 m large).

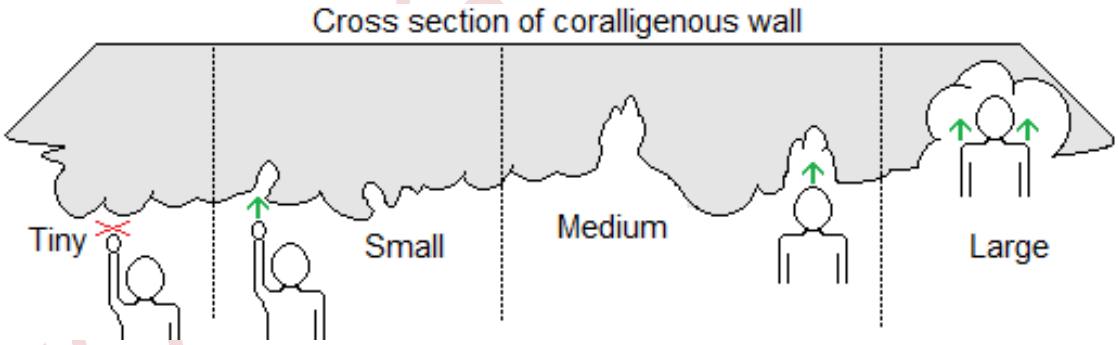


Figure 6: drawing of the four types of rugosity

When the segment is not uniform in terms of rugosity, we should retain the parameter of the largest holes, assuming that, in a big crevice, there can be intermediate rugosity.

- Cover: the method of estimation follows a logic that should allow easy understanding and less variability in the estimation of metrics. This method is simplier than the one given by Boudouresque (1971), which uses a more complex code and a large number of species. CIGESMED's protocole suggests realize this recording on 4 or 5 majoritary taxa (3 at least) with indication of relative abundance of encrusting or erected species (Figure 8) according to the code of Table 1 (examples on Figure 7).

-

Table 1: code of cover and abundance of encrusting and erected species

Code	+	++	+++
Encrusting species	0 % < cover < 20 %	20 % < cover < 50 %	Cover > 50 %
Erected species	1 to 4 individuals	Uniforme cover, but sparse (more than 4 individuals)	Either high density covering at least 50 % of the segment, or uniforme cover and not sparse

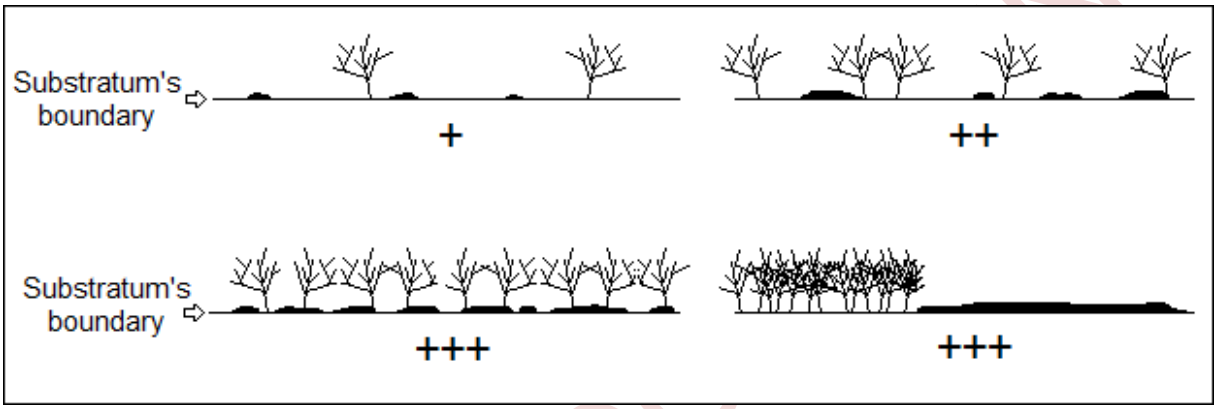


Figure 7: example of the different possibilities of cover and abundance

In addition, it is important to note the remarkable features such as: exceptional presence of one species, exceptional individuals (size, shape…) and/or remarkable stands.

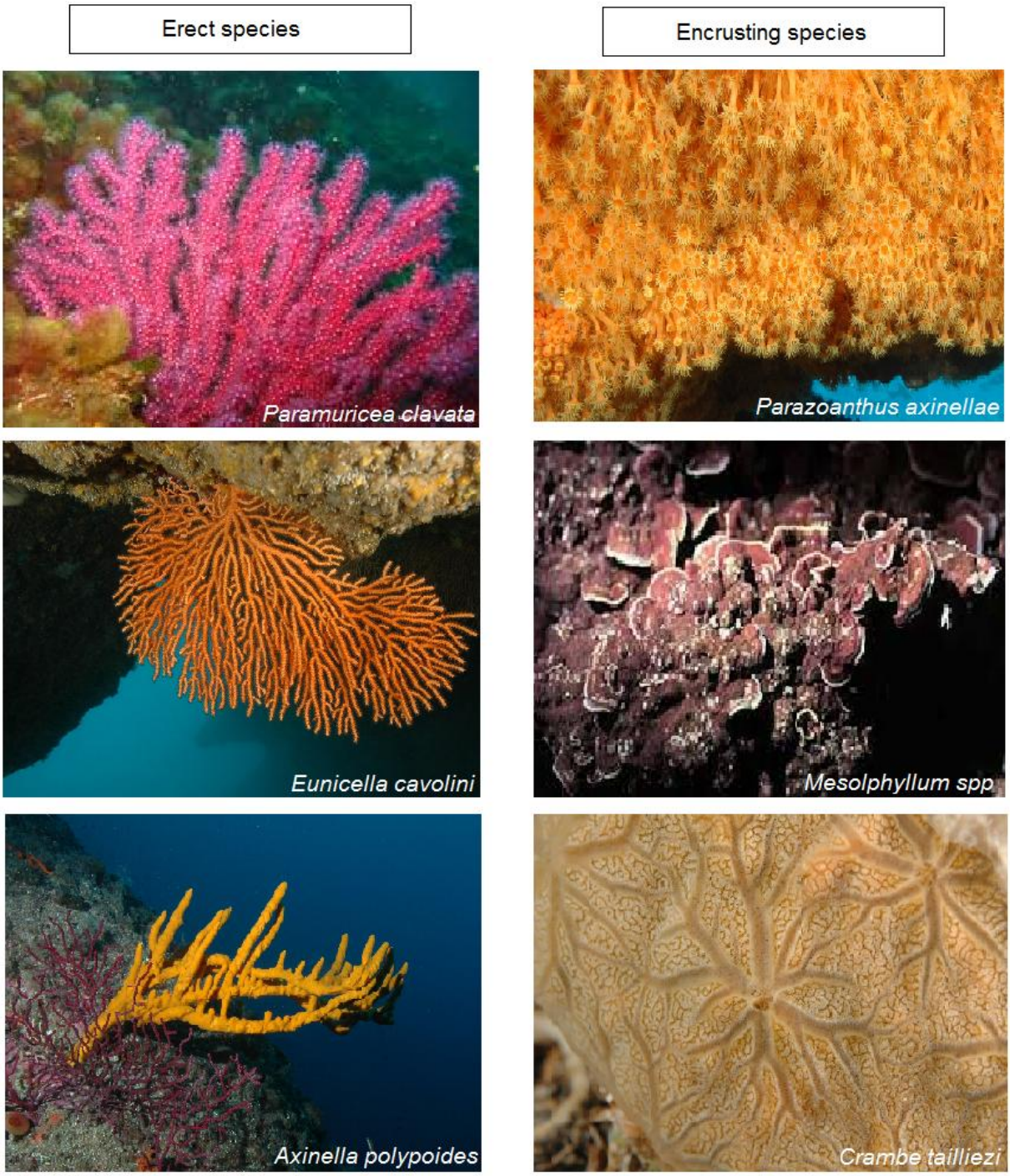


Figure 8: examples of encrusting or erected species

⚠ If the wall is vertical but situated under an overhang above (in and out of the water), the stand can represent a stand from overhang instead of a stand from vertical wall. Think to point out these characteristics on the note-pad.

3. Sampling

a. *Operations*

- Time per 5 m unit: 1 minute (at 30 m deep).
- Calibration at the beginning, then each 10 records (1 minute each) X (number to the appreciation of the diver) x fin's strokes number to make 5 m. An error of 1 m on average is possible without training. With training, the mean error should be around 10 % (this estimation is to attest).
- Notation should be as short as possible on the note-pad, since it's done during the dive (Figure 9). If the diver wants to change the typology of notes written on the note-pad, this shouldn't be a problem but he must use the typology presented here when inputing the data on computer, and he shoulf caption the note-pad to explain how to convert his notes into the common typology.
- Example of organization of the note-pad records.

Covering readings CIGESMED

Site: …… Date: …./…./…….. Depth: …….. m Observer: ……

N°	ORI	INC	RUG	*ES*	*EC*	*PC*	*CR*	ERA	FRA	EGA	FGA	Sponge	Codium	Bryozoa	Ascidac	Others	Remark ind	Remark pop
1																		
2																		
3																		
4																		

Figure 9: note-pad for the profiles' recording

The list of acronyms and their metrics is shown in the Table 2.

Table 2: list of the different metrics and their acronyms

Metric	Possible values	Signification
Time	Digital positive integer	Time in minutes on the Timer
Orientation	N, NE, E, SE, S, SO, O et NO	North, Northeast, East, Southeast, South, Southwest, West, Northwest
Inclination	VIFC	Vertical, Inclined, Flat, Ceiling
Rugosity	0, +, ++, +++	No rugosity, Low rugosity (fist), average rugosity (head), large rugosity (body)
Upper stratum[1]	CR, EpD, EC, ES, PC	*Corallium rubrum*, Erected sponge *Eunicella cavolinii, Eunicella singularis, Paramuricea clavata*
Basal stratum[2]	ARE, AVE, ARD, AVE, Bryo, Cod, Ep, Pey, Turf	Encrusting red algae, Encrusting green algae, Erected red algae, Erected green algae, Bryozoan, *Codium spp*, Sponge, *Peysonnelia spp*, Turf
	Halim, Lepto, Litho, Meso, Paraz,	*Halimeda tuna, Leptosamia pruvotii, Lithophyllum cabiochae, Mesophyllum alternans, Parazooxanthus axinellae*
Remarkable species	According to diver's knowledge	The diver must specify his knowledge fields on the form
Remarkable stands	According to diver's knowledge	The diver must specify his knowledge fields on the form
Solid waste	Objets and sizes	The diver must precise the object's type and its size (50 cm, 1 m, several metres…)

[1] Within CIGESMED, it is the highest stratum corresponding to erect species. In this stratum, the competition between species is mainly above the substrate. NB: some species may be part of two strata.

[2] Within CIGESMED, it is the lowest stratum corresponding to encrusting and turf species. In this stratum, the competition between species is mainly for the substrate. NB: some species may be part of two strata.

Diving scenario for the records:

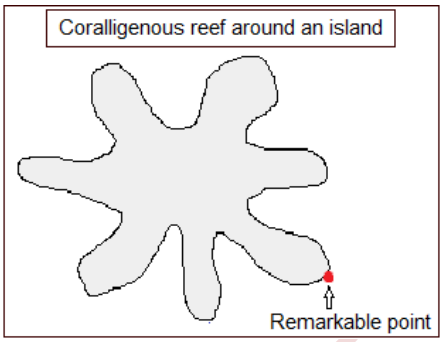


Figure 10: choice of the notable point

- First of all, while diving, look for the study site. Find a notable point (special sock, pipe, thermometer) at a peak or a corner (Figure 9). Descibe this point, photograph it, and note its depth. This should take 1 to 2 min. Make someone who is on the boat record the GPS coordinates, thanks to divers' bubbles if there is no current (1 or 2 minute after immersion).

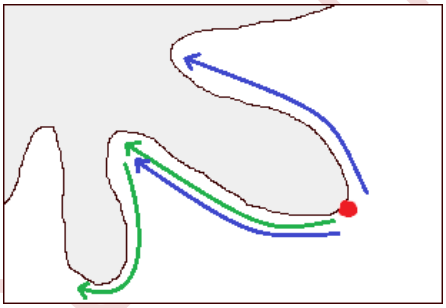


Figure 11: choice of transects

- Once identified, the notable point will serve as starting point for the transect(s). Two possibilities. Either by making a long transect along the coralligenous in one direction (see green arrows). Or by making a smaller first transect in one direction and then going back to the notable point and making a second transect in the other direction (see blue arrows), (Figure 10).

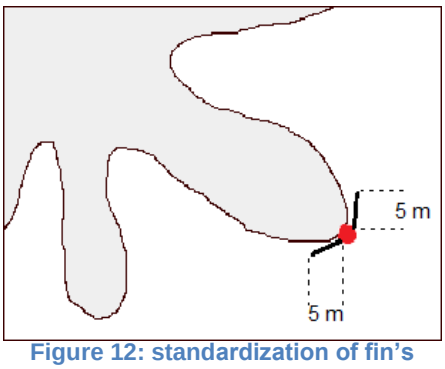


Figure 12: standardization of fin's strokes

- Before any manipulation, remove materials from nets, roll out the semi-decametre (make sure that it does not fall). To do this, deploy the semi-decametre in the direction of the first transect from the notable point. If a second transect is provided in the opposite direction, leave the end of the decametre on the starting point to make it visible for the passing on the second transect (Figure 11). The diver who is taking notes for the cartography must calibrate his kicking (more efficicient if slow and steady). The purpose of the standardization is to determine the number of fin's strokes required (which may vary with the current) to achieve 5 m. The time required for the standardization is about 1 or 2 min. If the current is too high, repeat the standardization step at each major orientation change (e.g. before going back to the starting notable point).

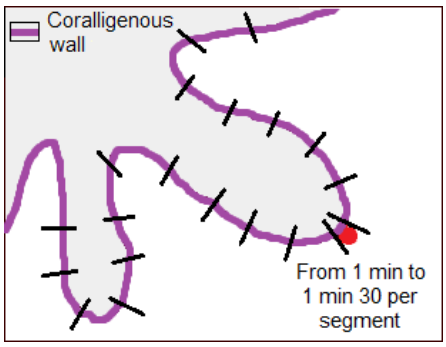


Figure 13: transects' segmentation

- Once the standardization is completed, the diver splits the transect in segments of 5 m, measuring the distance thanks to its number of fin's strokes standardized (Figure 12). He identifies a maximum of the segment's features and notes his records (time, depth, orientation, inclination, rugosity, cover, plus remarkable species and stands if there are) making back glances to not miss anything. Dives are done within a divers

group of two partners minimum. The first diver stays at expected depth (28 or 43 m) and records, as stated above, the profiles of each segment. The second diver films each segment setting back and about 2 m above the first diver (safety precaution, this also limits his blood saturation). The film enables to correct data afterwards if the diver has doubts. If a third diver is present, he can follow the first diver, and collect the samples, thus it spares an extra dive. Each record of profile takes 1 to 1,5 min, after this time the informations noted may provide extra precisions for the dataset.

- In the event that the chosen site has discontinuous coralligenous walls, transects are divided, thus segments are separated (Figure 13). The goal is to cover a maximum of orientations and inclinations.

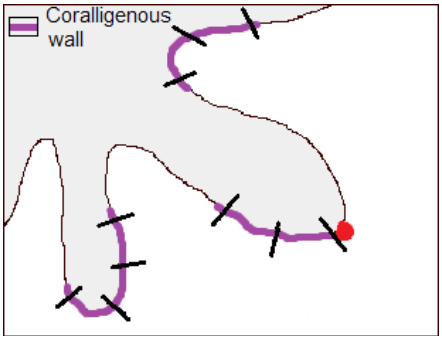


Figure 14: case of discontinuous coralligenous walls

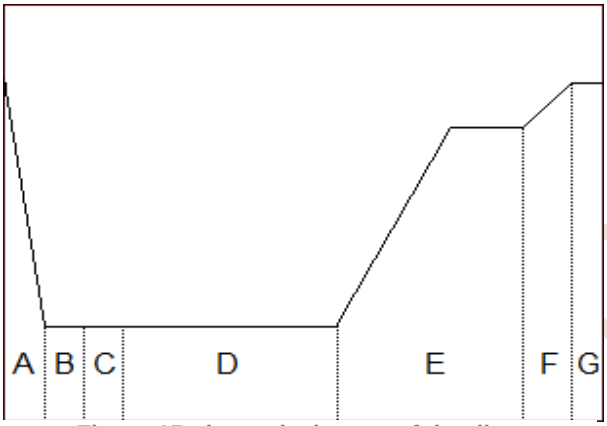


Figure 15: theoretical curve of the dive

A: descent on site (1 min). B: detection and identification of the notable starting point (2 min). C: material installation and **calibration**[1] (1 to 2 min). D: data collection (max: 16 min diving). E: rise (2 min). F: decompression stop (3 to 5 min). G: end of the dive (Figure 14).

According to the French law for professional diving, the maximum diving time at 30 m deep without mandatory decompression stop is 15 min. For safety reasons, and in order to not overstep this rule, dives' depth is set at 28 m. The ideal time to make a dive at this depth is thus 15 min maximum without decompression stop (this rule must be observed for the French citizens if the closest hyperbaric chamber is situated at more than a 2 h journey). In addition, we recommend to make a decompression stop of 5 min instead of 3 min. Deeper dives (beyond 40 m) will be realized with oxygen decompression within CIGESMED. If a collaborator wishes to implement it, he must ensure the availability of all necessary safety elements (a maximum of 25 min'dive at 45 m implies a decompression stop of 3 min at 9 m and 15 min at 6 m with O_2. 15 min at the bottom allows you to dive with a decompression stop of 15 min maximum with air).

[1] Operation that consists in measuring the number of fin's strokes needed to go 5 m (one segment) depending on the direction and strength of the current.

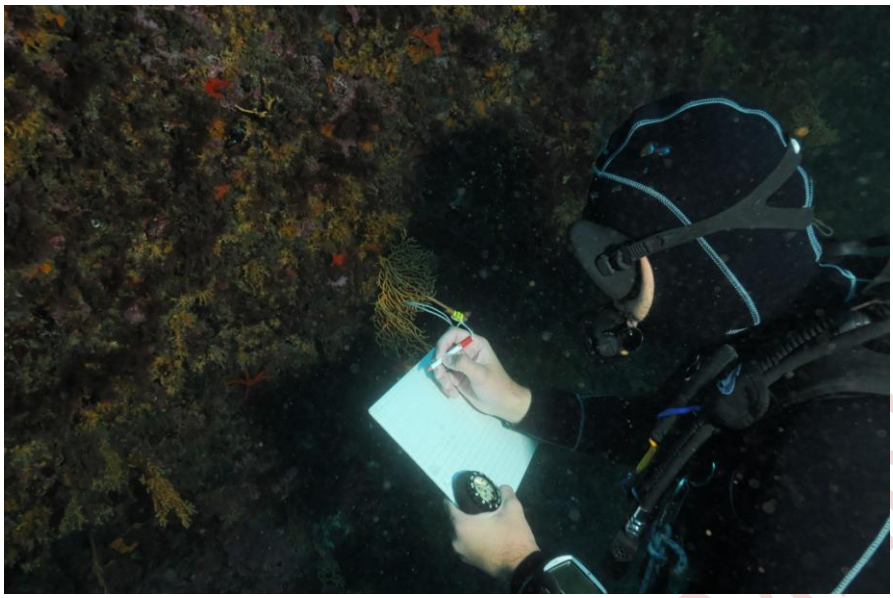


Figure 16: Diver in the process of mapping the coralligenous

b. *Data conditioning*

All note-pads used should be scanned (digital archiving) and photocopied (paper archiving). Data must be coded as follows:

[program]_[site]_[date] _**D**[depth]_[doer]

Example : CIGESMED_TME_20140121 _D1_AA01

Data must be sent as soon as possible to the program's manager or the person in charge of the database (for France: romain.david@imbe.fr).

c. *Material list*

- Conventional diving equipment.
- A compass attached to the note-pad.
- A pencil plus a spare one.

d. *Work time quantification*

The number of dives depends on the number of divers. Possible cases:

- Either 2 dives with 2 divers on 2 sides of the site. This makes 8 man-dives per site.
- Or 1 dive with 3 divers on 2 sides of the site. This makes 6 man-dives per site.

4. Deliverables

a. *Expected data*

Recorded profiles' data collected must be entered into a table (Table 3) and will be used to draw the sites' cartography (Figure 17).

Table 3: example of profiles' records on dive (complete table in appendix)

Date YYYYMMDD	Site (3 caracters)	n° segment	Depth type	Real depth (m)	Observer	Orientation	Slope	Rugosity	*ES*	*EC*
20140411	LPD	1B	D1	28	RD01	ORI_E1_N	INC_B_INCLINED	RUG_D_LARGE		Medium
20140411	LPD	2B	D1	28	DG01	ORI_D1_NW	INC_B_INCLINED	RUG_D_LARGE		Medium
20140411	LPD	3B	D1	28	RD01	ORI_C1_W	INC_B_INCLINED	RUG_D_LARGE		Medium
20140411	LPD	4B	D1	28	DG01	ORI_D1_NW	INC_B_INCLINED	RUG_C_MEDIUM		Medium
20140411	LPD	5B	D1	28	RD01	ORI_E1_N	INC_B_INCLINED	RUG_C_MEDIUM		Medium
20140411	LPD	6B	D1	28	DG01	ORI_E1_N	INC_B_INCLINED	RUG_C_MEDIUM		Medium
20140411	LPD	7B	D1	30	RD01	ORI_D2_NE	INC_B_INCLINED	RUG_C_MEDIUM		Medium
20140411	LPD	8B	D1	30	DG01	ORI_C2_E	INC_B_INCLINED	RUG_C_MEDIUM		Medium
20140411	LPD	9B	D1	28	RD01	ORI_C2_E	INC_B_INCLINED	RUG_D_LARGE		Medium
20140411	LPD	10B	D1	28	DG01	ORI_C2_E	INC_B_INCLINED	RUG_C_MEDIUM		Medium
20140411	LPD	1A	D1	28	FZ01	ORI_C2_E	INC_C_VERTICAL	RUG_D_LARGE		Medium
20140411	LPD	2A	D1	28	FZ01	ORI_C2_E	INC_C_VERTICAL	RUG_D_LARGE		Medium
20140411	LPD	3A	D1	30	FZ01	ORI_C2_E	INC_B_INCLINED	RUG_C_MEDIUM		Medium
20140411	LPD	4A	D1	32	FZ01	ORI_D2_NE	INC_B_INCLINED	RUG_C_MEDIUM		Medium
20140411	LPD	5A	D1	30	FZ01	ORI_D2_NE	INC_B_INCLINED	RUG_A_TINY		Medium
20140411	LPD	6A	D1	29	FZ01	ORI_E1_N	INC_A_FLAT	RUG_A_TINY		Medium
20140411	LPD	7A	D1	29	FZ01	ORI_E1_N	INC_A_FLAT	RUG_A_TINY		Medium

Figure 17: example of cartography of the coralligenous walls in Moyade island's site

b. *Data processing perspectives*

Data will be processed using QGIS software in order to create a map of the coralligenous profiles observed.

5. In brief

« Profiles and stands cartography » protocol

Method

Profiles recordings on two sides of each site:

- ✓ By 5 m units (segments) from a geo-referenced notable point
- ✓ At two depths: 28 m and 43 m (+/- 1 m)

Based on the following typology:

- ✓ Orientation (North, Northeast, East, Southeast, South, Southwest, West, Northwest)
- ✓ Inclination (VIFC: Vertical, Inclined, Flat, Ceiling)
- ✓ Roughness (+: hand; ++: head; +++: body)
- ✓ Majority species covers: 3-5 taxa and their relative abundance (0-20 %; 20-50 %; >50 %)
- ✓ Remarkable elements (individuals' and stands' size)

Expected data

- ✓ 2 to 4 fullfilled note-pads per site
- ✓ Typology of the digital data's nomenclature: [program]_[site]_[date] _**D**[depth]_[observer]

Questionnaire on module 1

« Profiles and stands cartography»

1) Your opinion on this protocol proposal « Profiles and stands cartography » :

a) The methods proposed in this module « Profiles and stands cartography » : do they seem feasible on your CIGESMED's site ? Why

b) Do you have at your disposal all the skills and means necessary to implement the protocol ? Detail the skills and means lacking.

c) What skills/means/work can you offer to any partner in order to complete the sampling ?

d) What changes can you propose to make this protocol operational on your CIGESMED's sites? (Taking care to maintain consistency with the other observers of the network)

e) What improvements would like to make on the descritpion of the protocol « Profiles and stands cartography » ?

f) What improvements would you like to make on the methods of the protocol « Profiles and stands cartography » ?

2) Your opinion on the feasibility of this protocol Profiles and stands cartography » :

a) Are you familiar with this type of research?

b) On what occasions do you apply this type of protocol ?

c) What are your difficulties of implementation ? which steps are not clearly described in the document ?

d) Do you have any alternative to proprose ?

3) Your investment in CIGESMED:

a) Are you ready to commit yourself to participate to the sampling of « Profiles and stands cartography »?

b) If you do not intend to apply this protocol « Profiles and stands cartography », please explain why.

c) Are you ready to commit yourself to improve and document the methods proposed by this protocole « Profiles and stands cartography »?

d) Are you interested in a meaningful participation in the writting of scientific paper describing the results obtained from this protocol « Profiles and stands cartography » ?

e) For this type of research are you ready to commit to participate as long-term observer applying this protocol « Profiles and stands cartography »?

If you use another protocol, please communicate your detailed protocol to romain.david@imbe.fr

MODULE 2

PROTOCOL OF CHARACTERIZATION BY PHOTO-QUADRATS

C. Module 2 : Protocol of « Characterization by photo-quadrats »

1. Principle

Monitoring networks of marine habitats are established, at best, nationally, and indexes often vary from one place to another (Borja *et al*., 2009). However, the variations observable on a range of species from the coralligenous habitats may be relatively subtle across two decades, and events on other taxa may result in large natural interannual variations of the metrics without any sign of degradation of coralligenous habitats (Teixidó *et al*., 2011).

The general principle of this protocol of the sites characterization is based on the analysis of photographs of coralligenous studied habitats, via a dedicated image processing software.

Several methods of photographic sampling and image processing will be tested in order to select, depending on the conditions, the most efficient method to better understand the variability of a number of metrics. Measure the variability induced by the choice of a particular method or equipment shall allow a better understanding of the efficiency of each experimental combination. This step is the phase of methods' inter-calibration. The objective is to achieve a standardized database of metrics on the whole Mediterranean coralligenous.

Photo-quadrats[1] are photographs taken throught a frame (the **quadrat**[2]) whose dimensions are fixed. They enable to measure a set of parameters more finely than the profiles surveys. These parameters are metrics such as occurrences, abundances, cover rates, dominances, size of populations and individuals, boundaries types between stands, fractionation… Metrics variability depends on:

- natural conditions, fluctuating in time (seasonal, annual or longer) and space,
- observers (scuba diving experience, underwater photography, fitness and physical abilities),
- conditions and equipment used for these observations (torches and flash, cameras and lens, quadrats' type and size),
- operators' knowledge and practice,
- qualities of the software and techniques used by the operator,
- and their variability due to anthropogenic pressures.

This phase of sites characterization by photo-quadrats is planned only in the region of Marseilles so far. If the results are positive, other partners may possibly try it out.

[1] Photograph taken through a quadrat, defining a specific area. Photographic sampling unit..
[2] Physical frame used to frame the photograph (taken during the dive in our protocol). It may be independent or integral with the shooting device (picture or movie camera).

a. *Inter-calibration on permanent transect*

The principle of this protocol is to achieve first of all a phase of **inter-calibration**[1] of methods/tools/observers to better understand the variability related to these experimental parameters: inter-methods variability, inter-observers variability, inter-operator variability, intra-site variability…

b. *Comparison of metrics variability between a permanent transect and random transects*

The establishment of a continuous transect is not always possible: on one hand it is quite difficult to install (setting landmarks in the rock), on the other hand, it can be quickly either deteriorated (by divers, nets) or covered by sessile species. Finally, their installation may not be allowed (in marine protected area).

Moreover, when studying the temporal variability, it is difficult to sample exactly the same locations as part of a large-scale protocol (i.e. you cannot always set a remarkable point, and the implementation shoud be as light as possible eventually). Thus it's best to identify metrics whose robustness is not brought into play by the accuracy of the location of the transect. Random sampling is more accessible to a wide variety of observers. One of the preliminary objectives of this protocol will be to identify the robust metrics, to be measured during the random sampling in the coralligenous areas.

To do this, we will compare the values obtained during the implementation of the permanent sampling to the ones obtained on the random sampling. At the end of this protocol's implementation, it would be possible to do without permanent transects, that is to implement the whole photo-quadrat sampling randomly.

A study will be required to check if the robustness of the metrics measured in Marseilles' sites is equivalent in other localities.

c. *Contextualisation of the metrics measured during the implementation of random transects*

Some metrics can be very robust when all contexts are mixed up, but gain **relevance**[2] and **efficiency**[3] in a specific context. For example, cartography allows us to study separately data from the image processing depending on the profile.

This approach is then used to better understand the natural variability depending on the context (i.e. variability inter-sites).

---

[1] Consists to confront techniques making only one parameter vary (in the best case) to compare the magnitude of variations related to this parameter.

[2] Said of an indicator if it varies mainly depending on the parameter studied / monitored. An effective indicator is relevant if it shows something about the system that you need to know.

[3] An indicator is efficient if its value range corresponds to the observable part of the phenomenon studied, and if it varies homogeneously regardless of the magnitude of the phenomenon studied.

Identify typical profiles of a context and analyse their simultaneity is the phase of **contextualization**[1]. Metrics from the analysis of photo-quadrats may also be elements of context for the molecular approach.

d. *Choice of the studied species*

The list of "species" refers to taxa that are considered a priority in the analysis of photo-quadrats, and for which operators must have a minimum of knowledge. This list will be used to determine which are the common taxa between the eastern and western parts of the Mediterranean Sea. It will help to compare the profiles of the habitats on which they occur, and thus discover the parameters that seem to further their settlement. Moreover it will be a basis for methodological guides that will be developed for networks of participating science. Some taxa will be handled at a supra pecific level (such as genus, family).

Principles of methodology for species selection of the list:

- Rely on an official list,
- Select species that are recognizable while diving
- Select species which are not too sparse

The list chosen and validated during CIGESMED kickoff is the one defined by the RAC SPA (UNEP – MAP – RAC/SPA, 2009) which was completed by French ecologists. Greek and Turkish ecologists following the same logic should supplement it. Possible synonymy has been checked thanks to the websites Worms and Algaebase in early February 2014. In France, validation of names and brief description is made by available experts.

Sélection of taxa is done throught an allocation of three types of qualifiers for each species:

- Quantification of the abundance of the taxon for each ecoregion (3 in france, X in Turkey, Y in Greece) in the form of 3 letters (A: low, that is populations or individuals rare or isolated; B: medium, that is dispersed populations, C: abundant, that is abundant dense population). This typology is based on the typology of abundance of marine ZNIEFF (REFERENCE), used by Natura 2000 then (A VERIFIER), and is therefore included to facilitate comparisons with such inventories. Abundance is estimated thanks to the existing studies on marine protected areas, and thanks to an assessment on areas and transects studied within CIGESMED. The expert community will validate it during the second workshop.

- A figure corresponding to the identification is given by divers from each ecoregion (confusion about a taxon is possible in some regions but not in others. The expert community will validate it during the second workshop. (1: clear identification, no current confusion by divers; 2: identification requires training about possible

[1] Within CIGESMED, the contextualization corresponds to assigning a profile to a sample in order to study the dataset by focusing only on one type of profile.

confusions, but identification is possible when diving ; 3: identification impossible when diving, requires an expert analysis).

To be added: AN EXAMPLE FOR EACH CASE with pictures (dorian)

- A note of sensitivity ranging from 4 to 12, according to index SSIS (Sensitive Species/Indifferent Species) from the IndexCor indicator, is also assigned when existing. This metric takes in account a species list of different sensitivity to anthropogenic impacts (increase of organic matter, fine particles inputs, physical impacts) and to impacts of global warming. The note of sensitivity classifies species according to their level of sensitivity (indifferent, tolerant, sensitive).

A final validation work is expected to consolidate the qualification of species.

2. Method

This protocol, using transects, not only produce metrics to make ecological analysis (about structure and functioning of the habitat), but also makes possible to identify metrics for the contextualization of the sites for the other approaches.

At the end of the sampling phases, we will have photo-quadrats for each sites (on two sides), on two determined profiles, at two depths.

Photo-quadrats are digital photographs, taken while diving throught a quadra (frame), whose dimensions are known and whose surface is a quadrilateral. Dimensions may vary according to the operator. These will be qualified with the variables describing the conditions of the areas where the photographs are taken (profile). Photo-quadrats represent the core samples. They will be treated thanks to PhotoQuad software, and datasets produced will be analyzed statistically (univariate and multivariate statistics, data mining).

On Figure 18, we can see a diver maintaining a frame: the part of the photograph located within this framework is what we call the photo-quadrat.

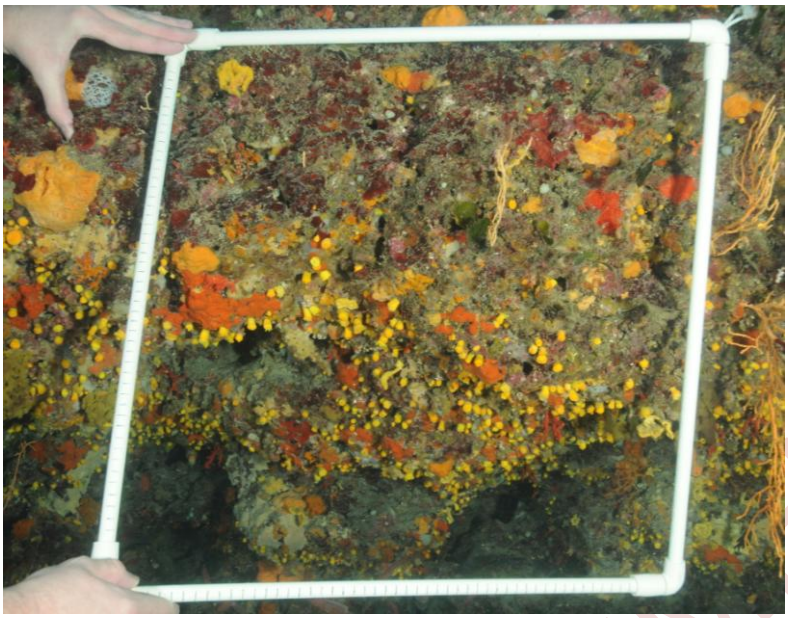


Figure 18: example of making a photo-quadrat

The drawing (Figure 19) illustrates the three sampling methods of photo-quadrats. The permanent transect method (1) consists in starting from a physical mark and take the photographs in a continuous way, moving the quadrat, at constant depth. We decided to make permanent transect 10 m long, which enables to make 20 photo-quadrats of 50 cm x 50 cm. The random sampling method (2) consists in conducting the photographing randomly at constant depth, focusing on some preset profiles.

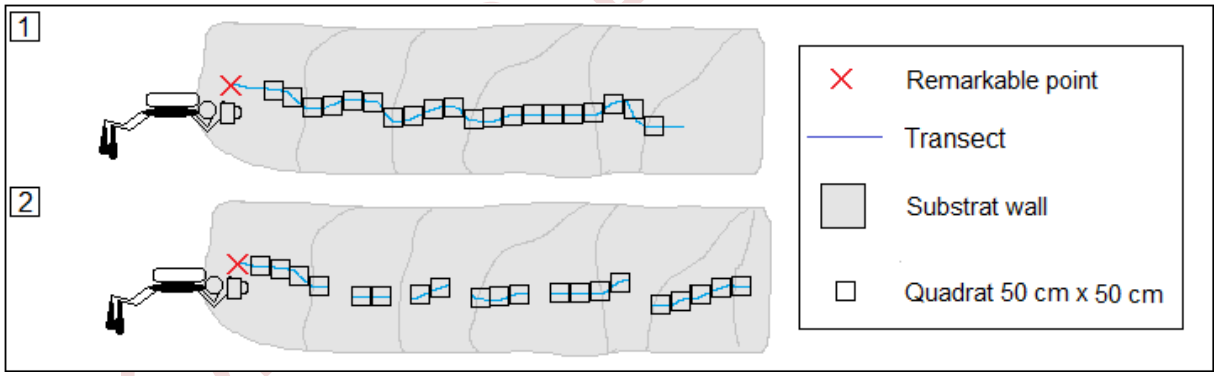


Figure 19: permanent and random transect

The movement of divers along transect is subjected to its buoyancy control: it is recommended to be careful to not generate a margin of error superior to 1 m compared to the target depth (Figure 20).

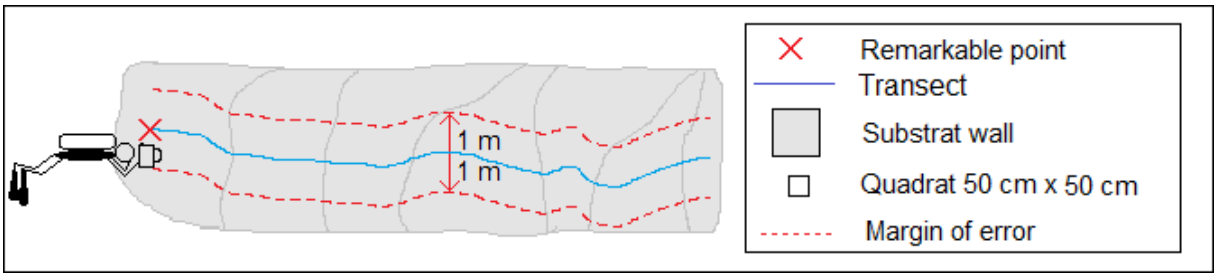


Figure 20: margin of error of a diver's trajectory

Another method will be tested: using **positioning frame**[1] for the quadrat to place it accurately from a fixed mark or a remarkable point (easy to find again) in the substrate. The positioning frame is a frame whose sides extend 15 cm at each corner to prop up 9 photo-quadrats accurately, respecting the horizontality (Figure 21). The way to ensure horizontality remains to find, but the use of a plumb-line could be fine. This system greatly reduces the variability related to the trajectory of the diver on a long transect.

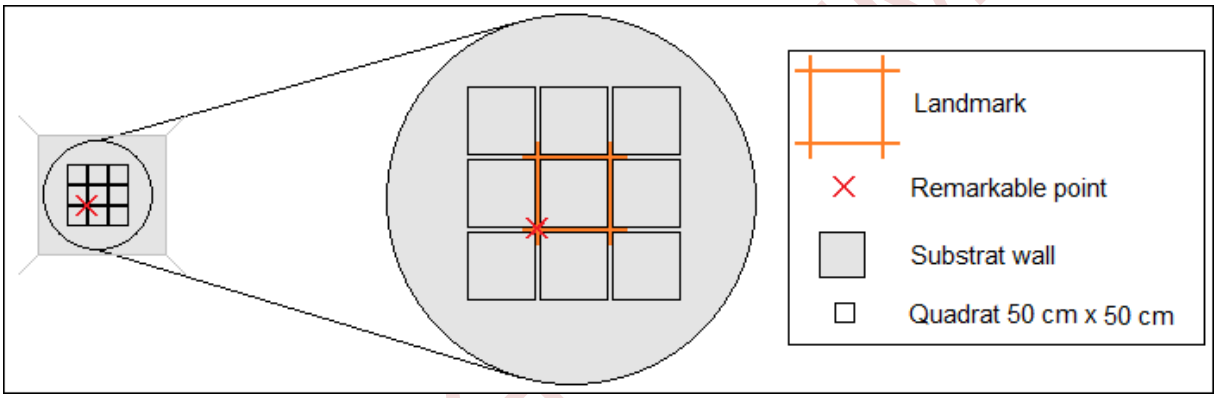


Figure 21: Photo-quadrats's sampling method with a positioning frame

a. *Inter-calibration on permanent transect*

Inter-calibration methods, tools, operatos, and observers, is done during a first sampling phase.

The objective is to separate natural variability from those due to the methods, tools, observers or operators. In this natural variability we look for metrics that can help to distinguish sites or conditions in a **relevant**[2] way.

To do so, we seek to identify, step by step, the different variability related to observers and operators, their skills, their equipment, techniques oand observations

[1] Within CIGESMED, it's a frame to position nine photo-quadrats from a fixed point, so that minimize the variability. The corners of the frame are actually crosses around which the quadrats prop up.
[2] Parameter whose evolution enables the evaluation of a situation. An indicator is reliable if it is relevant, effective, responsive and robust.

conditions. In order to perform this inter-calibration, **variability factors**[1] will be compared in turns, making only on factor vary at each comparison. Photographic samples (photo-quadrats) will be collected on **permanent transects**[2] (i.e. physically marked). For example, on a permanent transect we will keep the observer and the operator and make vary in turns: intensity of light (measured in lumen, but also in colour temperature), size and type of quadrat. The influence of each parameter will be demonstrated by the comparative study of metrics usable in each case.

A permanent transect should be easier to find and repeat because it's marked. We set up three permanent transects, 10 m long, in a study site, marked by threaded rods. On each transect, 20 quadrats of 50 cm x 50 cm (instead of 25 cm x 25 cm as Kipson *et al.*, 2011) adjacent, will be photographed. This outline is to adapt if other regions choose to experiment it. (Reminder: permanent transect must be located in one of the main sites. In Marseilles, the main site is "Frioul-Tiboulen du Frioul" (FTF)).

NB: The threaded rods marking transects deteriorate the substrate very locally only, and healing is very fast (about few months).

b. *Comparison of the variability of metrics values measured on a permanent transect and the ones obtained on a random transect*

Photographic samples (photo-quadrats) are this time collected on **random transect**[3] (i.e. not physically marked), at constant depth, on two distinct orientation, and two homogeneous profiles determined thanks to the cartography. In Marseilles region, the elected depths are 28 m ± 1 m and 45 m ± 1 m? For the others, the depths are to adapt according to the local ecology and the selected monitored profiles. The deeper depth could be adjusted according to the site's characteristics if needed (in case of a 40 m deep wall for example).

This means that to obtain a sufficient number of photographs for the statistical analysis, it will be necessary to take the photographs that constitute the random transect, outside the limits of the permanent transect (but at same depth). Photo-quadrats will be made on the two profiles previously determined, at same depth than the permanent transect. That is to say that the observer will place the quadrats randomly (but keeping the target depth) on the areas corresponding to the profiles targeted. As a precautionary measure, we realize more photographs than the number set (20 photo-quadrats by profile at least at each depth and on each site) in order to compensate for possible errors of profiles recognition. *NB: a randomization of the photo-quadrats collected on the permanent transect can also simulate the results of a random transect.*

[1] Factors related to measurement conditions, observer, operator or measured system, having an influence on the measurement accuracy.
[2] Within CIGESMED, said of a trajectory followed for taking pictures of quadrats, which is physically marked by a fixed threaded rod. These transects must be done at constant depth.
[3] Within CIGESMED, said of a trajectory followed for taking pictures of quadrats, which is not physically marked. These transects can therefore be done at constant depth.

This phase of contextualization should reinforce the hypothesis that the profiles help to identify robust metrics for the differentiation of two sites. The comparison between metrics of sites' characterization (depth, pressur, exposure…) and the ones related to the photo-quadrats content will help the identification of the conditions leading to the settlement of one species or population, and to better understand the coralligenous habitat at small-scale.

c. *Contextualization of the metrics measured during the implementation of tandom transects*

The first contextualization of the data from the photographs will be made using the profiles. Reminder: a profile consists of the following metrics: orientation, slope, rugosity, major covering stands, and remarkable elements such as exceptional size of individuals or stands. Cf part B. Cartography). Initially we will be limited to the identification of two profiles. These will be selected based on their recurrence, their orientation, and their geographic availability in CIGESMED studied area (France, Greece, Turkey), and their orientation. Thus preference will be given to one or two common profileswith sufficient possibility to sample *Myriapora truncata* and *Pseudolithophyllum cabiochiae*. These profiles will be studied about inter-specific diversity, intra-specific diversity, and population connectivity.

Additional contextualisation will be made based on elements determined thanks to the photographs (cavities, exposed areas, zone of sedimentation…) and/or from contextual elements of the acquisition (weather, depth, visibility, diver's comments). For this purpose, it would be interesting to acquire contextualization data on a large number of sites to better highlight ther inter-site variability.

The study of natural variability observed on the two profiles chosen will be even more accurate if the surveys of context explain this variability.

3. Sampling

a. *Operations*

Depending on the ressources available for each partners, one main site will be taken as model for the inter-calibration of methods or or observers on one hand, and as site of exercise at real depth on the other hand.

(Note: In Marseille, the main site is Frioul-Tiboulen du Frioul.)

Placing of the marks for the permanent transect

The mark should be fixed in the rock: a threaded rod is sinked, then fixed with resin in the wall. Three methods are applicable. Here they are presented from the lightest to the heaviest implementation work:

- 1st possible method: use an existing hole, clean encrusting species by scraping with a tool, then apply a resin in the hole and around the threaded rod, inserting the rod inside.

- 2nd possible method: use a chisel and a hammer to make the hole, then same process as in the 1st method.

- 3rd possible method: use a pneumatic immergeable drill (Rodcraft 4200 type) supplied with compressed air *via* a scuba tank and a drill bit. Using a hammer may also be necessary for the installation of the rod, which requires approximately 0.8 to 1 m^3 of air (a tank of 12 L at 200 bars contains 2,4 m^3). It is possible to use a standard pressure regulator to sypply the drill, but this requires the adjustement of the pressure to 6 bars.

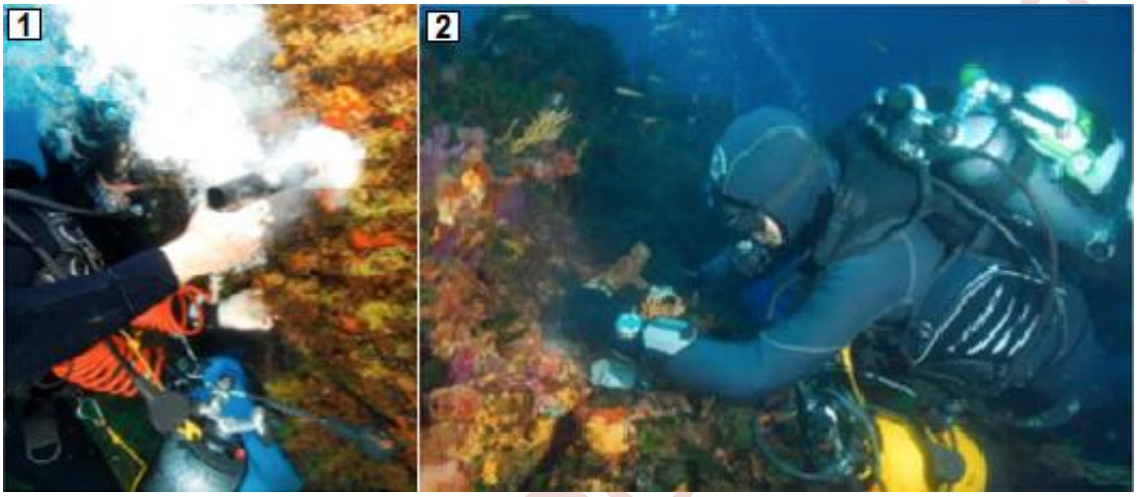


Figure 22: drilling of the wall with the Rodcraft drill gun (1) and use of a hammer (2)

On average, regardless of the method used, the placement of a rod takes about 10 min. Thus, in one dive it is possible to place 3 to 5 rods nearby.

Figure 23: examples of marked used as starting point for the permanent transects

A mark may be attached to the rod. It may be very noticeable, but be careful that it doesn't attract divers, like a golf ball may do. A label can be used as a mark, but it should be cleaned regularly to prevent its covering up.

Note: the resin needs several hours to solidify (the warmer is the temperature, the quicker it solidifies).

Photo-quadrats achievement: generalities

Photo-quadrats achievement can be carried out either by a single diver equipped with a camera mounted on the quadrat, or by two divers: one maintaining a basic frame and the other taking pictures through. Tha camera can be of high quality, or a simple GoPro: the two devices will be compared in order to choose the one that offers the best compromise between performance and constraints. Note that positioning the frame in the middle of erected species or crevices may be complicated and give photographs hard to operate. Besides, divers should always be careful to respect the depth targeted while moving along the rock wall. The team of the marine station of Endoume (Marseilles) will mainly use quadrats of 50 cm x 50 cm, but will also test quadrats of 25 cm x 25 cm, and possibly those used within the IndexCor program (40 cm x 60 cm) which should come up to the same results than the quadrats 50 cm x 50 cm.

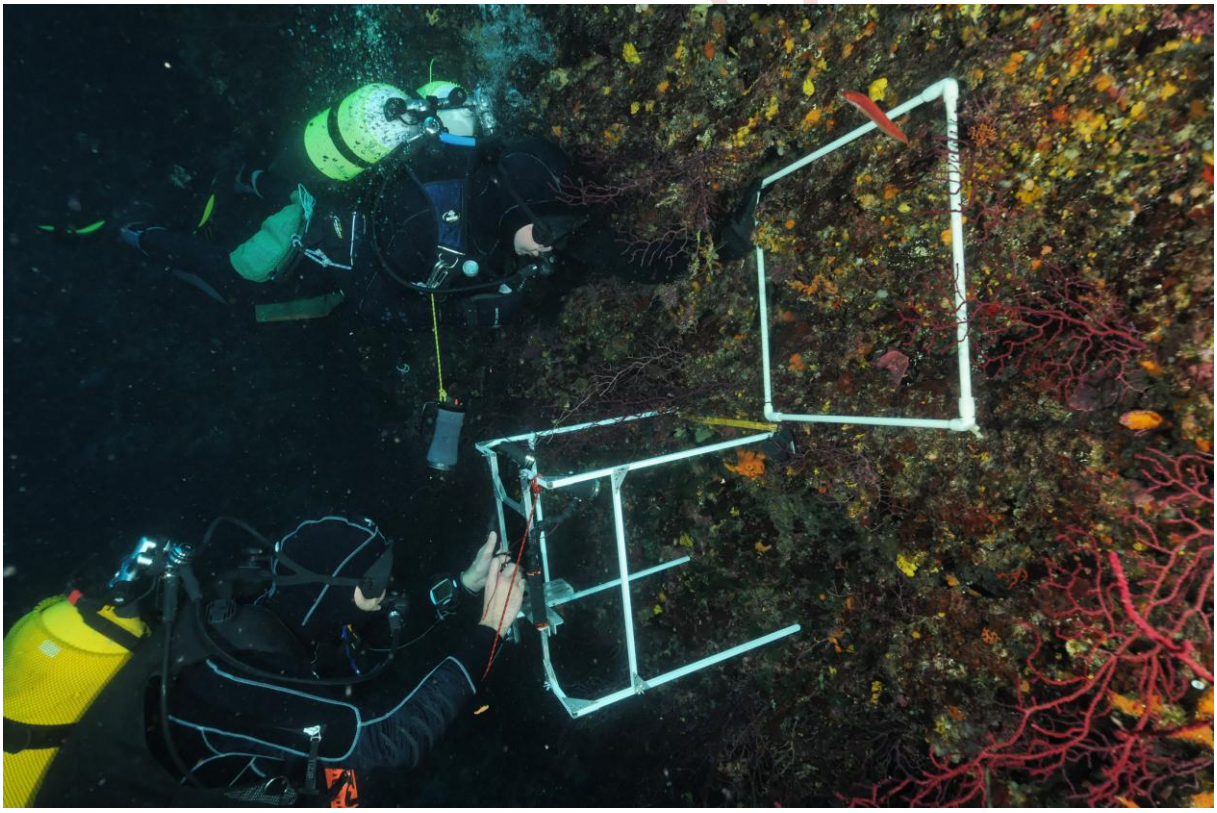


Figure 24: Illustration of the two types of quadrat used and a photograph diver making a photo-quadrat

Photo-quadrats achievement on permanent transect for the inter-calibration phase

The diver who maintains the quadrats moves along the permanent transect starting from the mark, while unrolling the decametre and monitoring its depth, in order to achieve 20 photo-quadrats on a 10 m long transect. The other diver takes a picture at each position of the quadrat.

Sampling of photo-quadrats on random transect for the phase of comparison of the variability of metrics values between permanent transect and random transect

This sampling is done on the same site, on a comparable place to the one of the permanent transect (avoid taking two locations that are obviously very different). It will be necessary to dive again there to achieve the photo-quadrats randomly. An additional dataset can be provide later on, using the photo-quadrats achieved on the permanent transect and select some of them randomly.

Random sampling of photo-quadrats on a determined profile for the phase of data contextualization

Divers look for the two determined profiles, and focus on the photography on these profiles.

Slope and rugosity have a significant part (light) in the presence of species. Sampling should be done on random transect selected for their homogeneity of slope and rugosity: one transect on a vertical zone, one transect on an horizontal zone, and one transect on a zone under overhang. Since all combinations can't be tested, only the 2 or 3 most frequent combinations in each region will be.

b. *Data conditioning*

Nomenclature and photos archiving

Each photograph (photo-quadrat) should be labeled according to a precise nomenclature defined below:

[program]_[site]_[date] _**D**[depth]_**T**[n°transect]_**Q**[n°quadrat] _[doer]

Example : CIGESMED_CAS _20140123_ D1_T02_Q08_AA01

Photo's labels must be written on the notepad used during the dive for the complementary observations by the diver. Each notepad must be archived in paper mode (photocopy) and digital mode (scan) and respect the same nomenclature typology as the photographs.

All these data must be entered on computer respecting the nomenclature typology. Then they should be sent to the program manager or the database manager (romain.david@imbe.fr in France).

c. *Material list*

Marking the permanent transect requires the following equipment:

- For driling:
  - Scraper
  - And/or chisel = hammer
  - or drill « Rodcraft 4200 » type + its tank of compressed air + drill bit
- Threaded rods
- Two-component polymerizing resin

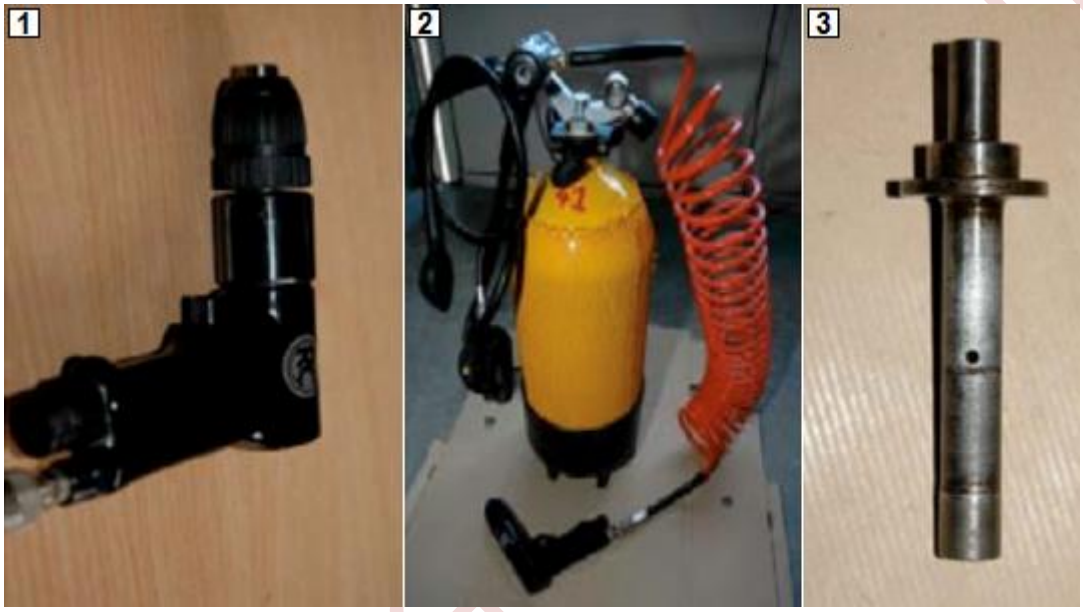


Figure 25: Rodcraft drill (1), drill connected to a tank of compressed air (2), diamonded drill bit (3)

Sampling will require:

- Quadrat of 50 cm x 50 cm (Marseilles region) or other
- Diving camera
- Diving torch of sufficient quality to light up the area photographed and make colours visible and species identification possible
- GPS on the boat to locate the starting point

Image processing requires:

- PhotoQuad software (open source)

Photos of the equipment to add

d. *Work time quantification*

Current tests of implementation show that it's possible to achieve in a single dive (15 min): two sampling transects of 10 m each, or quadrats photographed.

4. Delivrables

a. *Expected results*

Marseilles:

The three sampling methods will be tested (random sampling, permanent sampling, and sampling using a positioning frame). This implies the following number of photo-quadrats expected:

1) Inter-calibration phase:
   1 transect on the main site x 3 sampling methods (permanent / random / random positioning frame) x 3 replicates x 20 photos = 180 photo-quadrats

2) Contextualization phase:
   2 transects x 2 methods (random / random positioning frame) x 3 replicates = 240 photo-quadrats.

180 photo-quadrats (1) + 240 photo-quadrats (2) = 420 photo-quadrats

Therefore, in Marseilles, 420 photo-quadrats are expected for the whole 3 sites. Each additional sampled site will add 3 transects of at least 9 photos each made by the positioning-frame method. That is 27 photo-quadrats per additional site.

Each photograph must comply with the established nomenclature's typology (cf. part III.3.b).

Other regions (in France or foreign):

Only the contextualization phase will be processed, which implies:

2 transects x 2 methods (random sampling / random sampling with a positioning frame) x 3 replicates x 20 photos 240 photo-quadrats.

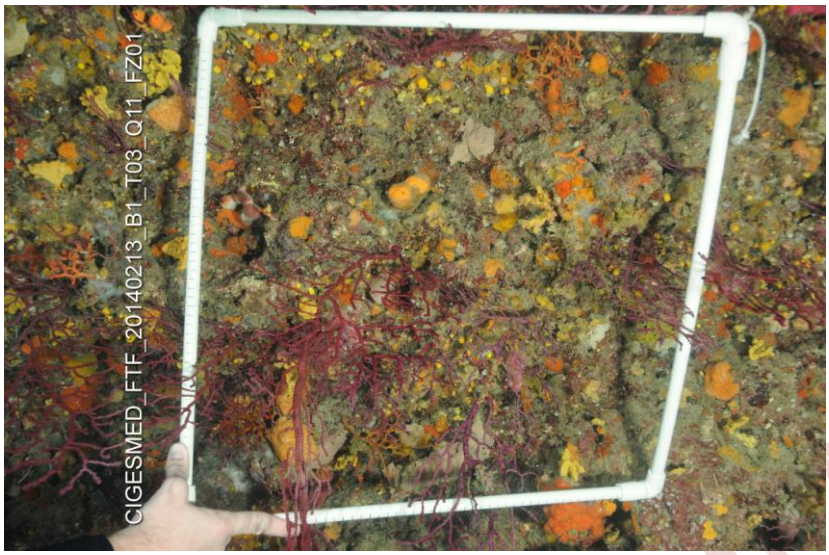


Figure 26: Example of photo-quadrat labelled with the correct shared nomenclature

b. *Data analyses envisaged*

Image processsign with the software PhotoQuad

Image processing will be carried out through the software PhotoQuad. (http://www.mar.aegean.gr/sonarlab/photoquad/index.php). This software of 2D image analysis is equipped with tools of spatial analysis (such as cover measuring), and counts of species.

The identification of species observed on the photograph, will be done first manually. For this, it's possible to start by applying a grid on the photo. In Marseilles, we think of using a grid of 20 cells x 20 celles on the photo-quadrat (which is 50 cm x 50 cm), so 400 cells of 2.5 cm x 2.5 cm. The resolution depends on the **efficiency optimum**[1] of the observers. Different resolution will be tested. The analysis will focus on the following metrics: count of taxon, percentage of surface covered by each taxon, size of individuals or surface covered by one taxon, boundaries between species covering, and spatial organization of the communities according to the substrate type.

[1] Refers to an observation device when its efficiency is optimal, in other words when the ratio between its cost and its effective appropriation from a wide range of observer is maximal.

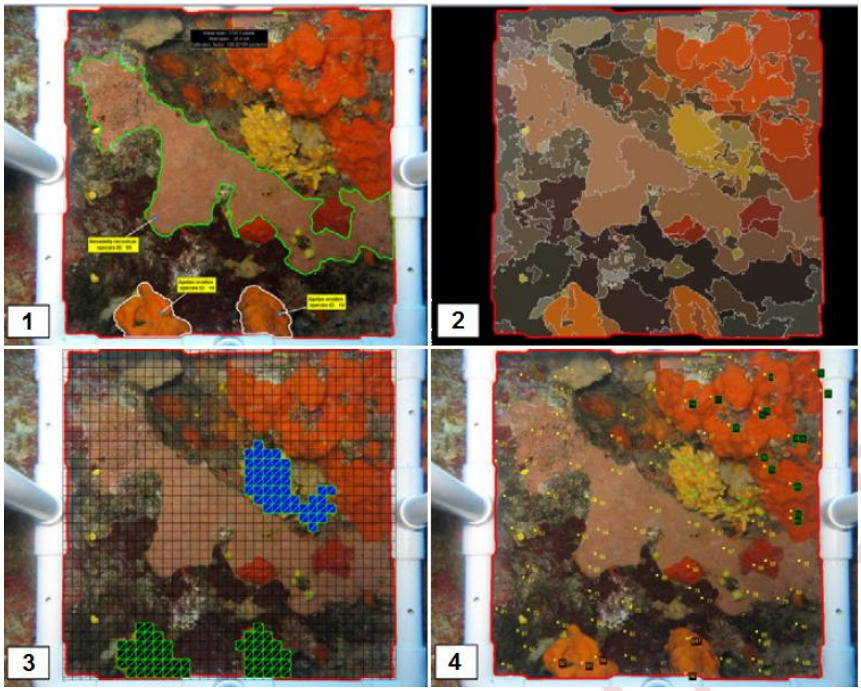


Figure 27: example of image processing with PhotoQuad (Trygonis & Sini, 2012).
1: Species markers, freehand drawn regions and calibration marks. 2: Image map and region boundaries. 3: Grid cell counts. 4: Stratified random point counts.

There will be an analysis of the major species by cell. The determination of the celles size is important sincethe significance of the results depends on it. But cells size may be adapted according to the analysis done and the operator's available time. Different cells forms will be tested also (e.g.: hexagonal versus square). The cells should be preferable to the points because they are more representative of the surfaces. Cells must be small enough to be representative of the species cover according to their size and forms. Moreover cells mustn't be too small or the time of analysis will be too long.

When processing photographs, non-exploitable zones of the pictures should not be taken in account for the analysis of metrics: such as dark corners, unidentifiable species…

Photographs should be of sufficient quality to enable the identification of species: this level of quality is to determine during the beginning of the protocole implementation. It should represents the best quality/cost ratio (in terms of resolution, brightness, colour, …)

Statistical analysis

A statistical analysis will be performed using the R software particularly. We will focus on taxa's presence/absence, percentage of taxa's covers, taxa assemblages, and relationship between taxa's abundances. These metrics will be confront with environmental data.

5. In brief

CHARACTERIZATION BY PHOTO-QUADRATS

Method

- Inter-calibration on permanent transect
  - ✓ 3 transects of 10 m long
  - ✓ Materialised by threaded rods fixed on the substrate
  - ✓ 20 photo-quadrats of 50 cm x 50 cm per transect

- Comparison of results obtained with permanent transect and with random transect
  - ✓ 20 photo-quadrats randomly sampled
  - ✓ at same depth than the permanent transect compared

- Contextualization of the metrics measured during the implementation of random transects
  - ✓ Between two selected type of profiles

Expected results

- 1) Inter-calibration phase:
  1 transect on the main site x 3 sampling methods (permanent / random / random positioning frame) x 3 replicates x 20 photos = 180 photo-quadrats

  2) Contextualization phase:
  2 transects x 2 methods (random / random positioning frame) x 3 replicates = 240 photo-quadrats.

  => 180 photo-quadrats (1) + 240 photo-quadrats (2) = 420 photo-quadrats

- Typology of the labels put on the photo-quadrats:
  [program]_[site]_[date] _**D**[depth]_**T**[n°transect]_**Q**[n°quadrat] _[observer]

Questionnaire on module 2

« Characterization by photo-quadrats»

1) Your opinion on this protocol proposal « Characterization by photo-quadrats » :

a) The methods proposed in this module « Characterization by photo-quadrats » : do they seem feasible on your CIGESMED's site ? Why

b) Do you have at your disposal all the skills and means necessary to implement the protocol ? Detail the skills and means lacking.

c) What skills/means/work can you offer to any partner in order to complete the sampling ?

d) What changes can you propose to make this protocol operational on your CIGESMED's sites? (Taking care to maintain consistency with the other observers of the network)

e) What improvements would like to make on the descritpion of the protocol « Characterization by photo-quadrats » ?

f) What improvements would you like to make on the methods of the protocol « Characterization by photo-quadrats » ?

2) Your opinion on the feasibility of this protocol Characterization by photo-quadrats » :

a) Are you familiar with this type of research?

b) On what occasions do you apply this type of protocol ?

c) What are your difficulties of implementation ? which steps are not clearly described in the document ?

d) Do you have any alternative to proprose ?

3) Your investment in CIGESMED:

a) Are you ready to commit yourself to participate to the sampling of « Characterization by photo-quadrats »?

b) If you do not intend to apply this protocol « Characterization by photo-quadrats », please explain why.

c) Are you ready to commit yourself to improve and document the methods proposed by this protocole « Characterization by photo-quadrats »?

d) Are you interested in a meaningful participation in the writting of scientific paper describing the results obtained from this protocol « Characterization by photo-quadrats » ?

e) For this type of research are you ready to commit to participate as long-term observer applying this protocol « Characterization by photo-quadrats »?

If you use another protocol, please communicate your detailed protocol to romain.david@imbe.fr

MODULE 3

PROTOCOL OF SAMPLE COLLECTION FOR METABARCODING

D. Module 3 : Protocol « sample collection for metabarcoding x 4 »

1. Principle

The study of interspecific biodiversity (allowing inference of classical indices of biodiversity: species biodiversity, phylogenetic diversity, etc.) will allow characterizing the community composition variability for each site, for each ecological profile, but also will allow establishing a comparison of specific diversity per phylum, among sites. Owing to the recent and impressive technological progress of genomics, the meta-barcoding approach, once calibrated with traditional taxonomy, could replace this traditional approach advantageously, being more cost-effective and time-effective, and thus allow temporal monitoring to be done.

2. Method

Collection season: Scrapings must be carried out at well marked seasons : in France we chose february, then the end of the summer (august-september) to avoid the high variability during recrutement seasons (example shift this year in 2013 in France where spring was very clement).

Four areas of scraping are determined on the adequate profiles, at depth 30 or 45 m, as explained below.

To determine the 4 quadrats to scrape, we follow these steps (Figure 28):

- (1) Choose an arbitrary point in the middle of a profile (choose a place sufficiently homogeneous without avoiding large erect species).
- (2) Place the extremity of a PVC tube on this point, scrape the other extremity on a 10cm x 10cm area (for vertical or overhang areas, a second diver may be needed to hold the tube while scraping is done).
- (3) Move the tube of a 90° angle, one end remaining at the central point, and scrape the new surface at the new extremity. Two divers can scrape simultaneously. Repeat this step until four places are scraped forming a square) (photo scraping quadrat)
- (4) In case the area is not « scrapable », shift following the tube's direction, externally. Be careful not to avoid large species! When large individuals are encountered, do not collect the whole specimen but a piece of a few cms (note these cases on report ?). However, it is necessary to collect all **epibionts**[1].

[1] Non parasite organisms using external animal surfaces larger than themselves as "substrate".

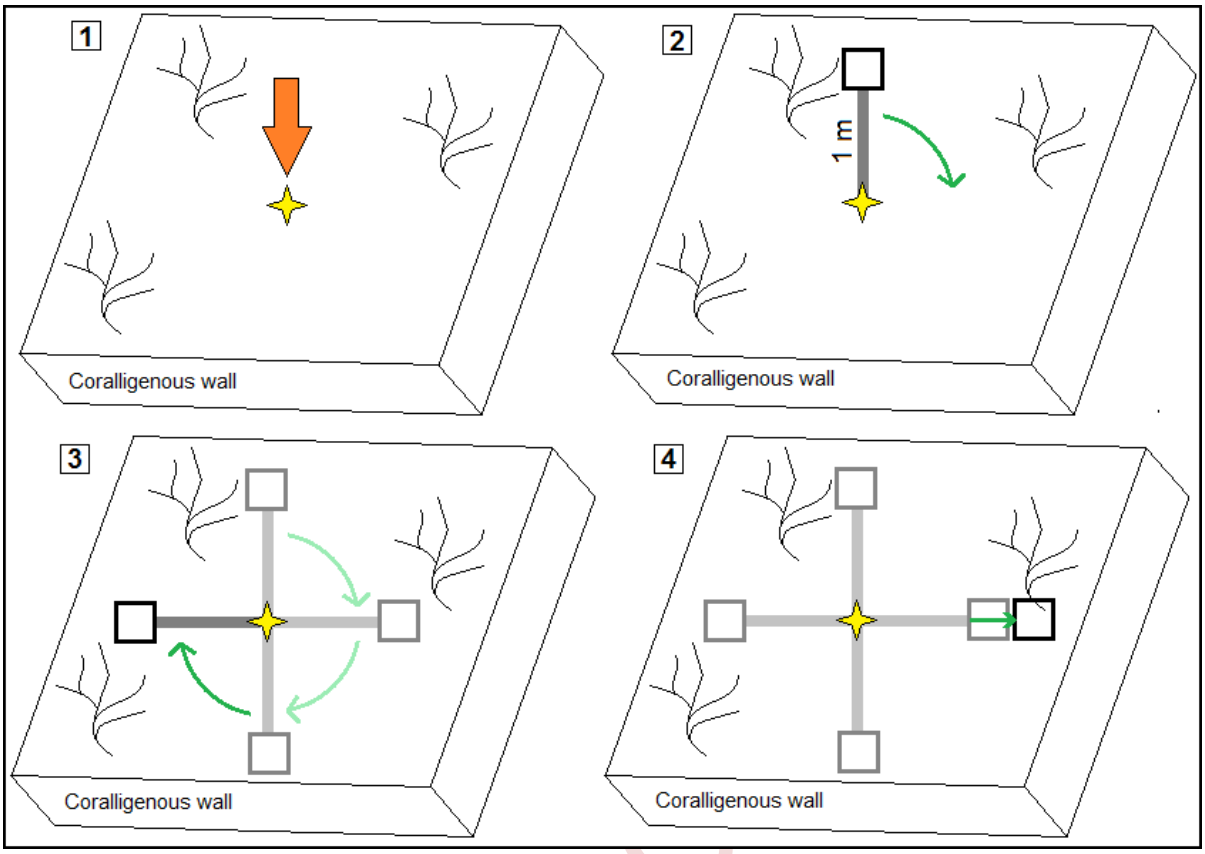


Figure 28 : methodology of localization of the samples scraped

Thus 4 scrapings are done and replicates ca be named « top », “bottom”, “right” and “left”.

3. Sampling

a. *Operations*

The obtention of authorizations by the Park (des Calanques) is ongoing. The document for such requests is in annex, but it should be adapted according to each country/MPA’s rules.

The number of replicates is a compromise between statistic robustness and environment preservation. A maximum of caution will be used to avoid habitat perturbation (Figure 26).

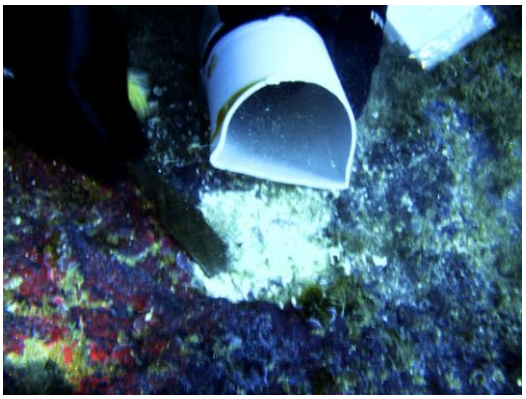


Figure 29 : raclage d’une paroi de coralligène

b. *Sample conditioning*

Each country must calibrate metabarcoding with traditional taxonomy (faunistic/algal) on a site at least. This consists in a visual analysis, just after the dive, by persons who know the algae, the fauna, of the content of a scraping of each profile and each orientation, if possible, each depth… All other scrapings will be exploited by meta-barcoding but the

content of scrapings must be photographed in each case (once spread in a large white basin/tray). Scrapings will be conserved in 95° ethanol.

Back to the lab:

1) The content of each scraping is spread in a white tray with sea water, in a fresh and well lightened place, to be rapidly sorted until conditioned in ethanol 95°. Label using the scraping code ‘see below).

2) Take a picture of tray content, zooming when appropriate, allowing (potentially) a posteriori identification. Samples and photographs must be labelled as follows:

[program]_[site]_[date] _**D**[depth]_[profil]_[position] _[doer]

Exemple: CIGESMED_CAS_20140121 _D1_V_TOP_AA01

3) Try to determine the number of visible species per phylum (though rapidly, since each dive provides usually 16-32 units to process).

4) Disentangle (separate as much as possible) the biocontructed coralligenous, brush with a hard toothbrush in the tray seawater.

5) Proceed to fractioning using the sieves (3 fractions as in the DEVOTES WP5 protocol). Wear gloves:
   a. Fraction > 2mm
   b.
   c. Inferior fraction (inferior to 0.5 mm): collect with an ethanol dropping bottle what remained on the sieve

c. *Material list*

Field material:

- PVC cross
- 10cm x 10 cm quadrats
- Scraping tool (photo d’un couteau menuisier)
- System to collect the scrape material
- Pre-labelled plastic bags

Conditioning/packaging material:

- White trays (size > A4)
- Sieves (precise the mesh size)
- Dropping bottles
- Ethanol 95%
- Falcon tubes or ependorff 5 ml-50 ml (to precise)

d. *Work time quantification*

Positioning takes about 2-3 min, then each scraping takes about 1-2 min. Finding of a new location for the central point, corresponding to the requested ecological profile takes, again, about 1-2 min (it is important to take the time to find an ecologically homogeneous profile). Each diver can thus obtain about 8 scrapings (2 quadriplicates) in a dive (maximum).

Amount of diving-work per site: 2 profiles X 2 orientations X 2 depths x 4 replicates = 32 scrapings, ideally for 2 seasons. Thus 2 4-8 dives-man per site (3 sites minimum, as in Marseilles, up to 8, ideally) thus x-y dives man.

The time requested to analyze the samples (with molecular tools) depends on the number of phyla or classes… (i.e. nb of primer pairs) to be amplified by PCR.

4. Delivrables

a. *Expected results*

32 samples will be scraped per site (2 profiles x 2 orientations x 2 depths x 4 replicates) (assuming a single season will be sampled). At least 3 sites will be studied, i.e. 96 samples.

b. *Envisaged analyses of data*

It is possible in NGS platforms to label 184 samples with distinct tags, thus we will be able to analyze in a single run 184 scrapings. For each we will obtain hundreds of thousands of DNA sequences to link to species identity using databases and sequences from reference species obtained by ourselves. For some taxa, more than one molecular marker will be used (in 95% of the cases we will use the COI mitochondrial gene).

Those phyla could be studied:

- Fish: The detection of eggs or juveniles of a species would reveal a role of the coralligenous habitat as a nursery (contributing to protection, eventually also feeding), potentially generator of a **spill-over**[1] effect.
- The vagile macrofauna which adults have reduced mobility : echinderms, bivalves (excluding lithophagous ones), nudibranchs, gastropods, crustaceans (we may call them **résident**[2] species, although this may need a precise definition).
- Opportunistic or transient macrofauna : we may find species absent in the coralligenous when adults, but which have a young stage in this habitat.
- Bioncontructors : corallinales (*Lithophyllum*, *Mesophyllum*, some Peyssonnelliaceae), bryozoans, madreporans, gorgonians, tube worms (*Bispira* sp, *Sabella* sp, *Protula* sp, *Serpula* sp.).

[1] Said of a phenomenon observed in the marine reserves, a transfer of adult and juvenile biomass to outlying areas.
[2] All sessile species from an habitat and motile species found mainly in the designated habitat.

- Other non seasonal species (to define): green algae (*Codium* sp, *Flabellia*, *Halimeda* + *Palmophylum*), brown algae (*Halopteris*) in particular), worms (*Bonelllia* sp, polychaetes annelids), cliones, erected sponges (*Acanthella* sp, *Axinella* sp) and ascidians (aplidiums, clavelines, *Halocynthia*), other cnidarians (*Parazoanthus* sp, *Leptosamia* sp.).

5. In brief

Protocol « collect of samples for metabarcoding x 4 »

Method

32 scraping: 4 replicates

- ✓ on 2 profiles
- ✓ on 2 orientation of the site
- ✓ at 2 depths: 28 m and 43 m

Using this method:

- ✓ quadrats of 10 cm x 10 cm
- ✓ on 4 zones each at 1 m from a central point in a zone of a given relatively homogeneous facies.
- ✓ materialized by a 1m long tube moved to determine each scraping quadrat.

Expected results

- ✓ 96 scraping samples (minimum) + 32 per additional site
- ✓ Typology of labelling of photo-quadrats:
  [program]_[site]_[date] _**D**[depth]_[profile]_[position]_[doer]

Questionnaire on module 3

« Collect of samples for metabarcoding »

1) Your opinion on this protocol proposal « Sampling protocol for population genetics»» :

a) The methods proposed in this module « Sampling protocol for population genetics » : do they seem feasible on your CIGESMED's site ? Why

b) Do you have at your disposal all the skills and means necessary to implement the protocol ? Detail the skills and means lacking.

c) What skills/means/work can you offer to any partner in order to complete the sampling ?

d) What changes can you propose to make this protocol operational on your CIGESMED's sites? (Taking care to maintain consistency with the other observers of the network)

e) What improvements would like to make on the descritpion of the protocol « Sampling protocol for population genetics » ?

f) What improvements would you like to make on the methods of the protocol « Sampling protocol for population genetics » ?

2) Your opinion on the feasibility of this protocol Sampling protocol for population genetics » :

a) Are you familiar with this type of research?

e) On what occasions do you apply this type of protocol ?

f) What are your difficulties of implementation ? which steps are not clearly described in the document ?

g) Do you have any alternative to proprose ?

3) Your investment in CIGESMED:

a) Are you ready to commit yourself to participate to the sampling of « Sampling protocol for population genetics »?

b) If you do not intend to apply this protocol « titre du protocole »**, please explain why.**

c) Are you ready to commit yourself to improve and document the methods proposed by this protocole « Sampling protocol for population genetics »?

d) Are you interested in a meaningful participation in the writting of scientific paper describing the results obtained from this protocol « Sampling protocol for population genetics » ?

e) For this type of research are you ready to commit to participate as long-term observer applying this protocol « Sampling protocol for population genetics »?

If you use another protocol, please communicate your detailed protocol to romain.david@imbe.fr

MODULE 4

SAMPLING PROTOCOL FOR POPULATION GENETICS

E. Module 4 : Sampling protocol for population genetics (32 individuals per species)

1. Principle and presentation of the studied species

The aim of this study is to understand the population structure of the coralligenous species by studying the intraspecific diversity of the populations and their connectivity.

The principle of the study of their connectivity within the Mediterranean Sea is based on the analysis of the dissimilarity of genetic markers. At least thirty specimens (for each studied species) are collected from different sites. They are compared with each other to reveal the population structure within each site, and also to unveil if a **contextualization factor**[1] would permit a partial explication of this structure. The sampling is equally performed on different ecological profiles of each site in order to know the structure of the population according to the profile and thus the species ecology.

a. *Species selection*

The studied species are found throughout the Mediterrannean Sea, which implies the possibility to discover cryptic species. For this reason, the barcoding method of Cytochrome c oxidase subunit I or COI (or eventually other alternative or complementary markers) would permit to detect the possible presence of cryptic species within the studied ones.

The choosen species in this study are the erect and tree-like bryozoan Myriapora truncata, and one species of bioconstructing corallinale algae Lithophyllum stictaeforme/cabiochiae, both of which are identifiable in situ. They were selected as they are have a widespread occurrence in the coralligenous, on all the facies and at all depths (even at very low luminosity).

b. *Species presentation*

Myriapora truncata

Figure 30 : photo de *Myriapora truncata* (Pallas, 1766)

Myriapora truncata (Pallas, 1766) (Figure 27) is a common bryozoan of the coralligenous throughout its distribution area (the Mediterranean Sea and a part of Eastern Atlantic). It's a species which was impacted by the warming of 1999, 2003 and 2006 in North-Western Mediterranean and it was studied to investigate the effects of global warming and ocean acidification (Lombardi *et al.*, 2011; Rodolpho-Metalpa *et al.*, 2010). Despite its

reproduction pattern (a **lecitotrophic**[1] and brooded larvae with low dispersion), it is widespread on mediterranean areas with low luminosity. We can ask ourselves wether this happens to be only one species in different basins, or there are cryptic species, even possibly in **sympatric**[2], as other bryozoans. Therefore it is interesting to verify with genetic markers the possibly presence of cryptic species or local adaptation depending on geography (basins, regions), ecology (comparing the different "profiles" *sensu* CIGESMED of a same locality) and the population structure (quantification of gene flux, construction of matrices of connectivity).

Description of *Myriapora truncata*

Myriapora truncata is an erect and tree-like species and is bright orange. Its branches are round, its extremities are truncated and the ramification is dichotomic (i.e. one branch give two branches which have the same thickness as the original branch). The zooidal lophophore is pale orange.

Possible confusions:

- *Corallium rubrum* : red with white polypes
- *Adeonella calveti* : round and flattered branches

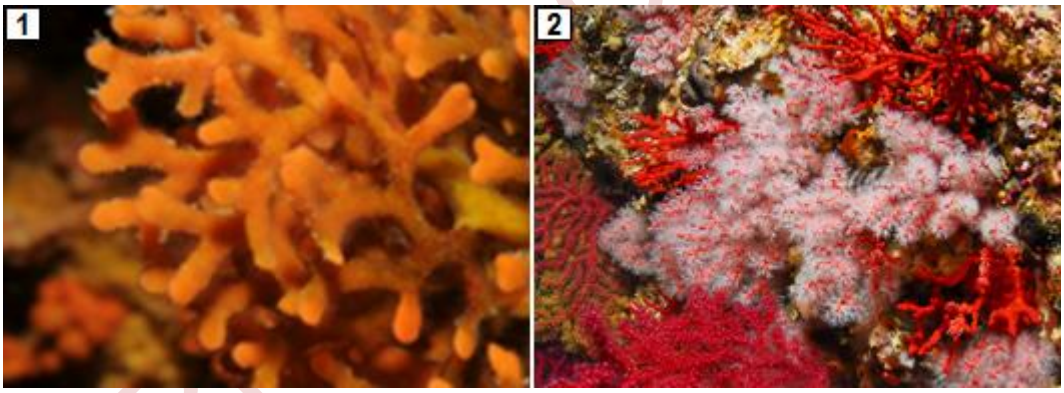


Figure 31: *Adeonella calveti* (1) and *Corallium rubrum* (2)

Lithophyllum stictaeforme/cabiochiae

Red calcareous algae of the order Corallinales are the main coralligenous builders. (Laborel, 1961; Laubier, 1966; Sartoretto, 1996; Ballesteros, 2006). In the frame of CIGESMED project the species complex of Lithophyllum stictaeforme/cabiochae (Areschoug) Hauck / (Boudouresque & Verlaque) Athanasiadis are chosen for the study of their genetic and specific diversity, and also to define the populations' structure and connectivity among the different study areas.

[1] Se dit d'une larve qui ne s'alimente pas directement et qui vit sur ses réserves
[2] Désigne la coexistence de deux espèces phylogénétiquement proches, sur un même territoire, mais ne s'hybridant pas.

These species along with others like *Neogoniolithon mamillosum* (Hauck) Setchell & L.R.Mason*, Sporolithon ptychoides Heydrich, Lithothamnion* spp. And various Peyssonneliales (M*etapeyssonnelia feldmannii*, *Peyssonnelia* spp*., Polystrata* spp*.),* are the encrasting red algae which contributes the most to the bioconstruction (Feldmann, 1937; trentaineHong, 1982; Sartoretto,1996; Athanasiadis,1999; Ballesteros, 2006).

c. *Sampling method*

For this approach, we compare the frequencies of different genetics variants, so it is important to have enough speciments in order to be able to conclude on the possible presence of significant differences of the genetic frequencies. Every locality's sampling needs about thrity individuals (Porter, Ryland, & Carvalho, 2002; Schwaninger, 1999).

The sampling should be on two sides of the study site, if possible opposites to another and at depth of 28 ± 1 m, in a way that the collected samples would come from all possible orientations. In each side, we choose two different profiles (in terms of the inclination and the rugosity of the sustrate) and the majority of segments should be where the studied species are found. These profiles would have been firstly determined by the results of the cartography.

Each colony of *Myriapora truncata* (Pallas, 1766) consists on the same genetic individu (the colony is formed from one cell) . The collected colonies must be separated by about 1 m of each other to not collected the same one several times. They must tie in an ecologic profile well defined and listed at the time of collection. Therefore the numerous of the segment in which the sample is collected has to be in the name of each individual (Figure 32).

Ideally, a minimu of 8 samples of each species should be collected in every one of the two pre-selected profiles (for two well distingued orientations and at two depths), in a way to provide replicas for the analysis. For this reason, the sampling would be in the 5 m segments (predefined by the cartography for the principal studied sites of Marseilles region, or chosen in relavence to their conformity for the secondary sites or for the localities where it wasn't priory possible to perform the cartography). In every segment where the sampling is possible, a fragment of each species should be collected at a minimum distance of 1 m so that a good repartition of the samples would be achieved

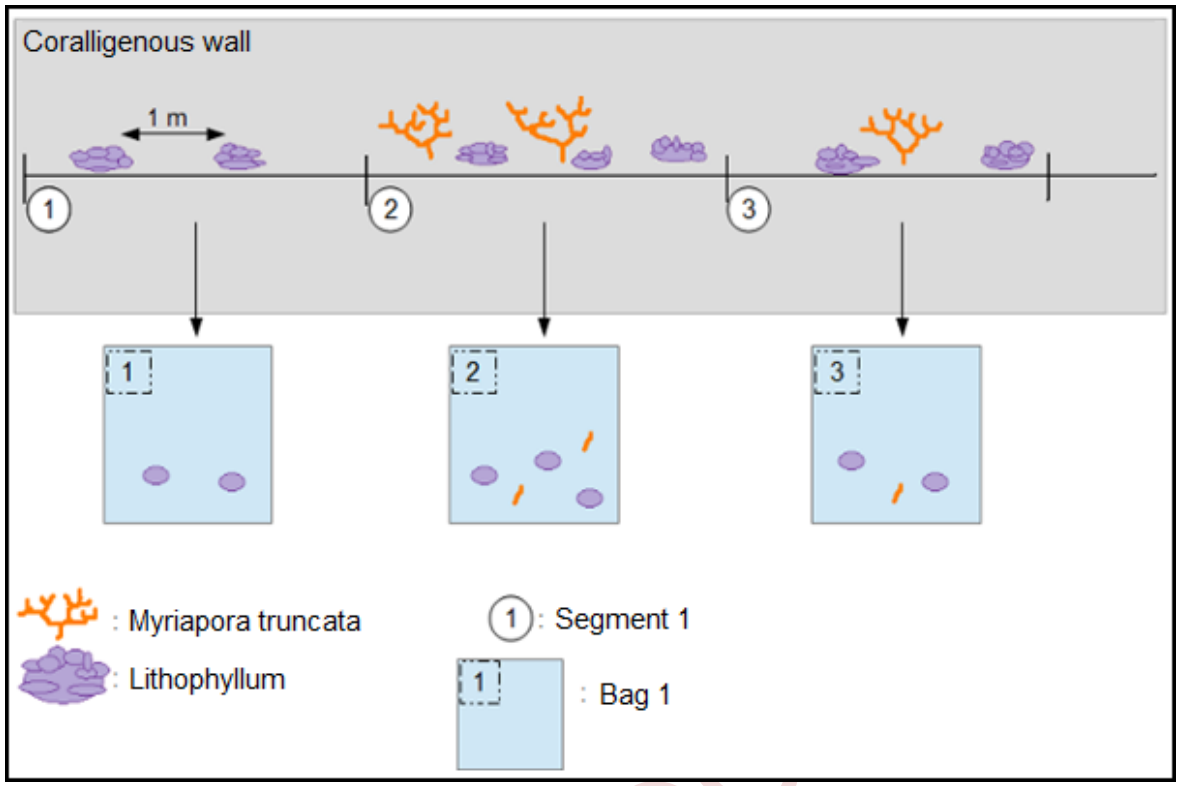


Figure 32: sampling method for *M. truncata* and *L.cabiochiae*

Ideally, samples of the studied species should also be collected at more or less bigger distances: at least two other regions in order to be able to attempt a correlation in the genetic and the spatial distance. This would permit to deduce a « distance of dispersion within the generations »:

- From the Western Mediterranean Sea, with example some study sites between Nice and Montpellier, but should also make an object of prospecting at out neighbors Italians and Spanish.
- From the Eastern basin, which would be possible with the collaboration of our CIGESMED partners from Greece and Turkey.

d. *Methodology of identification of Lithophyllum stictaeforme/cabiochiae*

The taxonomy of this group of algae is very difficult to determine and the valid scientific name of the species is constantly changing (

Table 4).

Table 4: Current scientific naming of the main bioconstructing species: *Lithophyllum* and *Mesophyllum* of coralligenous (http://www.algaebase.org - january 2014)

Valid name	Synonyms
Genus *LITHOPHYLLUM*	

Lithophyllum cabiochiae (Boudouresque & Verlaque) Athanasiadis	*Pseudolithophyllum cabiochiae* Boudouresque & Verlaque 1978 *Lithophyllum frondosum* f. *cabiochiae* (Boudouresque & Verlaque) Babbini & Bressan 1997
Lithophyllum stictaeforme (Areschoug) Hauck	• *Lithophyllum expansum f. agariciforme* Hauck • *Lithophyllum expansum f. stictaeformis* (Areschoug) Foslie • *Lithophyllum frondosum* (Dufour) G.Furnari, Cormaci & Alongi • *Lithophyllum grandiusculum* (Montagne) Woelkerling, Penrose & Y.M.Chamberlain • *Melobesia frondosa* Dufour • *Melobesia stictaeformis* Areschoug • *Pseudolithophyllum expansum f. decumbens* Foslie • *Pseudolithophyllum expansum f. strictaeforme* Philippi
Genus *MESOPHYLLUM*	
Mesophyllum alternans (Foslie) Cabioch & M.L.Mendoza	*Lithothamnion philippii* f. *alternans* Foslie
Mesophyllum expansum (Philippi) Cabioch & M.L.Mendoza	*Lithophyllum expansum* Philippi *Stereophyllum expansum* (Philippi) Heydrich *Pseudolithophyllum expansum* (Philippi) sensu Me.Lemoine *Crodelia expansa* (Philippi) Kylin *Lithothamnion expansum* (Philippi) Foslie *Lithophyllum expansum* f. *genuinum* Foslie *Tenarea expansa* (Philippi) Kuntze *Hyperantherella expansa* (Philippi) Heydrich *Crodelia incrustans* var. *expansa* (Philippi) Heydrich
Mesophyllum macroblastum (Foslie) Adey	*Lithothamnion macroblastum* Foslie

Verification of the right samples identification:

From yet unpublished data (Line Le Gall, pers. Com.) there is a hint that *Lithophyllum cabiochae* is a species complex containing at least 7 different species. The species *L. stictaeforme* and *L. cabiochiae* are quite difficult to be distinguished *in situ*. One should start by being able to differenciate *L. stictaeforme/cabiochiae* from *Mesophyllum* spp.. All those

species are red calcareous algae with horizontal blades of colour, which may vary from pink to purple (Figure 33).

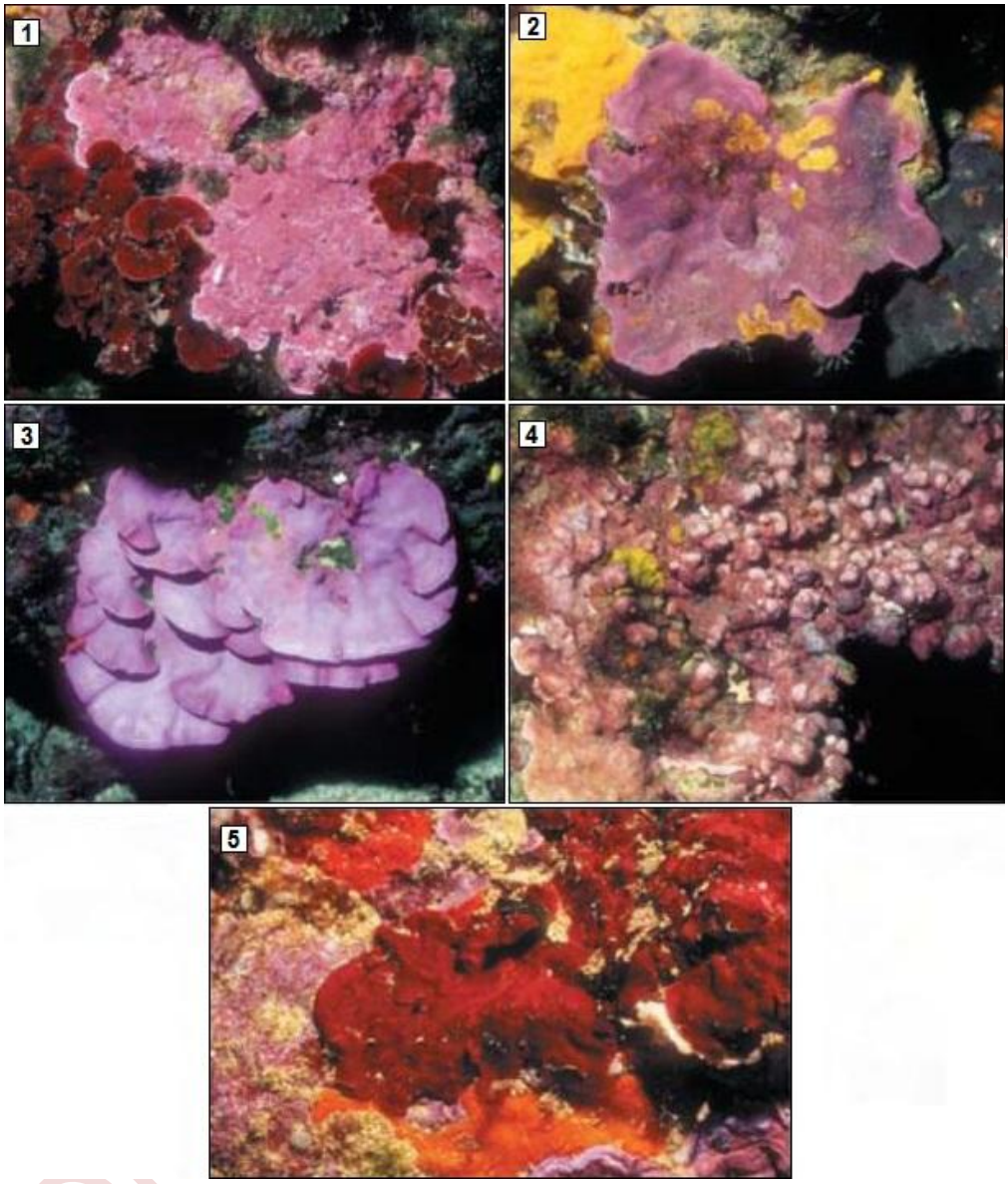


Figure 33: (1) *Mesophullum alternans* and *Peyssonnelia*, (2) *Lithophyllum stictaeforme*, (3) Lithophyllum cabiochiae, (4) *Neogoniolithon mamillosum*, (5) *Peysonnelia rosa-marina* (Ballesteros, 2006)

For the identification of species from te inferior surface of the thalle there are :

- Inferior surface smooth and whitish for *Mesophyllum expansum.*
- Inferior surface rose and finely striped for *Lithophyllum stictaeforme/cabiochiae*.
- Concentric stripes for *Lithophyllum stictaeforme.*
- Radial and concentric stripes for *Lithophyllum cabiochiae.*

On the upper face *Mesophyllum expansum* is pink-orangish with a white border (1) and its inferior face is rather smooth and whitish (2). The upper surface of *Lithophyllum* is pink-

purple rather mat and without white border, (3) and its inferior surface is pink, rather rough with white stripes, and often with tubers (4) (Figure 34).

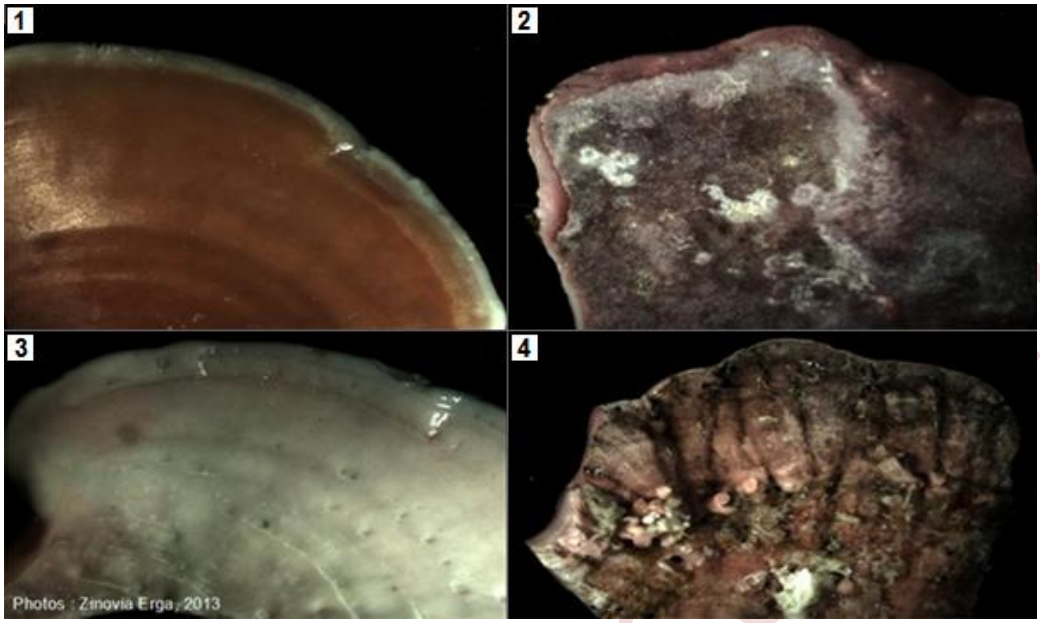


Figure 34 : External differences between *Mesophyllum* and *Lithophyllum*

In case where the morphological identification of these species is too complicated (i.e. Colour, individuals with white border but inferior surface more or less rough,...), it's the study of the cellural structure that will permit us to identify *Lithophyllum* from *Mesophyllum*.

The study of the anatomy of an encrusting corallinale's species should be effectuated on vertical radial sections following in the growth pattern of the calcareous blades. The species of the complex *Lithophyllum stictaeforme/cabiochiae* have a basis of basal cells (hypothallus) which creates vertical cellular lines (perithallus) slightly sloping (*stictaeforme)* or slightly sloping in the centre of the blades and strongly sloping near the edges (*cabiochiae*). The cells of the perithallus are never alined and arranged (in the contrary to the *Mesophyllum*) and communicate with each other lateraly by secondery synapsis (small holes). There are never lateral cell fusions (larges communications between the cells) like are present in *Mesophyllum*. The cells have rounded lateral walls. For this observation a stereo microscope is necessary.

To be added : les schemas et photos du diaporama de M Verlaque

Cells of *M. expansum* are ranged concentrically with locally cells fusions (1 and 2). Cells of *Lithophyllum* (3 and 4) have rounded lateral walls and communicate laterally only by secondary synapsis (Figure 35).

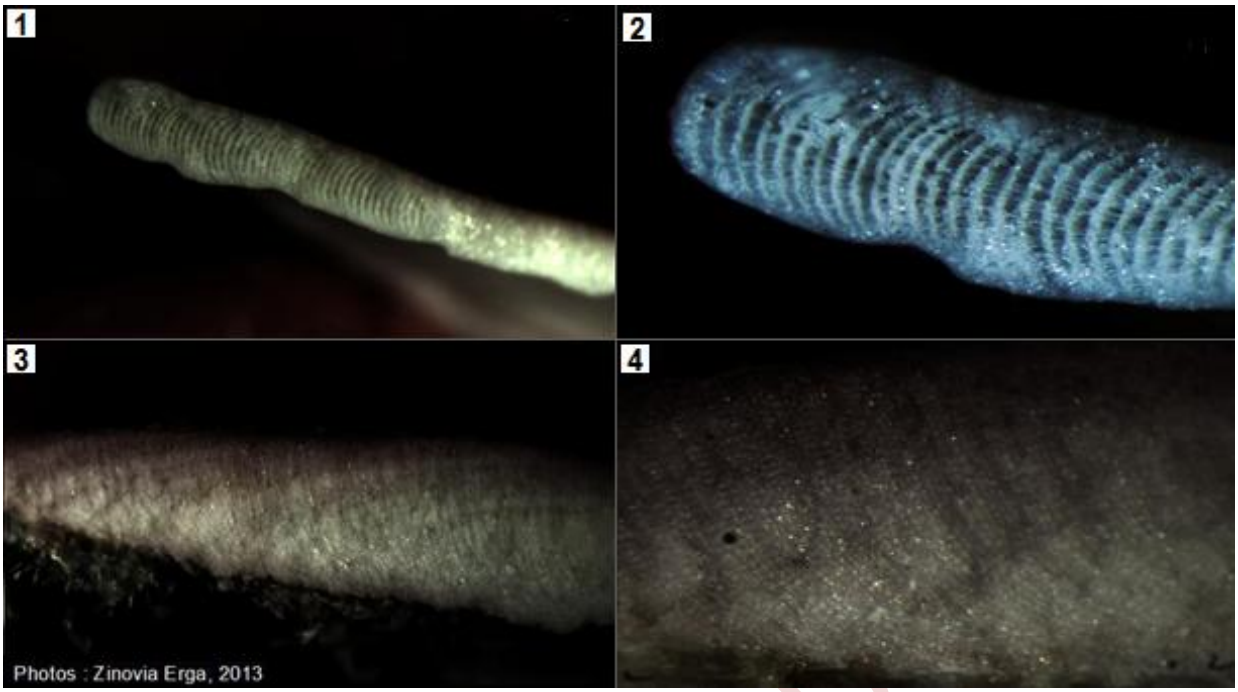


Figure 35: Photos of radial vertical section of the thallus of *Mesophyllum expansum* (1 and 2) and *Lithophyllum stictaeforme / cabiochiae* (3 and 4)

2. Sampling

a. *Operations*

Preparation of the bags which would be used for the sampling of *Myriapora truncata* and *Lythophyllum spp.* done by the means fo scuba diving

The zip bags should be numbered in a redundant way: the numerotation should be indelible, and situated at two places of the bag in order to avoid to be erased (1). In order to be able to refind the samples' origine the correspondance between the number of the bag and the segment should be respected.

The preparation of the bags should preserve the samples without any hesitation during the dive. Dangle an elastic on a hook in a way to make a slip-knot (2). Slip the elastic loop into the hole of each bag by starting with the last one (3). During the transport, the loop should be secured into the hook and thus to avoid loosing the bags (4). During the dives, the loop could be unhooked and placed around the wrist. The bags are so blocked/secured, by the hook on the one side and the diver's wrist on the other, which makes them more accessible (5) (Figure 36). By liberating the elastic loop from the wrist and pulling the first bag the diver will be able to open it and put the samples inside. The bags are then placed in the diver's mesh. If none of the species are not present on a segment, then pull out the numbered bag so that the correspondance between bags and segments/cartography will not be lost.

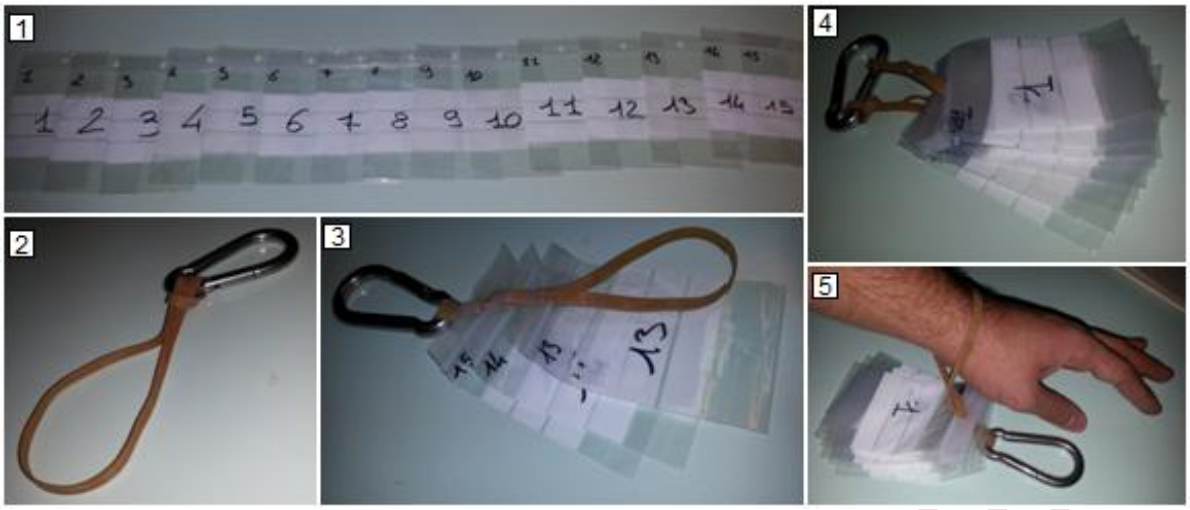


Figure 36: Methodology for the samples' collection

Sampling of *Myriapora truncata* and *Lithophyllum cabiochiae* done in diving:

While Scuba diving are collected: 2 to 3 fragments of Myriapora truncata (Pallas, 1766) sized about 3 cm, by carefully braking a piece from the top of the colony (becarefull not to break all the colony) as well as 3 to 4 fragments of Lithophyllum (in order to compensate a possible confusion with Mesophyllum) sized around 3 cm. A mean of 16 samples of every species should be collected from each side of the site, thus 32 samples in total from every site for the given depths. This number was choosen so that the analysis results would be statistically significants (Porter, Ryland et Carvalho, 2002; Schwaninger, 1999).

The same protocol would be used for the sampling at 45 m. If at any case it could not be operational for that depth, it would be adapted according to the technical possibilities for the depths between 40 m et 50 m. The importance is to have a minimum of 10 m difference between the two studied depths.

b. *Samples conditioning*

At the end of the sampling, the specimens should be placed directly into a portable fridge for their protection until the arrival at the laboratory. In case that the immediate conservation is not possible, the samples could be stabilised in the laboratory aquariums, but not for more that 48 h.

The specimens should be sent at the project responsible or the analysis responsible (anne.chenuil@imbe.fr).

Myriapora truncata

The manipulation of the fragments must be done with pliers, on a proper surface. The colony must be cleared of epibionts with binocular microscope (if it's necessary) to eliminate alien DNA which could distort the genetic results. The fragments must be placed in Falcon 10 or 20 ml tubes with 95% ethanol to ¾ of the tube to preserve them. Ethanol must be changed

after one hour to eliminate the water and the pigments. If it's not possible, the samples must be rinsed with ethanol in a beaker before put them into their individual tube. If the samples are too big for the tube, you can break them.

We write on each tube an individual code with a permanent marker and we write the same code on tracing paper with a pencil which is placed inside the tube (Figure 37):

[program]_[site]_[date]
**D**[depth][species]_**T**[n°transect]_**S**[n°segment]_**R**[n°replicate]_[observer]

Exemple : CIGESMED _CAS_20140123 _D1_truncata_T02_S07_R01_AA01

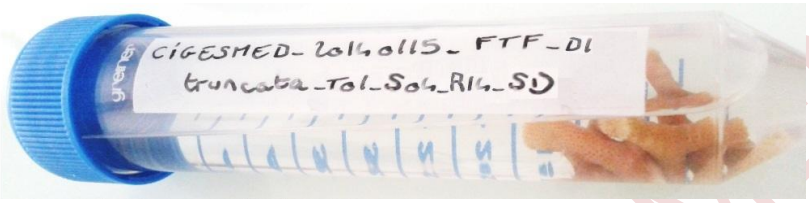


Figure 37: photo of the conditioning of *M. truncata* en tubes of 20 mL

⚠ Each tube/sample must have this code. Then, the samples are stored in a cold room at 4°C.

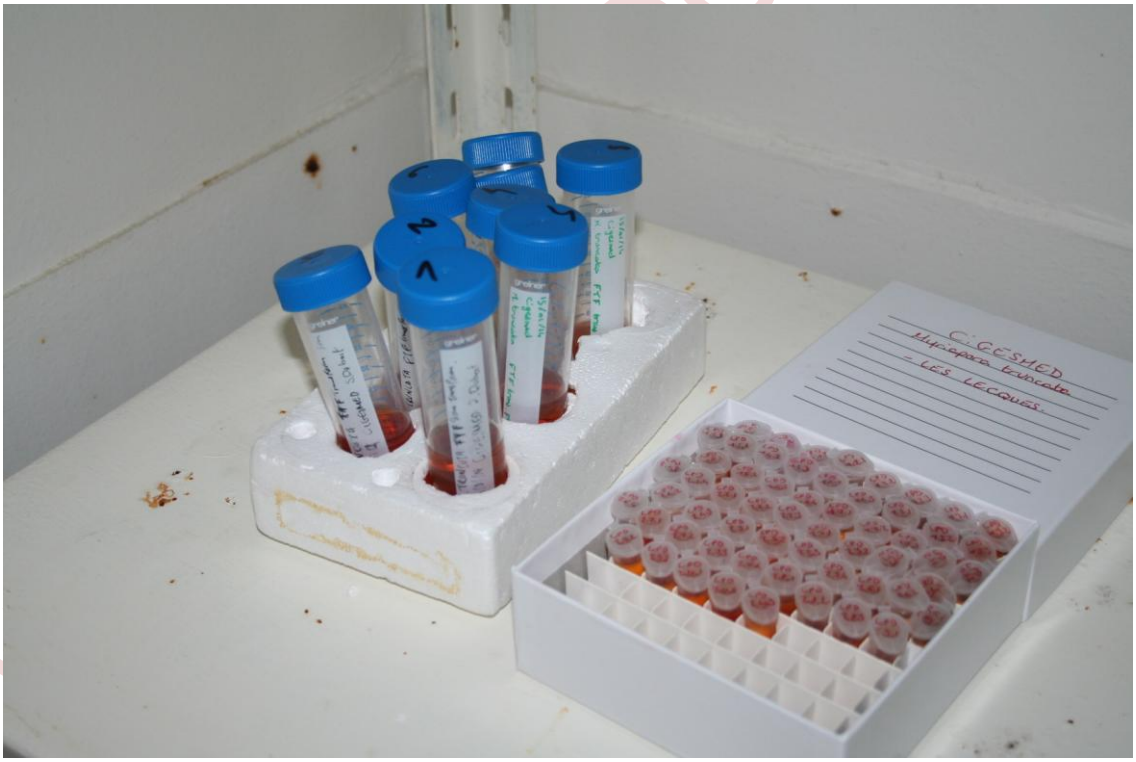


Figure 38: Samples stored in a cold room

Data must be saved in computer with the same code as the sample. Then they are sent to the program's responsible or to the database's responsible (in France, romain.david@imbe.fr).

Lithophyllum stictaeforme/cabiochiae

So that the morphological traits, like the inferior surface of the thallus, would be visible by the operator, it is often necessary to clear as much as possible the specimens from their epibiotic organisms (sponges, bryozoans, other algae, sponges, mollusc, etc). Once the identification of Lithophyllum stictaeforme/cabiochiae is confirmed (look at section b."Methodology of identification of Lithophyllum stictaeforme/cabiochiae"), dry well the specimen with laboratory paper. Note with permanent marker, on the bag, and with a pencil on transparent paper, which should be place into the bag, the specimens' code:

[program]_[site]_[date]_
D[depth]_[species]_**T**[n°transect]_**S**[n°segment]_**R**[n°replicate]_[doer]

Example: CIGESMED _CAS_20140123 _D1_cabiochiae _T02_S07_R01_AA01

The bags are then fulled with **"silicagel"**[1] enough to cover the specimen (quantity may vary with size) (Figure 39). The samples are then classify by site, and placed in a dry and dark place so that their DNA would be best conserved. The conservation methodology of corallinales into "silicagel" is detaily described in Broom *et al*. (2008).

Figure 39: photo of the conditioning of *Lithophyllum stictaeforme/cabiochiae*

⚠ Be very careful not to consider a broken sample as two different ones.

The samplings should be then listed on an excel file with each code corresponding to the appropriate specimen. This information should be sent along with the samplings to the project responsible or at the responsible of the projects' database (For France, romain.david@imbe.fr).

[1] Silicat gel in form of small granules serve at the conservation by dehydrating the speciments of corallinale's algae. These granules could be regenerated/dehydrated by some hours in the furnace at 70°C..

c. *Material list*

- 16 zipped plastic bags, numbered on two places (16 is the max number of segments of a transect but eventually a greater number could be considered).
- 1 rubber band long enough
- 1 hook
- 1 picnic transport fridge

d. *Work time quantification*

For each site, 2 divers should perform 2 dives: which makes a total of 4 man's dives.

3. Delivrables

a. *Expected results*

In order to facilitate the task of the ones charged with the samples analysis, the samples should be classified and packed in clear and methodological way. Which could mean for example to regroup all the samples of a transect in a bag, using a box per site, and in the meanwhile never forgetting to well indicate their origin. Each packing should carry an etiquette based on the proposed norm.

b. *Data analyses envisaged*

4. In brief

SAMPLING PROTOCOL FOR POPULATION GENETICS
(32 x individuals per species)

Method

- 2 target species which have a good natural range on all the sites sampled on the two selected profiles att both depths (28m et 43m) :
  - ✓ *Myriapora truncata*
  - ✓ *Lithophyllum cabiochiae*
- 2 to 3 fragments of *Myriapora truncata* with a size of 3 cm minimum, taken on top of colonies
- 3 to 4 fragments of *Lithophyllum cabiochiae* with a size of 3 cm minimum

Expected results

- ✓ 32 samples of *Myriapora truncata* (2 profiles x 2 orientations x 8 replicates) collected at 28 m deep. Potentially the same number of samples at 43 m.
- ✓ Typology of labelling samples of *Myriapora truncata*: [program]_[site]_[date]_D[depth]_[species]_T[n°transect]_S[n°segment] _R[n°replicate] _[doer]
- ✓ 32 samples of *Lythophyllum cabiochiae* (2 profiles x 2 orientations x 8 replicates) collected at 28 m deep. Potentially the same number of samples at 43 m.
- ✓ Typology of labelling samples of *Lithophyllum cabiochiae*: [program]_[site]_[date]_ D[depth]_[species]_T[n°transect]_S[n°segment]_R[n°replicate]_[doer]

Hors CIGESMED mais à combiner : Pour le suivi du cycle de reproduction : Une étude du cycle de reproduction de cette espèce est en cours à Marseille (collaboration Ostrovsky-Ereskowski) avec la fin du premier suivi annuel en février 2014. Il serait intéressant d'avoir des individus fixés pour l'histologie provenant d'autres régions de Méditerranée. Pour une telle étude, il suffit de prélever des fragments ramifiés de 2 cm environ dans 3 à 5 colonies, avec un fragment terminal et un fragment plus basal pour chaque colonie. Ces prélèvements doivent être placés dans un pilullier contenant 4% de formaldehyde dans de l'eau de mer filtrée. On peut alternativement fixer au Bouin qui est encore mieux pour l'histologie mais dans ce cas, attention, il faut laisser le tube ouvert une journée (évaporation du CO_2) sinon cela explose! Ensuite fermer avec du parafilm et le bouchon.

Questionnaire on module 4

« Sampling protocol for population genetics»

1) Your opinion on this protocol proposal « Sampling protocol for population genetics»» :

a) The methods proposed in this module « Sampling protocol for population genetics»» **: do they seem feasible on your CIGESMED's site ? Why**

b) Do you have at your disposal all the skills and means necessary to implement the protocol ? Detail the skills and means lacking.

c) What skills/means/work can you offer to any partner in order to complete the sampling ?

d) What changes can you propose to make this protocol operational on your CIGESMED's sites? (Taking care to maintain consistency with the other observers of the network)

e) What improvements would like to make on the descritpion of the protocol « Sampling protocol for population genetics»» ?

f) What improvements would you like to make on the methods of the protocol « Sampling protocol for population genetics»» ?

2) Your opinion on the feasibility of this protocol "Sampling protocol for population genetics»» :

1. **Are you familiar with this type of research?**

2. **On what occasions do you apply this type of protocol ?**

3. **What are your difficulties of implementation ? which steps are not clearly described in the document ?**

4. **Do you have any alternative to proprose ?**

3) Your investment in CIGESMED:

a) Are you ready to commit yourself to participate to the sampling of « Sampling protocol for population genetics» »?

b) If you do not intend to apply this protocol « titre du protocole »**, please explain why.**

c) Are you ready to commit yourself to improve and document the methods proposed by this protocole « Sampling protocol for population genetics»»?

d) Are you interested in a meaningful participation in the writting of scientific paper describing the results obtained from this protocol « Sampling protocol for population genetics»» ?

e) For this type of research are you ready to commit to participate as long-term observer applying this protocol « Sampling protocol for population genetics»»?

If you use another protocol, please communicate your detailed protocol to romain.david@imbe.fr

F. CIGESMED's Work Package 3 (Indicator and test)

The aim of the 3rd work package of CIGESMED (WP3) is to offer and standardize a method for assessing the conservation state of coralligenous. This method relies on the program INDEX-COR working on the French coasts for the agency of protected marine areas. The link between INDEX-COR and CIGESMED is at two levels: (i) the test of the spatio-temporal variability of metrics involved in the INDEXCOR's index (particularly on local and/or intrasite scale), and (ii) the adaptation of metrics and the method testing in other Mediterranean regions (in Greece and Turkey). Modification concerning the semantics of metrics.

1. General principles

INDEX-COR's index is based on the general principles described by Borja *et al*. (2008) for the establishement of a biological index:

- Definition of a spatio-temporal scale for the application of the index,
- Selection of the metrics used in the composition of the index,
- Definition of the combination of metrics (index),
- Validation of the index (datasets and/or acquisition of new datasets).

Selection of the metrics involved in the index takes in account various parameters fluctuating with the level of impacts (environmental and anthropogenic) on coralligenous:

- Ratio between species sensitive to impacts and indifferent species (« bio-indicator » notion)
- Characterization of species richness associated with coralligenous
- Characterization of the structural complexity of coralligenous bottom (hypothesis: complexity's simplification depending on the growth impacts)

a. Description of the metrics

INDEX-COR index is composed of three metrics:

→ Metric M1 or **ESEI**[1] ratio: characterizes the ratio betweenn **species sensitive**[2] and **species indifferent** [3] to impacts. For this, a reference table has been established for the French coast. It includes sixty benthic species for which a sensitivity rating (1-3) is associated with 4 main types of impacts: (i) organic matter input, (ii) fine particles input, (iii) physical impacts (fishing, anchoring, diving…) and (iv) sensitivity to temperature (global

[1] Metric participating to the IndexCor indicator. It corresponds to the ratio of the number of sensitive species on the number of indifferent species.
[2] Within IndexCor, species whose sensitivity level on a scale of 1 to 3 is 3, compare to a sensitivity criteria included in the index ESEI of IndexCor.
[3] Within IndexCor, species whose sensitivity level on a scale of 1 to 3 is 1, compare to a sensitivity criteria included in the index ESEI of IndexCor.

change). An overall rating summing the four rating is calculated, ranging from 4 to 12. Three major species groups are thus formed: 1st group (indifferent species, rating ranging from 4 to 6), 2nd group (tolerant species, rating ranging from 7 to 9) and 3rd group (sensitive species, rating from 10 to 12).

→ Metric M2 or **RSO**[1]: characterizes the specific observable richness in sessile and motile benthic macro-species. It includes all species recognizable without sampling, thanjs to pictures and videos in situ.

→ Metric M3 or **ICS**[2]: index of structural complexity of coralligenous bottom. It is based on a land approach ("forestry approach") which takes in account the existence of 3 main layers: (i) a **basal layer**[3] consisting of benthic organisms whoe thickness does not exceed 5 cm (Corallinacea, encrusting Peyssonnelaicea, encrusting sponges…), (ii) an **intermediate layer**[4] consisting of organisms whose thickness is between 5 cm and 15 cm (bryozoans, bushy sponges, sea squirts…) and (iii) an **erect layer**[5] consisting of species whose size exceeds 15 cm (large gorgonians, erect sponges…).

b. *Composition of the INDEX-COR index and reference*

INDEX-COR index is the aggregation of three metrics described previously. The final formula is being defined and may correspond to a weighted sum of metrics. The value obtained for this global index will be the **state of reference**[6] of the studied site at time T0 (relative baseline). This reference will be the one taken in account to estimate the conservation state of the habitat, rather than an absolute reference.

2. Acquisition method

a. *Proceedings*

Divers wear open-circuit aqualungs. They work in the coralligenous environment in team of two divers: (i) one supplier-observer diver, plus (ii) one photographer diver. Field operations require a minimum of knowledge about the site, this can be provided by a preliminary cartography, or at least few exploration dives. The work starts by marking the site with a buoy in surface located by a GPS. Two 15 m long transects will be settled on the bottom on comparables facies, with same orientation and depth.

[1] Metric of the IndexCor indicator. It corresponds to the number of species observed.
[2] Index calculated from a set of metrics characterizing the stratification of coralligenous stands.
[3] Within IndexCor, the basal layer is the layer of coralligenous animals that live between 0 and 5 cm from the substrate.
[4] Within IndexCor, the intermediate layer is the layer of coralligenous animals that live beyond 5 cm from the substrate and 15 cm below.
[5] Within IndexCor, the erect layer is the layer of coralligenous organisms that live beyond 15 cm from the substrate.
[6] Ecological status that corresponds to the steady state and has not suffered from any pressures other than natural ones.

Along these transects, 15 photos will be taken (so 30 per site in totality). A quadrat of 50 cm x 50 cm will be used. The photographer diver will take photographs during a first passing. During its second passing on the transects, the diver will film using the same camera, with a running time of 4 min per transect at least.

The supplier-observer diver materializes the transects by unrolling decametres, notes the environmental parameters: depth, orientation, type (bench, semi-inclined, wall) and main facies. He also notes parameters related to impact evaluation (solid waste, fishing line, broken colonies, traces of ancient necrosis, etc.). The diver must assess: (a) the general condition of the habitat, and (b) the general condition of the upper stratum, thanks to a pre-filled grid (sedimentation, richness of cryptic fauna, **invasive species**[1], colours…). Finally, he notes all species observed along both transects (Figure 40).

The time required for data acquisition is about 25 min, with a team of divers used to the protocol. This time of diving implies a decompression time of 20 to 25 min maximum if using pur oxygen: air decompression stop at 9 m, and pur O^2 decompression stop at 6 m. Diving tables used in France: MT92. Other tables are possible depending on the country and regulations: US Navy, …) We can reasonably estimate that between 0 and 45 m deep only one dive in necessary, and beyond this depth two dives are needed.

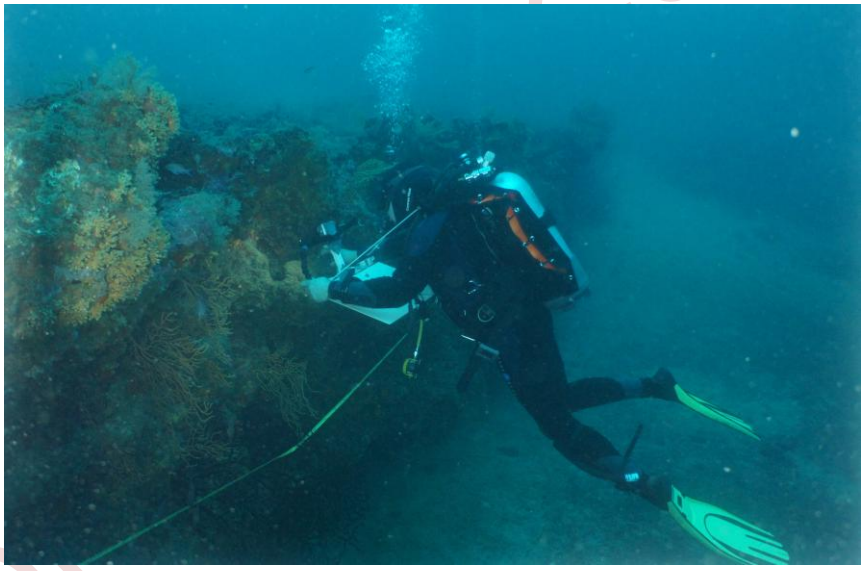


Figure 40: a diver recording profiles on one segment

b. *Material list*

The necessary equipment will included:

- Two decametres

[1] Species colonizing an area unconnected to its region of origin and whose range is expanding rapidly and durably (often due to humans), able to reproduce without human assistance, and pose ecological issues (Boudouresque, 2012).

- A note-pad provided with a GoPro camera to take pictures and video of the site (Figure 41- 2)
- A frame (quadra) equipped with a camera in a box and torchs (Figure 41 - 1)
- A system of localisation in surface (galiteau)
- A GPS
- A decompression line (buoy + a line to fix a tank of decompression)
- A scuba tank of O^2 pur (6 L or 9 L) provided with a set of pressure regulators

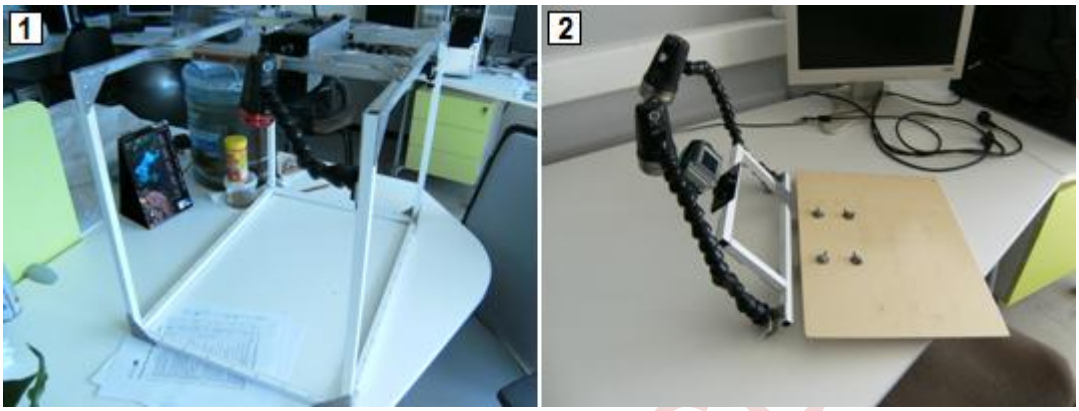


Figure 41: camera with flash mounted on a frame (1) and a note-pad provided with a GoPro and two torchs (2)

3. Data processing

The evaluation of metric M1 is done using the software PhotoQuad. 30 photographs will be processed per site. On each photograph 100 stratified points are appended. Each point is associated with a species or a category (rock, sediment, holes, etc.) from a library predefined and refillable as and when data processing with PhotoQuad (Figure 42).

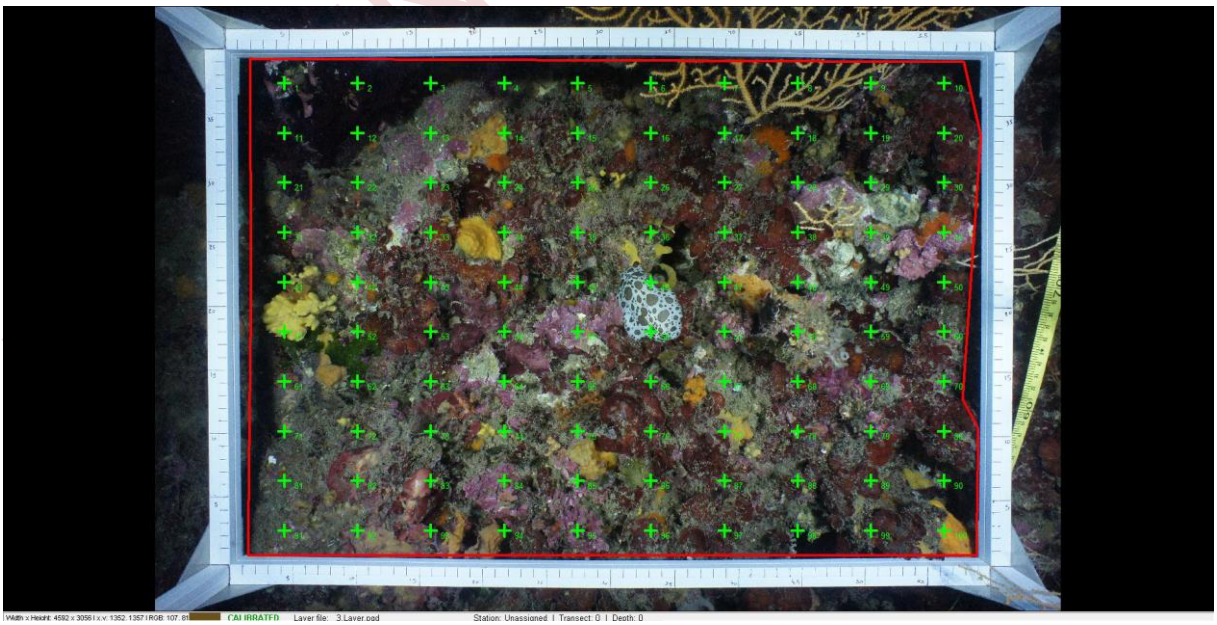


Figure 42: photograph processing with the software PhotoQuad

Metric M2 is obtained by the sum of species observed on the photographs, videos (VLC processing, an image every 5 s) and by the “logistics” diver. Finally, regaring metric M3, several tests were performed to process information related to the basal and intermediate stratums on PhotoQuad.

4. Planning

Phase 1 resulted in the definition and test of metrics. It will lead to a further processing of metric M3 (surface, grid, point). Metrics 1 and 2 must be a consolidation (species list and data aggregation). Phase 2 will be devoted to the adaptation and test of metrics in the eastern Mediterranean basin. This point will be discussed in IZMIR.

5. Delivrables

At the end, various documents will be produced:

- A methodological guide incorporating the method of acquisition processing of the data.
- A guide to species (by region or country)
- Species list of metric M1
- The library associated with the processing in PhotoQuad.

MIND MAPPING to added

Pour chaque manip, les Plongeurs IMBE s’occupent de la profondeur 30 m, IFREMER de la profondeur 45 m (on retient 15 m de différence) -> cela n’exclus pas que l’on se donne des coups de mains pour former une équipe à une date (Romain + Stéphane, Christian + Stéphane…)

IV. Protocole discussion

This part (IV) will be translated when more elements will be available.

A. Reproductibility of the protocol

Afin que le travail d'échantillonnage soit facilement reproductible, il est important de favoriser la méthode dite optimale, c'est-à-dire à mettre en place une méthode qui soit d'appropriation et de mise en œuvre faciles : les exigences en terme de qualification des plongeurs et de sophistication du matériel doivent être suffisamment accessibles pour être à la portée de la plupart des organisations, tout en fournissant des résultats suffisamment robustes et reproductibles (on cherche à trouver le minimum nécessaire).

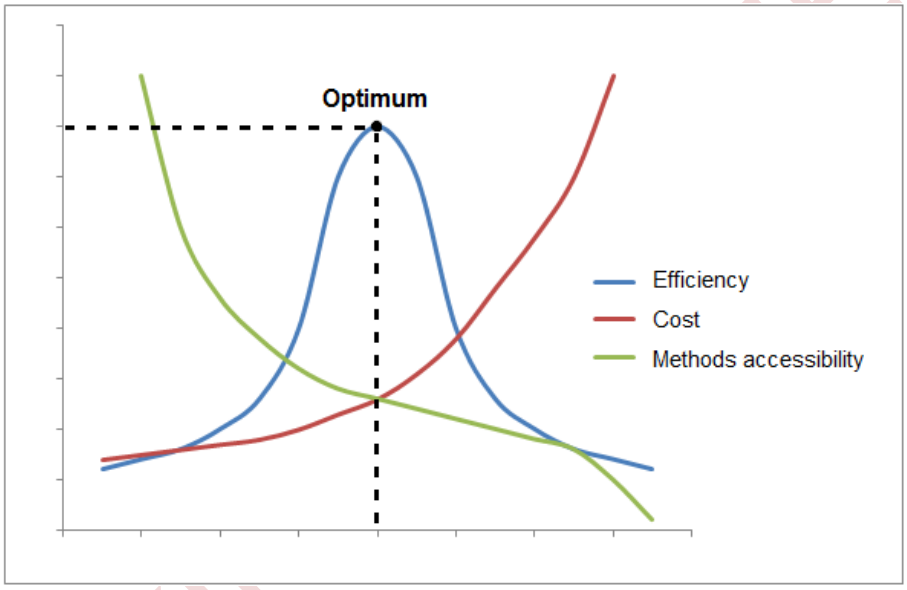


Figure 43 : optimum recherché pour le matériel

Schéma à faire évoluer (couts non rectiligne, optimum = asymptote de l'efficacité)

Un des résultats attendus permettra de qualifier la meilleure organisation en fonction des capacités des structures et des objectifs du réseau (Figure 43).

To be completed dependind on the discussions at Izmir

B. Sécurité

NB: Evolution souhaitable en France au CNRS et ailleurs, le passage à des plongées au Nitrox 40/60 permettrait d'envisager des plongées plus longues : profondeur équivalente 21 m soit 35 mn de plongée sans palier

SS : La réglementation actuelle permet l'utilisation des Trimix, Nitrox et théoriquement des recycleurs. Comme je l'ai signalé précédemment, ce n'est qu'une réglementation interne au CNRS qui limite les choses pour l'instant. Concernant les nitrox, c'est à adapté en

fonction de la profondeur d'intervention et le nitrox n'est pas exempt de danger. Donc, en conclusion, il serait bien qu'il y ait une harmonisation des réglementations entre les pays car peu ont une réglementation concernant la plongée professionnelle. Pour le reste, je ne pense pas que ce soit le rôle du document de préciser quel type de gaz utilisé, et comment.

To be completed dependind on the discussions at Izmir

C. Inter-calibration sur transect permanents

NB : les tiges filetées marquant le transect ne détériorent que très localement le substrat, et la cicatrisation de l'emplacement dégradé est très rapide (de l'ordre de quelques mois).

To be completed dependind on the discussions at Izmir

D. Comparaison de la variabilité entre transects permanents et transects aléatoires

To be completed dependind on the discussions at Izmir

E. Eléments de discussion lors de la première validation

Here put the elements that you would like to be discuss in the final document.

SS : je pense qu'il y a trop de faciès, idem pour celle du MNHN. Qui dit faciès, dit entité écologique et la prédominance de certaines espèces n'est pas forcément synonyme d'entité écologique)

SS : peut-on parler de faciès à Myriapora truncata qui est une espèce ubiquiste et très tolérante (sauf aux chocs physiques)?

SS : IV.3.1.i. Faciès à Eunicella singularis / Eunicella verrucosa (ce sont deux choses différentes. L'un est infralittoral et l'autre appartient au circalittoral profond)

SS : Poissons : l'éventuelle détection d'œufs ou de juvéniles montrerait le rôle du coralligène en tant que nurserie (ce serait une piste mais ça m'étonnerait qu'avec les raclages on puisse vraiment mettre en évidence cela (et tout dépend de la saison des échantillonnages). On aura peut-être la chance de trouver des traces pour certaines espèces benthiques (gobies, blennies,...) mais ensuite, c'est vraiment une étude spécifique qui pourrait permettre de mettre en évidence cela), potentiellement générateur d'un effet d'exportation (spill-over).

ZE : Le nomenclature Pseudolithophyllum cabiochiae n'est pas accepter. Sur le site de worms Lithophyllum stictaeforme est bien accepter et il y en a une grande liste de synonimised taxons (http://www.marinespecies.org/aphia.php?p=taxdetails&id=196013) entre autres Lithophyllum frondosum f. cabiochae qui etait proposé par les même mecs voir Boudou et Verlaque A part le faite qu'il est fortement conseillé par Mark Verlaque de

consulter le site algaebase.org pour des questions sur la nomenclature et la taxonomie des algues. D'ou Lithophyllum cabiochiae est bien accepter donc existant comme espèce ! D'allieur ces deux especes freres sont considerés comme Lithophyllum cabiochiae l'endemique de Mediterranee et L.Stictaeformae de l'Atlantic. Je comprends pas point de l'argument de cette phrase dont il n'y existe pas une discussion sur le sujet en moins pas encore.

ZE : Parce que c'est difficile de les distingué sous l'eau parce que il sont identifiés par leurs cotes inferieur donc caché. Et en plus qu' on cherche de trouvé des hybrides potentiels que on regarde tous les deux. Pourtant, il y a une paragraphe dans ma partie qui explique comment les identifié.

AC : Pourquoi cette formulation ? C'est pas du tout le langage écologique et statistique habituel : on teste l'effet des différents facteurs (ou variables) environnementaux c'est pas plus clair dit comme ceci ? C'est peut-être du jargon qui fait partie de la culture base de données, mais cela me trouble…

JPF : C'est pour cela qu'il vaut mieux parler d'habitats, au pluriel, pour le coralligène

JPF : plongée nitro et trimix non autorisée : Il faut citer les termes du décret. Il n'y a pas que le CNRS qui est concerné

Toujours l'écrire de la même façon IndexCor.

JPF : glossaire. définition de station à revoir. Proposition de définition pour station = Point ou localisation précise (coordonnées).
RD : station = aire de recouvrement continu par une même espèce.

On ne peut pas dire que ça éclaire ! (traits rouge comme transects).

NB : Un tirage aléatoire sur les données de transect permanent pourra aussi simuler les résultats d'un transect aléatoire.

TO BE NOTICED BY THE READERS:

This version of the protocol must undergo some improvements such as:

- **Make the corrections using highlighted text.**
- **Add illustrations**
- **Take into account more references (any suggestion is welcome)**
- **List the suggestions in the discussion part (you are welcome to participate)**
- **Add maps which will be made for Marseilles' sites**
- **Improve the list of species (the proposed values for the abundance may vary depending on the season, and the identification difficulty may depend on the presence of a similar species in the area.**
- **An additional booklet will complete the species list, describing biotopes, species, identification traps, description of similar species.**
- **Add illustrations for each species. You may be asked to help with your photos if you can complete ours.**
- **A legend will be add at the species table**

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Appendix

Figures table

Tables

Acronyms and Abbreviations

- MPAA [fr = AAMP]: Marine Protected Areas Agency
- ANR: National Research Agency
- ARMS : Autonomous Reef Monitoring Structures
- ASUs : Artificial Substrate Units
- CDB: Convention on Biological Diversity
- CIGESMED: Coralligenous based Indicators to evaluate and monitor the "Good Environmental Status" of the MEDiterranean coastal waters
- CNRS: National Centre for Scientific Research
- SSIS [fr = ESEI]: Sensitive Species on Indifferent Species ratio
- FAO: United Nations Food and Agriculture Organisation
- FFESSM : Fédération Française d'Etude et de Sports Sous-Marins
- GBIF: Global Biodiversity Information Facility
- GPS: Global Positioning System
- HCMR: Hellenic Center for Marine Research Institute of Marine Biology, Biotechnology and Aquaculture
- ISC [fr = ICS]: Index of Structural Complexity
- IFREMER: French Institute for Exploitation of the Sea
- IMBE: Mediterranean Institute of Biodiversity and marine and terrestrial Ecology
- IndexCor: program to develop an overall index of the state of conservation of coralligenous for environmental managers.
- MIO: Mediterranean Institute of Oceanography
- MNHN: (French) National Museum of Natural History
- DTM [fr = MNT]: Digital Terrain Model
- NMPZ: National Marine Park of Zakynthos
- OSU: Observatory of Sciences of the Universe
- OSR [fr = RSO]: Observable Species Richness
- PCR: Polymerase Chain Reaction
- RVA: Rapid Visual Assessment
- GIS [fr = SIG]: Geographic Information System
- SPE: Systems for Environment, coastal ecosystem
- SPN: Natural Heritage Service of the National Museum of Natural History
- MRU [fr = UMR]: Mixed Research Unit
- MSU [fr = UMS]: Mixed Service Unit
- MTU [fr = UMT]: Mixed Technological Unit
- RU [fr = UR]: Research Unit
- WGS 84: World Geodetic System 1984

Glossary

The definitions that have no source are proposals that may change during the construction of CIGESMED's ontology. It is thus awaiting enhancements/corrections.

Accessibility of an observation device [fr = accessibilité d'un dispositif d'observation]: ease of use of an observation device (material cost, skills requested, formation, observation and treatment times). It determines the size of the skilled observers panel.

Association [fr = association]: permanent aspect of a biocenosis with vegetal physionomic dominance where the species are linked by an ecological compatibility and a chorological affinity. (From UNEP, APM, RAC/SPA, 2006).

Biocenosis (or biocoenosis) [fr = biocénose]: a grouping of living organisms, linked by relationships of interdependence within a biotope with relatively homogeneous major characteristics; each biocenosis comprises mainly the phytocenosis, which includes flora, and the zoocenosis, which includes fauna. The notions of community or association in the phytosociological sense of the word are very close to the notion of biocenosis although they cannot exactly replace it (from PNUE, PAM, CAR/ASP, 2006).

Biotope [fr = biotope]: a geographical area with variable surface or volume subjected to ecological conditions where the dominant elements are homogeneous. (From UNEP, APM, RAC/SPA, 2006).

Positioning frame [fr = cadre de positionnement]: within CIGESMED, it's a frame to position nine photo-quadrats from a fixed point, so that minimize the variability. The corners of the frame are actually crosses around which the quadrats prop up. (photo to be added)

Calibration [fr = calibrage]: operation that consists in measuring the number of fin strokes needed to go 5 m (one segment) depending on the direction and strength of the current.

Complete mapping [fr = cartographie complète]: complete record of profiles detailing all fields as recommended by the protocol.

Partial mapping [fr = cartographie partial]: mapping limited to profiles interesting to be taken (i.e. predetermined profiles for which the profile frequency is proven, and the frequency of *Myriapora truncata* and *Lithophyllum cabiochiae* populations are sufficient to be sampled). This mapping will be mainly implemented for the samples on secondary sites, which are distant or for which there is no sufficient continuum of coralligenous bio-concretions to perform a complete mapping. This may also be the case for the second depth (around 43 m), less accessible, to shorten the intervention time.

Chorology [fr = chorologie]: explanatory study of the geographical distribution of living species and its causes (climatic and edaphic factors).

Circalittoral [fr = circalittoral]: The region of the sublittoral zone, which extends from the lower limit of the infralittoral to the maximum depth at which photosynthesis is still possible.

Contextualization [fr = contextualisation]: consists to provide a context for a data, assign a qualifier corresponding to recording conditions or biotic / abiotic special conditions of the measured data. Within the context of CIGESMED, it corresponds to an assignment of a profile to a sample, in order to study the dataset by focusing only on one type of profile.

Erect layer [fr = couche érigée]: within IndexCor, the erect layer is the layer of coralligenous organisms that live beyond 15 cm from the substrate.

Basal layer [fr = couche basale]: within IndexCor, the basal layer is the layer of coralligenous animals that live between 0 and 5 cm from the substrate.

Intermediate layer [fr = couche intermédiaire]: within IndexCor, the intermediate layer is the layer of coralligenous animals that live beyond 5 cm from the substrate and 15 cm below.

Objective document [fr = document d'objectif (DOCOB)]: planning document that is used in Natura 2000 sites within the European directives « Habitats » and « Birds ». Each site designated to be part of the network must equipped itself with this document. The objective document includes an inventory of the natural environment and human activities, an established management guidance that best suits to the marine area concerned and the financial means (source www.aires-marines.fr).

Ecotone [fr = écotone]: transition between two ecosystems or habitats or areas with different biotic and/or abiotic conditions.

Efficiency (of an indicator) [fr = efficacité (d'un indicateur)]: an indicator is efficient if its value range corresponds to the observable part of the phenomenon studied, and if it varies homogeneously regardless of the magnitude of the phenomenon studied. A AJUSTER (cf Dominique Pelletier 2005).

Resident species [fr = espèce résidente]: all sessile species from an habitat and motile species found mainly in the designated habitat (e.g. *Hypselodoris* and *Cacospongia, Peltodoris atromaculata and Petrosia ficiformis*...)

Epibionts [fr = épibiontes]: non-parasitic organisms using larger organism as support.

Epiphytes [fr = épiphytes]: photosynthetic autotrophic organisms using other plants as support.

ESEI (Sensitive species / Indifferent species) [fr = ESEI (espèce sensibles / espèces indifférentes)]: metric participating to the IndexCor indicator. It corresponds to the ratio of the number of sensitive species on the number of indifferent species.

Cryptic species [fr = espèces cryptiques]: species defined as such because isolated in term of reproduction and / or whose genetic line has a significant genetic differentiation, indicating an ancient divergence between one and the other, but that is not distinguishable from a morphological point of view.

Indifferent species [fr = espèce indifférente]: within IndexCor, species whose sensitivity level on a scale of 1 to 3 is 1, compare to a sensitivity criteria included in the index ESEI of IndexCor ((i) organic matter input, (ii) addition of fine particles, (iii) physical impact (fishing, anchoring,

diving...) and (iv) sensitivity with respect to temperature (Global Change)). This grade is assigned by an expert, by classifying all the species according to the three values of this criteria, and fairly (i.e. a comparable species number for each category).

Invasive species [fr = espèce invasive]: (exotic) species colonizing an area unconnected to its region of origin and whose range is expanding rapidly and durably (often due to humans), able to reproduce without human assistance, and pose ecological issues (Boudouresque, 2012). Biological invasions are now considered by the UN as one of the major causes of biodiversity loss, as well as pollution, ecological fragmentation of habitats/ecosystems.

Sensitive species [fr = espèce sensible]: within IndexCor, species whose sensitivity level on a scale of 1 to 3 is 3, compare to a sensitivity criteria included in the index ESEI of IndexCor ((i) organic matter input, (ii) addition of fine particles, (iii) physical impact (fishing, anchoring, diving...) and (iv) sensitivity with respect to temperature (Global Change)). This grade is assigned by an expert, by classifying all the species according to the three values of this criteria, and fairly (i.e. a comparable species number for each category).

Seasonal species [fr = espèce saisonnière]: species that does not persist during all seasons and therefore whose presence on a site or a transect can be ignored if the frequency of observation is not strong enough to be taken in account.

Tolerant species [fr = espèce tolérante]: species able to adapt to a wide range of salinities (euryhaline species), temperatures (euryterm species) and/or depths (eurybathic species).

Layering [fr = étagement]: marine layering depends on environmental factors. These factors are: wiping and light as main ones (climatic factors), but also hydrodynamism, salinity, substrate type and temperature, as secondary factors (edaphic factors), (Bellan-Santini et al., 1994).

Standardization [fr = étalonnage]: adjustment of a measuring device on a known scale of values, by estimating the uncertainty associated with the measurement using a standard.

Baseline (= initial state, zero point) [fr = état initial]: ecological status that corresponds to the measurements, made on the first state observed at the beginning of the habitat study, without knowledge of its antecedent state (which can be either better or worse). Define a baseline allows to highlight trends and/or set goals (maintain, improve a particular metric…)

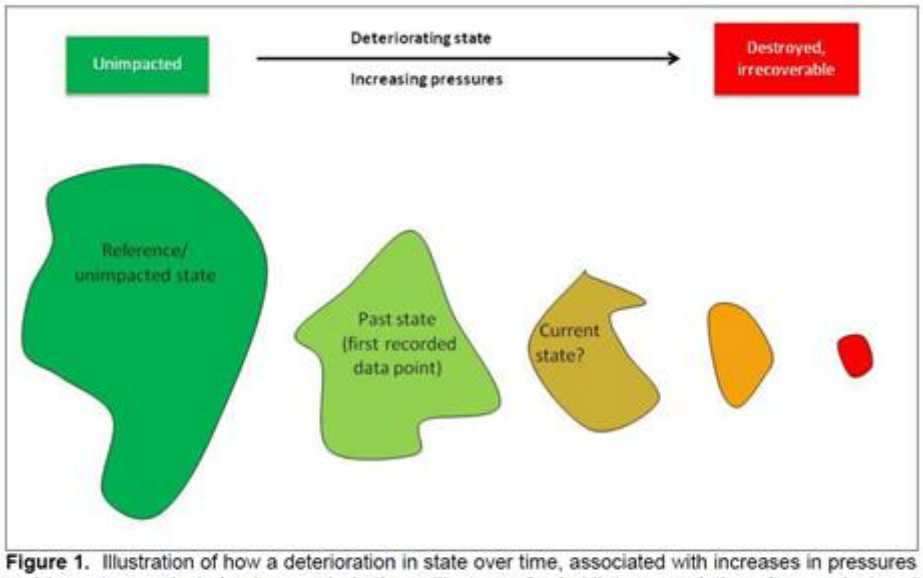


Figure 1. Illustration of how a deterioration in state over time, associated with increases in pressures and impacts, can include changes in both quality (e.g. of a habitat or population of a species) and quantity (e.g. habitat extent, population size) of a biodiversity component. Setting the baseline as 'current state' represents a very different scenario to using 'past state' or 'reference state'. Figure from Moffat *et al.*, 2011.

State of reference [fr = état de référence]: ecological status that corresponds to the steady state and has not suffered from any pressures other than natural ones. We can also talk of an optimum state. Since the natural conditions differ from one habitat to the other depending on the region, the optimum state is variable depending on the natural environment. The reference state may be founded on the MPA of the vicinity/surroundings that are in the as good as possible state in a given area.

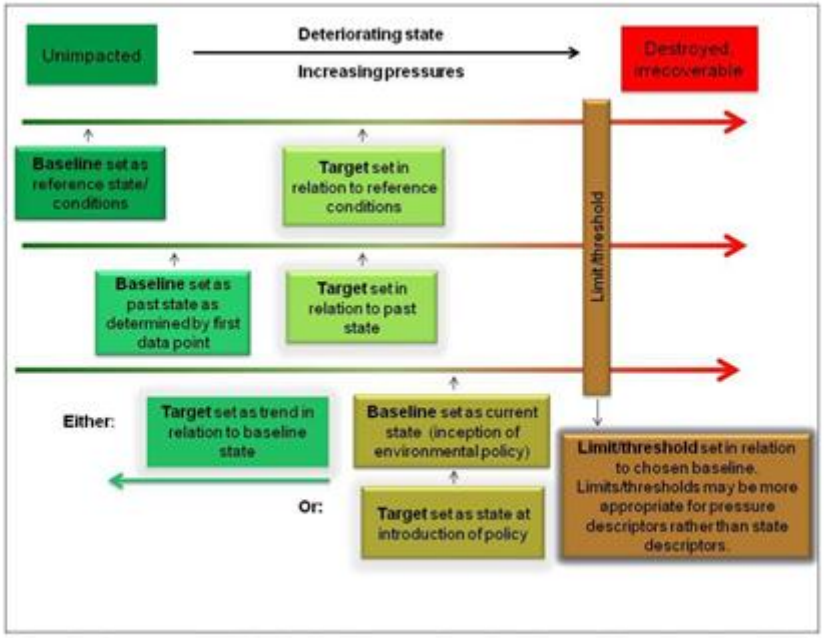


Figure 2. The conceptual relationship between various baseline conditions, targets and limits. Figure from Moffat *et al.*, 2011.

Facies [fr = faciès]: An aspect exhibited by a biocenosis when the local predominance of certain factors causes the prevalence of either one or a very small number of species, essentially animal ones.

Contextualisation factor [fr = facteur de contextualisation]: context categorized according to a specific typology, with as less as possible ambiguous values to evaluate the measure's environment by a biotic or abiotic parameter.

Variability factors [fr = facteurs de variabilité]: factors related to measurement conditions, observer, operator or measured system, having an influence on the measurement accuracy.

Reliability (of an indicator) [fr = fiabilité]: parameter whose evolution allows the evaluation of a situation. An indicator is reliable if it is relevant, effective, responsive and robust. A AJUSTER PELLETIER

Population genetics [fr = génétique des populations]: study of the distribution and changes in the frequency of gene versions (alleles) in populations of the same species. Population genetics allows for example to answer questions about the connectivity of two populations of the same species.

Habitat [fr = habitat]: An area distinguished by geographic, abiotic and biotic features (definition of EEC Directive 92/43). The definition of the habitat can be compared herein to that of a biocenosis, facies and association.

Infralittoral [fr = infralittoral]: in the mediterranean area, the infralittoral zone is the part constantly submerged whose upper fringe corresponds to the chart datum. Its lower limit is one that is compatible with life of photophilous algae and marine phanerogams.

Inter-calibration [fr = inter-calibration]: consists to confront techniques making only one parameter vary (in the best case) to compare the magnitude of variations related to this parameter.

ICS (Structural Complexity Index) [fr = indice de complexité structurale]: index calculated from a set of metrics characterizing the stratification of coralligenous stands. This index is used in the conception of the indicator IndexCor.

Isobath [fr = isobathe]: imaginary line, at constant depth, along a submarine relief.

Lecithotrophic [fr = lécithotrophe]: said of a larva that does not feed directly and lives on its reserves. (these larvae are often consider having lower dispersion capacity).

Locality [fr = localité]: geographic region where sampling sites of one partner are grouped (e.g. Marseilles locality).

Metric [fr = métrique]: parameter describing a phenomenon or a variable, biotic or abiotic.

Digital Elevation Map [fr = modèle numérique de terrain]: 3D digital cartography of the elevation.

Observer [fr = observateur]: responsible person for the acquisition of the data *in situ*, following a protocole.

Operator [fr = opérateur]: person who operates the observations and field data (often in digital form) received from the observer.

Efficiency optimum [fr = optimum d'efficacité]: refers to an observation device when its efficiency is optimal, in other words when the ratio between its cost and its effective appropriation from a wide range of observer is maximal.

Group of diving partners [fr = palanquée]: group of divers having the same dive parameters (direction, depth, duration, decompression time).

Relevance (of an indicator) [fr = pertinence]: said of an indicator if it varies mainly depending on the parameter studied / monitored. An effective indicator is relevant if it shows something about the system that you need to know. A AJUSTER (cf Dominique Pelletier).

Phylogeography [fr = phylogéographie]: the study of processes that explain the distribution of genealogical lineages within the same species (may go as far as speciation processes).

Polysemy [fr = polysémie]: said of a term when it has different meanings depending on the region, discipline or subject considered. This is one of the main obstacles to interdisciplinary studies.

(Environmental) Profile [fr = profil]: within CIGESMED framework, a profile is a situation, mixing the exposition (i.e. slope + orientation + rugosity) and the covering type. Two profiles will be selected for their representativeness on two sides of the island studied and on two depths, in order to achieve the different monitoring (sampling for genetic analysis, observations, photographs, videos…). Profile = covering + orientation + slope + rugosity.

Quadrat [fr = quadra]: physical frame used to frame the photograph (taken during the dive in our protocol). It may be independent or integral with the shooting device (picture or movie camera).

Photo-quadrat [fr = quadra-photo]: photograph taken through a quadrat, defining a specific area. Photographic sampling unit.

Photophilous [fr = photophile]: refers to an organism, animal or plant, which grows preferentially in areas sufficiently exposed to light.

Data qualification [fr = qualification de la donnée]: metadata (site description, viewing conditions, observation device, observer) or enrichment of this data by an attribute from an ontology. This qualification can be specified in the database or in associated index. It makes possible to sort the data and / or analyze them with holistic approaches (multivariate) or non-statistical ones (data mining).

Robustness (of an indicator) [fr = robustesse]: an indicator is considered to be robust if it retains a constant value during the repetition of the same event in identical conditions.

RSO (observed species richness) [fr = richesse spécifique observable]: metric of the IndexCor indicator. It corresponds to the number of species observed.

RVA (Rapid Visual Assessment) [fr = méthode d'évaluation visuelle rapide]: method to assess the coralligenous habitat, during the dive, based on the assessment of population stands on a surface … citer Gullia A DEFINIR

Sciaphilous [fr = sciaphile]: refers to an organism, animal or plant, which grows preferentially in the shadows. Sciaphilous is the contrary of photophilous.

Segment [fr = segment]: mapped length (spatial object) of 5 m to which is assigned a profile. A transect is composed of different segments (which are considered the pixels of our mapping). Smallest mapping unit used for mapping profiles in the CIGESMED protocol.

Sensitivity (of an indicator) [fr = sensibilité]: an indicator is said sensitive if it is precise and responsive to the variations studied. It must therefore consist of sufficiently distinct qualifying or quantificative categories so that there is no uncertainty in the allocation of its value.

Silica gel [fr = gel de silice]: it is a granular, vitreous, porous form of silicon dioxide made synthetically from sodium silicate which has a strong affinity for water molecules. It is used as a desiccant to control local humidity to avoid spoilage or degradation of some goods. Such granules are used for the preservation of the coralligenous samples by drying. They regain their desiccation capacity if put in a steriliser at 70°C (343.15 K).

Spill over effect [fr = effet de "spill-over"]: said of a phenomenon observed in the marine reserves, a transfer of adult and juvenile biomass to outlying areas. The spill-over effect contributes to improving the production of fished species near the marine reserve, due to the distinct increase of juveniles and adults inside (source : http://www.aires-marines.fr/Glossaire/Spillover).

Sympatry [fr = simpatrie]: refers to the coexistence of two species phylogenetically close, on the same territory, and that do not hybridize.

Site [fr = site]: within CIGESMED, it can be an island, a small island, a shoal, from the coastal fringe or coralligenous fringe, as long as it has a maximum of different orientations and at least two that are diametrically opposed.

Basal stratum [fr = strate basale]: within CIGESMED, it is the lowest stratum corresponding to encrusting and turf species. In this stratum, the competition between species is mainly for the substrate. NB: some species may be part of two strata.

Upper stratum [fr = strate supérieure]: within CIGESMED, it is the highest stratum corresponding to erect species. In this stratum, the competition between species is mainly above the substrate. NB : some species may be part of two strata.

Substrate [fr = substrat]: natural support (usually the bottom) on which organisms develop.

Transect [fr = transect]: trajectory followed by the diver while he surveys (occurrence, abundance, biomass…), which can be done systematically or randomly. In our case these records will be made from photo-quadrat of the coralligenous bio-concretions.

Random transect [fr = transect aléatoire]: within CIGESMED, said of a trajectory followed for taking pictures of quadrats, which is not physically marked. These transects can therefore be done at constant depth.

Permanent transect [fr = transect permanent]: within CIGESMED, said of a trajectory followed for taking pictures of quadrats, which is physically marked by a fixed threaded rod. These transects must be done at constant depth.

Turbidity [fr = turbidité]: quantifiable characteristic of water whose transparency is limited by the presence of suspended solid matter, drawn by the currents and whirls.

World Geodetic System 1984 (WGS 84): global standard geodetic system, used especially by the GPS system.

Data typology

	Code
Note-pad (profiles description)	[program]_[site]_[date] _**D**[depth]_[doer]
***M.truncata* samples**	[program]_[site]_[date]_**D**[depth]_[species] _**T**[n°transect]_**S**[n°segment]_**R**[n°replicate]_[doer]
***L.cabiochae* samples**	[program]_[site]_[date]_**D**[depth]_[species] _**T**[n°transect]_**S**[n°segment]_**R**[n°replicate]_[doer]
Photo-quadrats	[program]_[site]_[date]_**D**[depth]_**T**[n°transect] _**Q**[n°quadrat]_[doer]
Samples from crapping	[program]_[site]_[date]_**D**[depth]_[profile]_[position] _[doer]

Computer files sorting in a folder use the position of letters from left to right. Our data typology helps to find data sought after.

[program] : name of the program for which the sample is taken (e.g. « CIGESMED »). Consists of 8 capital letters.

[date] : date of the sampling in the form YearMonthDay (e.g. « 20140121 » for January 21, 2014). Consists of 8 numbers: 4 for the year, 2 for the month, 2 for the day.

[site] : 3 letters code of the site sampled (e.g. « FTF » for « Frioul – Tiboulen de Frioul »). Consists of 3 capital letters:

- either 1 letter for the main site (ex : « F » for « Frioul »), followed by 2 letters of other precisions (e.g. « TF » for « Tiboulen de Frioul »)
- or the 3 first letter of the site (e.g. « CAS » for « Cassidaigne »)

[doer] : initials (first name followed by lastname) of the person in charge of the sampling, followed by its identifier ifrom the database (e.g. “AA00”). Consists of 2 capital letters. In order to avoid homonymy.

D[depth] : the letter « D » in capital, followed by the depth of the sampling (« 1 » for 28 m environ or « 2 » for 43 m).

[species] : name of the species sampled (e.g. « truncata » ou « cabiochiae »).

T[n°transect] : the letter « T » in capital, followed by the number (of two numerals) of the transect sampled (e.g: « T01 » for the transect number 1).

S[n°segment] : the letter « S » in capital, followed by the number (of two numerals) of the transect sampled (e.g: « S05 » for the transect number 5).

R[n°replicate] : the letter « R » in capital, followed by the number (of two numerals) of the transect sampled (e.g: « R04 » for the transect number 4).

Q[n°quadrat] : the letter « Q » in capital, followed by the number (of two numerals) of the transect sampled (e.g: « Q09 » for the transect number 9).

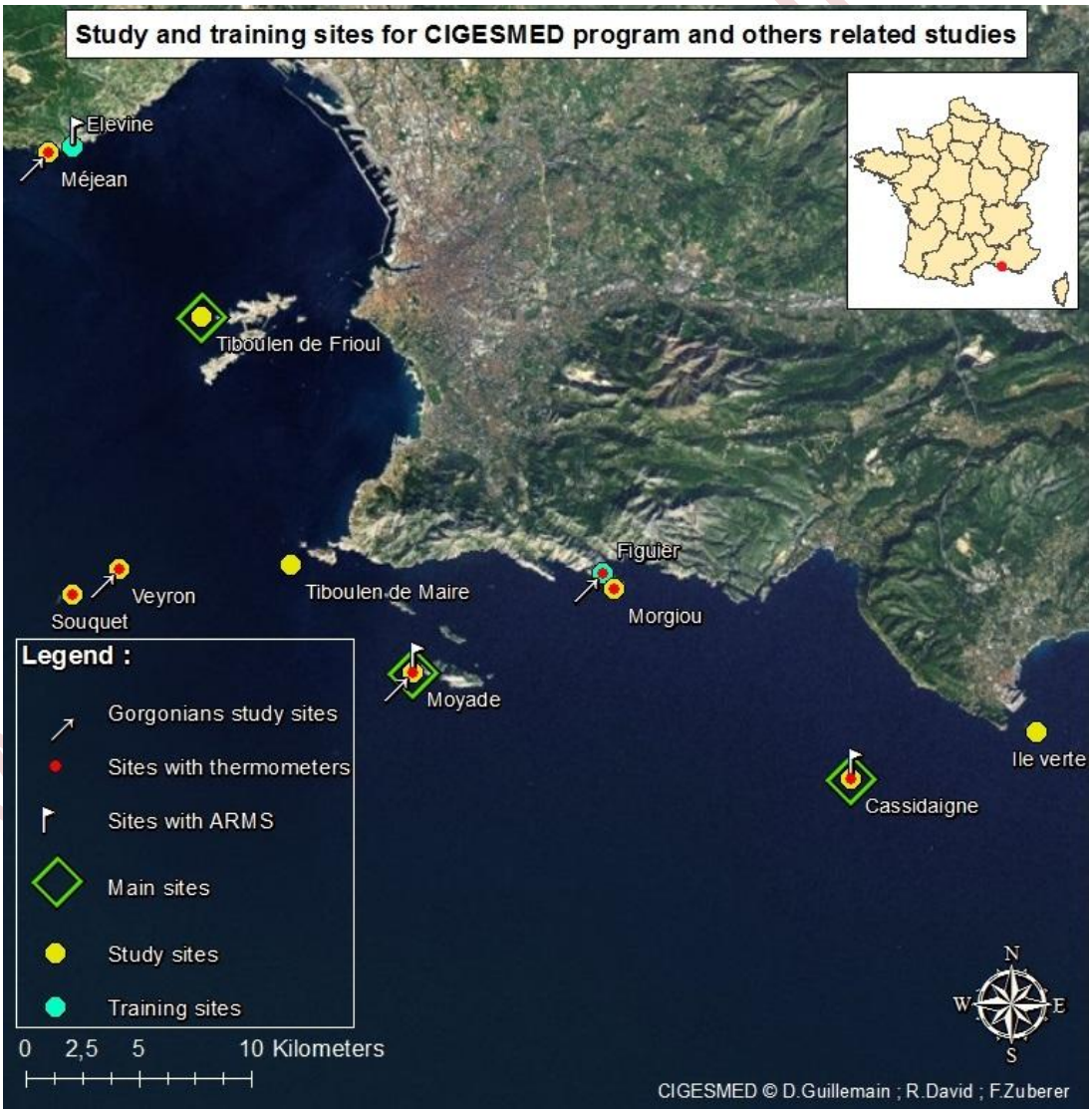
[profile] : the capital letter corresponding to the profile on which the scraping is done (e.g. « V » for « Vertical »).

[position] : location of scraping related to the starting point. Scraping is doable on 4 positions: TOP, BOTTOM, LEFT, RIGHT.

The choice of nomenclature was brought to international terms in order to facilitate data exchange between different countries.

Marseilles' study sites, and associated calendar

Location name	Site	Code site	Latitude (°)	Longitude (°)
Sormiou	Figuier	SFI	43,2055	5,4465
Maire	Tiboulen de Maire	MTM	43,2082	5,3217
Planier	Veyron	VEY	43,2069	5,2529
Planier	Souquet	PSO	43,1995	5,2347
Méjean	Méjean	MEJ	43,3283	5,2247
Morgiou	Morgiou	MOR	43,2010	5,4510
Frioul	Tiboulen du Frioul	FTF	43,2805	5,2859
Riou	Moyade	RMO	43,1767	5,3707
Cassis	Cassidaigne	CCA	43,1457	5,5457
	Ile verte		43,1593	5,6199
	Elevine		43,3297	5,2347



Calendar

- October to november : 3 dives x 4 islands, 30 m deep for IMBE-Pytheas divers = **12 dives**
- December : 2 dives x 4 islands for the collect of 32 samples of 3 species at 30 m deep = **8 dives**. Done by divers from IMBE-Pytheas.
- December-January : 3 dives x 4 islands, at 45 m deep = **12 dives**. Done by divers from IFREMER.
- February : 2 dives x 4 islands for the collect of 32 samples of 3 species at 45 m deep = **8 dives.** Done by divers from IFREMER.
- January- February : scraped samples at 30 m and 45 m deep. 4 replicates of scraped samples x 2 profiles x 4 orientations. 2 dives per island. **8 dives done by IFREMER, 8 dives done by IMBE-Pytheas**.
- In case of bad weather, one island won't be sampled (Ile Verte).
- Spring : 4 islands added for the cartography + collect of 32 individuals (5 dives per island and per depth) = **20 dives IFREMER and 20 dives IMBE-Pytheas.**
- Automn 2014 : scraped samples done on 8 islands = **16 dives IFREMER and 16 dives IMBE-Pythéas.**

This program is very ambitious. But we could improve the efficiency using nitrox (2 ives at 30 m deep and at 45 m deep per day). We also coul increase the number of divers.

Turkish study sites, and associated calendar

TO COMPLETE by turkish

Greek study sites, and associated calendar

TO COMPLETE by greek

Sites summary per country

Country	Zone	Cartography	Myriapora truncata	*Lithophyllum stictaeforme/cabiochiae*
France	Marseille	Entire[1]	2 sides on 3 main sites	2 sides on 3 main sites
	Marseille	Partial[2]	2 sides on 4 sites	2 sides on 4 sites
	Corse	Partial	2 sides on 3 main sites	2 sides on 3 main sites
Greece	Grèce 1	Partial	2 sides on 3 main sites	2 sides on 3 main sites
	Grèce 2	Partial	2 sides on 3 main sites	2 sides on 3 main sites
Turkey	Turquie 1	Partial	3 main sites	2 sides on 3 main sites
	Turquie 2	Partial	2 sides on 3 main sites	2 sides on 3 main sites

Country	Zone	Cartography	Permanent quadrat	Random quadrat	Inter-calibration
France	Marseille	Entire	1 site	3 sites	1 site
	Marseille	Partial	0 site	4 sites	NA
	Corse	Partial	NA	3 sites	NA
Greece	Grèce 1	Partial	?	3 sites	1 site
	Grèce 2	Partial	NA	3 sites	NA
Turkey	Turquie 1	Partial	NA	1 site	NA
	Turquie 2	Partial	NA	1 site	NA

[1] Complete record of profiles detailing all fields as recommended by the protocol.
[2] Mapping limited to profiles interesting to be taken (i.e. predetermined profiles for which the profile frequency is proven, and the frequency of *Myriapora truncata* and *Lithophyllum cabiochiae* populations are sufficient to be sampled). This mapping will be mainly implemented for the samples on secondary sites, which are distant or for which there is no sufficient continuum of coralligenous bio-concretions to perform a complete mapping. This may also be the case for the second depth (around 43 m), less accessible, to shorten the intervention time.

Typology of the data expected for the cartography

Data	Définition des données	Possiblilités ou exemple	Définition des possibilités ou des exemples
Date	Date on which the statements were made	20140324	25 march 2014
Site	Three letter code site	e.g : CCA	Cassis cassidaigne
Transect	The number of transect	e.g : T01	Transect number 1
Depth type	The depth to which would be due readings	D1	Corresponds to 28 meters
		D2	Corresponds to 43 meters
Real depth	Depth at which to took readings	e.g : 27	27 meters
Observeur	Oberver code (First letter of first name, first letter of name and two-digit number)	e.g : RD01	Romain David 01
N° segment	Segment numbers	e.g : S01	Segment number 1
Orientation (ORI)	Orientation of the wall	ORI_A1_S	South
		ORI_B1_SW	South-West
		ORI_B2_SE	South-East
		ORI_C1_W	West
		ORI_C2_E	East
		ORI_D1_NW	North-West
		ORI_D2_NE	North-East
		ORI_E1_N	North
Slope (INC)	Slope of the wall	INC_A_FLAT	Flat
		INC_B_INCLINED	Inclined
		INC_C_VERTICAL	Vertical
		INC_D_CEILING	Ceiling
Rugosity (RUG)	Rugosity of the wall	RUG_A_TINY	Holes do not allow the passage of a fist
		RUG_B_SMALL	Crevices allow the passage of a fist at most
		RUG_C_MEDIUM	A head can enter in one of the segment's hole at least
		RUG_D_LARGE	Crevices can contain at least, the upper body
ES (*Eunicella Singularis*)	Abundance d'*Eunicella singularis*	Low	0 % < cover < 20 % or 1 to 4 erect individuals
		Medium	20 % < cover < 50 % or more than 4 erect individuals
		Maxi	Cover > 50 % or either high density covering at least 50 % of the segment
EC (*Eunicella cavolini*)	Abundande d'*Eunicella cavolini*	Low	0 % < cover < 20 % or 1 to 4 erect individuals

		Medium	20 % < cover < 50 % or more than 4 erect individuals
		Maxi	Cover > 50 % or either high density covering at least 50 % of the segment
PC (*Paramuricea clavata*)	Abundance de *Paramuricea clavata*	Low	0 % < cover < 20 % or 1 to 4 erect individuals
		Medium	20 % < cover < 50 % or more than 4 erect individuals
		Maxi	Cover > 50 % or either high density covering at least 50 % of the segment
CR (*Corallium rubrum*)	Abundance de *Corallium rubrum*	Low	0 % < cover < 20 % or 1 to 4 erect individuals
		Medium	20 % < cover < 50 % or more than 4 erect individuals
		Maxi	Cover > 50 % or either high density covering at least 50 % of the segment
ERA	Encrusting red algae recovery	Low	0 % < cover < 20 % or 1 to 4 erect individuals
		Medium	20 % < cover < 50 % or more than 4 erect individuals
		Maxi	Cover > 50 % or either high density covering at least 50 % of the segment
FRA	Abundance of foliose red algae	Low	0 % < cover < 20 % or 1 to 4 erect individuals
		Medium	20 % < cover < 50 % or more than 4 erect individuals
		Maxi	Cover > 50 % or either high density covering at least 50 % of the segment
EGA	Encrusting green algae recovery	Low	0 % < cover < 20 % or 1 to 4 erect individuals
		Medium	20 % < cover < 50 % or more than 4 erect individuals
		Maxi	Cover > 50 % or either high density covering at least 50 % of the segment
FGA	Abundance of foliose green algae	Low	0 % < cover < 20 % or 1 to 4 erect individuals
		Medium	20 % < cover < 50 % or more than 4 erect individuals
		Maxi	Cover > 50 % or either high density covering at least 50 % of the segment

Sponges	Abundance of sponges	Low	0 % < cover < 20 % or 1 to 4 erect individuals
		Medium	20 % < cover < 50 % or more than 4 erect individuals
		Maxi	Cover > 50 % or either high density covering at least 50 % of the segment
Codium	Abundance of codium	Low	0 % < cover < 20 % or 1 to 4 erect individuals
		Medium	20 % < cover < 50 % or more than 4 erect individuals
		Maxi	Cover > 50 % or either high density covering at least 50 % of the segment
Bryozoa	Abundance of bryozoa	Low	0 % < cover < 20 % or 1 to 4 erect individuals
		Medium	20 % < cover < 50 % or more than 4 erect individuals
		Maxi	Cover > 50 % or either high density covering at least 50 % of the segment
Ascidaceae	Abundance of ascidaceae	Low	0 % < cover < 20 % or 1 to 4 erect individuals
		Medium	20 % < cover < 50 % or more than 4 erect individuals
		Maxi	Cover > 50 % or either high density covering at least 50 % of the segment
Others	Abundance of others spicies	Alcyo (Low)	*Alcyonium spp.* présent sur 0 à 20 % du segment
		Lepto (Medium)	*Leptosamia pruvoti* present sur 20 à 50 % du segment
		ParaZ (maxi)	Forte abondance de *Parazoxanthus axinella* sur au moins 50 % du segment
Remarkable individus	Name of remarkable individual on the segment	Corallium rubrum (Low)	Low density of *Corallium rubrum*
Remarkable population	Name of remarkable population on the segment	Parazoanthus axinella (Maxi)	High abundance of *Parazoanthus axinella*
Comment(s)	All comments on the data segment	Waste	< 33 cm
			From 33 cm to 1 m
			> 1 m
		Net	< 1
			From 1 to 5 m
			> 5 m

Example of a table reporting data for the cartography

Date YYYYMMDD	Site (3 caracters)	n° segment	Depth type	Real depth (m)	Observer	Orientation	Slope	Rugosity	*ES*	*EC*	*PC*	*CR*	ERA	FRA	EGA	FGA	Sponge	Codium	Bryozoa	Ascidaceae	Others	Remarkable individus	Remarkable population	Remarque(s)
20140411	LPD	1B	D1	28	RD01	ORI_E1_N	INC_B_INCLINED	RUG_D_LARGE		Medium			Medium	Medium	Low			Low	Medium					
20140411	LPD	2B	D1	28	DG01	ORI_D1_NW	INC_B_INCLINED	RUG_D_LARGE		Medium			Medium	Medium	Low			Low	Medium					
20140411	LPD	3B	D1	28	RD01	ORI_C1_W	INC_B_INCLINED	RUG_D_LARGE		Medium			Medium	Medium	Low			Low	Maxi					
20140411	LPD	4B	D1	28	DG01	ORI_D1_NW	INC_B_INCLINED	RUG_C_MEDIUM		Medium			Medium	Medium	Low				Maxi					
20140411	LPD	5B	D1	28	RD01	ORI_E1_N	INC_B_INCLINED	RUG_C_MEDIUM		Medium			Medium	Maxi	Low				Medium					
20140411	LPD	6B	D1	28	DG01	ORI_E1_N	INC_B_INCLINED	RUG_C_MEDIUM		Medium			Medium	Maxi	Low				Medium					
20140411	LPD	7B	D1	30	RD01	ORI_D2_NE	INC_B_INCLINED	RUG_C_MEDIUM		Medium			Medium	Maxi	Low				Medium					
20140411	LPD	8B	D1	30	DG01	ORI_C2_E	INC_B_INCLINED	RUG_C_MEDIUM		Medium			Medium	Maxi	Low			Low	Medium					
20140411	LPD	9B	D1	28	RD01	ORI_C2_E	INC_B_INCLINED	RUG_D_LARGE		Medium			Medium	Maxi	Low			Low	Maxi					
20140411	LPD	10B	D1	28	DG01	ORI_C2_E	INC_B_INCLINED	RUG_C_MEDIUM		Medium			Medium	Maxi	Low			Low	Maxi					
20140411	LPD	1A	D1	28	FZ01	ORI_C2_E	INC_C_VERTICAL	RUG_D_LARGE		Medium			Medium	Medium	Low				Maxi					
20140411	LPD	2A	D1	28	FZ01	ORI_C2_E	INC_C_VERTICAL	RUG_D_LARGE		Medium			Medium	Medium	Low				Maxi					
20140411	LPD	3A	D1	30	FZ01	ORI_C2_E	INC_B_INCLINED	RUG_C_MEDIUM		Medium			Medium	Medium	Low				Maxi					
20140411	LPD	4A	D1	32	FZ01	ORI_D2_NE	INC_B_INCLINED	RUG_C_MEDIUM		Medium			Medium	Medium	Low				Maxi					
20140411	LPD	5A	D1	30	FZ01	ORI_D2_NE	INC_B_INCLINED	RUG_A_TINY		Medium			Medium	Medium	Low				Maxi					
20140411	LPD	6A	D1	29	FZ01	ORI_E1_N	INC_A_FLAT	RUG_A_TINY		Medium			Medium	Medium	Low			Medium	Medium					
20140411	LPD	7A	D1	29	FZ01	ORI_E1_N	INC_A_FLAT	RUG_A_TINY		Medium			Medium	Medium	Low			Medium	Medium					

Species list

Species	Taxonomiste and date	French name(s)	Grec name(s)	Turc name(s)	Abundance (ZNIEFF typoloy)	IndexCor Sensibility	Level of taxonomy
ALGAE							
Codium bursa	(Olivi) C.Agardh, 1817	Béret basque, Codium boule	Ascos	Bask beresi	C	NA	1
Codium coralloides	(Kützing) P.C. Silva, 1960	Codium mamelonné			C	NA	1
Codium vermilara	(Olivi) Delle Chiaje, 1829	Codium fragile, Codium orvet, algue chou-fleur		Yesil kece yosunu	B	NA	2
Codium effusum	(Rafinesque) Delle Chiaje, 1829	Codium étalé			B	NA	2
Palmophyllum crassum	(Naccari) Rabenhorst, 1868	Palmophylle	Palmophyllo		B	8	1?
Pseudochlorodesmis furcellata	(Zanardini) Borgesen, 1926				B	NA	2?
Valonia macrophysa	Kützing, 1843	Valonie grosse bulle			A OU B	8	2
Halimeda tuna	(J. Ellis & Solander) J.V. Lamouroux, 1816	Monnaie de Poséidon, Halimède	Halimeda		C	6	1
Flabellia petiolata	(Turra) Nizamuddin, 1987	Udotée	Flabellia		C	6	1
Dictyota dichotoma	(Hudson) J.V.Lamouroux, 1809	Dictyote jaune	Kitrino dikti	Sari Dictyota	B	NA!	2
Dictyota fasciola	(Roth) J.V. Lamouroux, 1809				B	NA	2
Dictyopteris polypodioides	(A.P.De Candolle) J.V. Lamouroux, 1809	Fougère de mer, Parfum d'Antée			B	NA	2
Halopteris filicina	(Grateloup) Kützing, 1843				B	NA	2
Zanardinia typus	(Nardo) P.C.Silva, 2000				B	NA	2
Phyllariopsis brevipes	(C.Agardh) E.C.Henry & G.R.South, 1987	Algue petite feuille de tabac			A	NA	2
Cystoseira zosteroides	C.Agardh, 1820	Cystoseire profonde			A	spp 8	2?
Chrysymenia ventricosa	(J.V.Lamouroux) J.Agard, 1842	Algue branchue cartilagineuse, chrysyménia	Khrisiménia	Kartilajli catal yosun	A	NA	2? (*Halymenia floresii+Halymenia elongata*)

Halymenia elongata	C.Agardh, 1822	Halyménia allongée			B	NA	2? (*Halymenia floresii+Chrysymenia ventricosa*)
Gloiocladia repens	(C.Agardh) Sanchez & Rodriguez-Prieto, 2007	Gloioclade rampant			A	NA	1
Sphaerococcus coronopifolius	Stackhouse, 1797	Sphérocoque	Sfèrokokkos	Kirmizi yosun	A	NA	2
Peyssonnelia spp.		Peyssonnelia	fiki tou Pessonnel, Triantafullo tis thalassas	Deniz gülü	C	4-6	1
Lithophyllum cabiochiae	(Boudouresque & Verlaque) Athanasiadis	Feuille de pierre encorbellée	Lithophyllo		C	NA	3
Lithophyllum stictaeforme	(Areschoug) Hauck	Feuille de pierre encorbellée	Lithophyllo		C	NA	3
Mesophyllum expansum	(Philippi) Cabioch & M.L.Mendoza, 2003	Mésophylle	Mesofilo	Tas yaprak yosun	C	spp 9	1
Mesophyllum alternans	(Foslie) Cabioch & M.L.Mendoza, 1998				C	spp 4	3
Halymenia floresii	(Clemente) C.Agard, 1817	Halymène pourpre	Porfiri khaliménia	Kirmizi yosun	B	NA	2?
Wrangelia penicillata	(C.Agardh) C. Agardh, 1828	Algue duvet	Poupoulofikia	Kustüyü alga	B	NA	2?
MOLLUSCA							
Peltodoris atromaculata	Bergh, 1880	Doris dalmatienne, doris maculée, doris léopard	Gymnovrankhio dalmatinass	Dalmacyali doris	C	NA	1
Flabellina affinis	Gmelin, 1791	Flabelline mauve	Flavellina	Mor tüyuclu deniztavsani	B	NA	2
Flabellina pedata	Montagu, 1815	Coryphelle mauve	Korifella	Mor tüyuclu deniztavsani	B	NA	2
Felimare picta	Schultz in Philippi, 1836	Doris géante, hypselodoris??	Gigantio gymnovrankhio	Dev deniztavsani	A	NA	1
Janolus cristatus	Delle Chiaje, 1841	Antiopelle			B	NA	1
Pleurobranchus testudinarius	Cantraine, 1835	Pleurobranche tortue		Kaplumbaga deniztavsani	A	NA	1
Euthria cornea	Linaeus, 1758	Buccin veiné, buccin corné			A	NA	2?
Bittium circa	Moreno, 2006				A	NA	2?
Pinna nobilis	Linnaeus, 1758	Grande nacre, Nacre, jambonneau hérissé, Jambonneau de mer, Pinne noble, Pinne géante	Pinna		B	NA	1

Umbraculum umbraculum	Lightfoot, 1786	Ombrelle	Ombréla	Şemsiyeli deniztavşanı	B	NA	2
Aplysia spp.	Linnaeus, 1767	Lièvre de mer			A	NA	1
Thuridilla hopei	Vérany, 1853	Thuridille, thuridille de Hope, thuridille splendide, élysie rayée, thuridille rayée	Thouridilla		A	NA	2
Felimida britoi	Ortea & Pérez, 1983	Doris de Brito; doris d'Alberto Brito		Alberto Brito dorisis	A	NA	1
Felimida krohni	Vérany, 1846	Doris de Krohn, doris d'Auguste Krohn	Dooriss tou Krohn	August Krohn dorisi	A	NA	2
Felimida luteorosea	Rapp, 1827	Doris à tache d'or, doris tacheté mauve		Mor lekeli doris	B	NA	1
Felimida purpurea	Risso in Guérin, 1831	Doris Pourpre, doris rose		Pembe doris	A	NA	2
Felimare fontandraui	Pruvot-Fol, 1951	Doris de Fontandrau		Fontandrau dorisi	A	NA	2
Felimare tricolor	Cantraine, 1835	Doris tricolore		Uçrenkli doris	B	NA	2
Felimare villafranca	Risso, 1818	Doris de Villefranche		Villefrans doris	A	NA	2
Felimare orsinii	Vérany, 1846	Doris céleste		Göyüzü doris	B	NA	2
Felimare cantabrica	Bouchet & Ortea, 1980	Doris de Cantabrique, doris de cantabrique, Limace Gordini		Dev lacivert doris	A	NA	2
Doris verrucosa	Linnaeus, 1758			Siğilli doris	B	NA	1
Cratena peregrina	Gmelin, 1791	Hervia, hervia processionnaire, limace pélerine	Khervia	Gezgin deniztavaşanı	B	NA	2
Dondice banyulensis	Portmann & Sandmeier, 1960	Godiva, godive orange		Turuncu tüylü deniztavaşanı	A	NA	1
Flabellina ischitana	Hirano & Thompson, 1990	Flabelline d'Ischia		Ischia deniztavaşanı	B	NA	2
Doris pseudoargus	Rapp, 1827	Citron de mer, doris citron			B	NA	1
Callistoctopus macropus	Risso, 1826	Poulpe tacheté, pieuvre ou poulpe à longs bras, grande pieuvre, poulpe moucheté	Lefkostikto khtapodi		B	NA	1
Octopus vulgaris	Cuvier, 1797	Poulpe commun	Kino khtapodi		A	NA	1
Lithophaga lithophaga	Linnaeus, 1758	Datte de mer			C	NA	1
ANNELIDAE and others							
Eupolymnia nebulosa	Montagu, 1818	Polymnie nébuleuse, Térébelle filamenteuse, ver spaghetti	Polimnia	Cüce tüplükurt	B	4	2

Protula spp.	Montagu, 1803	Protule lisse	Kokkini protoula	Yasi tüplükurt	C	NA	1
Sabella spallanzanii	Gmelin, 1791	Spirographe	Spirografos	Semsiye tüplükurt, deniz lalesi	B	NA	1
Sabella pavonina	Savigny, 1822	Sabelle, sabelle paon	Sabella pagoni	Tavuskuşu tüplükurdu	A	NA	3
Bispira volutacornis	Montagu, 1804				B	NA	1
Serpula vermicularis	Linnaeus, 1767	Serpule, petite serpule, serpule commune	Mikri erpili	Kirmizi tüplüsolucan	A	NA	1
Salmacina spp / Filograna spp	Claparède, 1870 / Berkeley, 1835				C	NA	1
Bonellia viridis	Rolando, 1821	Bonelie verte, ver hérissé	Bonellia	Denizkurd	C	4	1
BRYOZOA							
Adeonella calveti	Canu & Bassler, 1930	Adéonelle	Adéonella	Adeonella	B	11	2
Adeonella pallasii	Heller, 1867	Adéonelle de Méditerranée orientale	Adéonella	Adeonella	B	NA	3
Reteporella spp.	Busk, 1884	Dentelle	Dandéla	Danteli	B	10	2
Smittina cervicornis	Pallas, 1766	Bryozoaire bois de cerf	Vriozo'o elaphokérato	Geyikboynuzu byrozoa	B	11	2
Turbicellepora avicularis	Hincks, 1860	Cellépore ramifiée, Turbicellépore cornu	Dendrodèss vriozo'o	Dallı byrozoa	C	10	1
Myriapora truncata	Pallas, 1766	Faux corail	Psefdocoralli	Yalanci mercan	C	7	1
Schizotheca serratimargo	Hincks, 1886	Schizorétépore	Schizorétépora	Schizoretepora	A	11	2
Schizomavella mamillata	Hincks, 1880	Schizomavelle mamelonnée	Schizomavella	Tepeli bryozoa	B	spp 6	2
Pentapora fascialis	Pallas, 1766	Rose de mer	Thalassio rodo	Turuncu byrozoa	C	9	1
Hornera frondiculata	Linnaeus, 1758	Hornère frondiculée	Ornéra	Hornera	A	NA	1
Frondipora verrucosa	Lamouroux, 1821	Frondipore verruqueux	Frondipora mè lépia	Siğilli bryozoa	A	NA	2
Gregarinidra gregaria	Heller, 1867				A	NA	1
Rhynchozoon spp.	Hincks, 1895	Rhynchozoon d'Europe			B	NA	3
Beania hirtissima cylindrica	Hincks, 1886	Béania enroulé, béania cylindrique, bryozoaire enroulé	Tilighméno vriozo'o	Sarılı bryozoa	B	NA	2
Caberea boryi	Audouin, 1826	Bryozoaire à fouet, Crisie de Bory, Cabérée de Bory			B	NA	2
Cellaria spp.	Ellis & Solander, 1786				B	NA	2
Diporula verrucosa	Peach, 1868	Diporula			B	NA	3

Idmidronea spp.	Canu & Bassler, 1920				B	NA	3
Dentiporella sardonica	Waters, 1879	Bryozoaire corne d'élan	Kerasforo vriozo'o	Geyik boynuzu bryozoa	B	NA	2
UROCHORDATA							
Clavelina spp.	Savigny, 1816	/	/	/	B	NA	2
Diazona violacea	Savigny, 1816	Diazone, Estouffat, Ascidie glauque, "grande claveline"			B	NA	2
Halocynthia papillosa	Linnaeus, 1767	Ascidie rouge, violet rouge, outre de mer	Kokkino askidio	Kirmizi tünikat, kirmizi deniz tulumu	C	11	1
Diplosoma spp.	Macdonald, 1859	Didemne gélatineux et transparent			A	NA	3
Phallusia mammillata	Cuvier, 1815	Ascidie blanche, vioulé blanc, ascidie mamelonnée, phallusie	Askdhio phalloss	Beyaz tünikat, deniz tulumu	A	spp 4	2
Phallusia fumigata	Grube, 1864	Ascidie noire, Phallusie noire			A	spp 4	2
Ciona edwardsi	Roule, 1884	Ascidie jaune, cione jaune d'Edwards	kitrinophouska	Sari tünikat	B	NA	2
Pyura dura	Heller, 1877	Violet à bouche rose, ascidieaux siphons roses	rhodosiphoni phouska	pembe agizli tünikat	A	NA	1
Aplidium undulatum	Monniot & Gaill, 1978	Aplidium café au lait			A	NA	3
Aplidium pseudolobatum	Pérès, 1956				A	NA	3
***Aplidium* spp.**	Savigny, 1816	Synascidies	Synaskidhia	Koloni tünikat	A	NA	3
Cystodytes dellechiajei	Della Valle, 1877	Ascidie coloniale pourpre			B	NA	3
Didemnum drachi	Lafargue, 1975	Didemne orange du coralligène			B	NA	3
***Polysyncraton* spp.**	Nott, 1892	Didemne			B	NA	3
Polyclinum aurantium	Milne-Edwards, 1841	Couille d'âne			C	NA	1
Pycnoclavella spp.	Garstang, 1891	Ascidie naine			C	NA	1
Aplidium proliferum	Milne-Edwards, 1841	Ascidie coloniale ovoïde			B	NA	1
CNIDARIA							
Aiptasia mutabilis	Gravenhorst, 1831	Aiptasie verte, anémone trompette, enémone de verre, aiptasie bleue, aitasie de Couch	Èptasia, anémoni trombéta	Yeşil tüp anemon	B		NA
Cereus pedunculatus	Pennant, 1777	Anémone solaire, anémone palmée	Anémoni tou hiliou, hiliaktida	Saplı anemon	C		NA

Cribrinopsis crassa	Andrès, 1881	Anémone charnue	Khondranémoni	Şişko anemon	B		NA
Anemonia viridis	Forskål, 1775	Anémone verte, ortique, actinie verte	Prasini anémoni	Moryeşil anemone, deniz sakayigi	B		NA
Condylactis aurantiaca	Delle Chiaje, 1825	Anémone soleil, anémone dorée	Khrisanémona	Altin anemon	C		NA
Alicia mirabilis	Johnson, 1861	Anémone feu, anémone d'herbier, anémone alicie	Tiiss fotiass	Ateş tüp anemon	B		NA
Cerianthus membranaceus	Spallanzani, 1784	Grande cérianthe	Membranodiss kyrianthoss	Tüp anemone, deniz lalesi	B		spp 4
Parazoanthus axinellae	Schmidt, 1862	Anémone encroutante jaune, mimosa de mer	Kitrini anémoni	Sari deri anemone	C		6
Epizoanthus arenaceus	Delle Chiaje, 1823	Anémone encroûtante beige	Kafé épipédi anémoni	Kahverengi deri anemone	B?		NA
Corynactis viridis	Allman, 1846	Anémone-bijou, anémone-perle	Anémonia kosmima	Inci mercan	C		NA
Caryophyllia (Caryophyllia) inornata	Duncan, 1878	Madrépore œillet, petite dent de chien	Koralli gariphallo	Karanfil mercan	B		6
Caryophyllia (Caryophyllia) smithii	smithii Stokes & Broderip, 1828	Dent de chien			B		NA
Balanophyllia (Balanophyllia) europaea	Risso, 1826	Dent de cochon	Dondi gourouniou	Domuz dişi mercan	B		NA
Hoplangia durotrix	Gosse, 1860	Corail nain	Khoplangia	Cüce mercan	B		NA
Leptopsammia pruvoti	Lacaze-Duthiers, 1897	Corail jaune solitaire	kitrino moniress koralli	Sarı düğme mercan	C		5
Madracis pharensis	Heller, 1868	Madracis, Madrace étoile	Pharos	Yıldız mercan	A		NA
Phyllangia americana mouchezii	Lacaze-Duthiers, 1897	Madrépore colonial sombre, corail d'Amédée Mouchez	Koralli nanos	Amédée Mouchez mercanı	A		spp 8
Cladocora caespitosa	Linnaeus, 1767	Cladocore	Cladocora	Taş mercan	B		10
Aglaophenia spp	Lamouroux, 1812	Hydraire plume	Ptéroïdrozo'o	Tüy hidra	C		NA
Eudendrium spp	Ehrenberg, 1834	Eudendriums de Méditerranée, hydraire dendriforme	Dendroïdrozo'o	Ağac hidra	B?		NA
Pteroeides griseum	Linnaeus, 1767	Pennatule grise	Féo ftéro tiiss thalassass	Gri deniz tüyü	B?		NA
Alcyonium acaule	Marion, 1878	Alcyon méditerranéen, magnotte	Alkionario, khéri tiiss thalassass	Deniz parmağı mercani	B?		NA
Alcyonium coralloides	Pallas, 1766	Alcyon encroûtant	Parasitiko alkionario	Deri mercan	B		NA
Eunicella verrucosa	Pallas, 1776	Gorgone verruqueuse	Gorgonia mè élièss	Siğilli gorgon	B?		7

Eunicella singularis	Esper, 1791	Gorgone blanche	Lefki gorgonia	Beyaz gorgon	C		10
Eunicella cavolini	Koch, 1887	Gorgone jaune	Kitrini gorgonia	Sarı gorgon, agacsi mercan	C		9
Paramuricea clavata	Risso, 1826	Gorgone rouge, gorgone pourpre	Kokkini gorgonia	Kırmızı gorgon	C		10
Leptogorgia sarmentosa	Esper, 1789	Gorgone orange	Portokali gorgonia	Portakal rengi gorgon	B		4
Corallium rubrum	Linnaeus, 1758	Corail rouge de Méditerranée, sang de Neptune, corail des bijoutiers, corail de Sardaigne	Tiss Mésoghiou	Kırmızı mercan	C		10
Savalia savaglia	Bertoloni, 1819	Anémone buissonnante, faux corail noir, Zoanthaire arborescent	Dendranémoni	Siyah mercan	A?		NA
Tubipora musica	Linnaeus, 1758	Orgue de mer			A		spp 8
ECHINODEMATA							
Antedon mediterranea	Lamarck, 1816	Comatule de Méditerranée	Komatoula	Tüy yıldızı	B?	NA	1
Astrospartus mediterraneus	Risso, 1826	Gorgonocéphale, tête de méduse	Gorgonoképhaloss	Gorgon denizyıldızı	B?	NA	1
Ophiothrix fragilis	Abildgaard, in O.F. Müller, 1789	Ophiure fragile, Ophiure à piquants, ophiure singe	Kinoss ophiouross	Kırlıgan yılanyıldızı	B?	NA	1
Ophioderma longicauda	Bruzelius, 1805	Ophiure lisse, ophiure serpent, ophiure brune	Méghaloss lioss ophiouross	Yassı yılanyıldızı	B?	NA	1
Echinaster sepositus	Retzius, 1783	Etoile de mer rouge	Kokkinoss astériass	Kırmızı denizyıldızı	C	NA	2
Hacelia attenuata	Gray, 1840	Etoile de mer lisse		Düz denizyıldızı	C	NA	2
Marthasterias glacialis	Linnaeus, 1758	Etoile de mer glaciaire, astérie glaciaire, étoile épineuse	Astériass toonn paghoonn	Dikenli denizyıldızı	C	NA	1
Ophidiaster ophidianus	Lamarck, 1816	Etoile de mer violette, Astérie serpent, astérie pourpre	Mégaloss kokkinoss astérias	Mor denizyıldızı	A	NA	2
Chaetaster longipes	Retzius, 1805	Etoile de mer aux longs bras	Makripodoss astériass	Uzunkollu denizyıldızı	A	NA	2
Holothuria (Holothuria) tubulosa	Gmelin, 1791	Holothurie tubuleuse, vit de marin	Kili olothouria, kilo angouri tiiss thalassass	Denizhıyarı	A	spp 4	2
Holothuria sanctori	Delle Chiaje, 1823	Concombre cracheur marron, bêche de mer, holothurie auréolée	Pholidoti olothouria, pholidoto angouri tiiss thaassass	Halkalı, denizhıyarı	B	spp 4	1
Holothuria (Panningothuria) forskali	Delle Chiaje, 1823	Holothurie noire, bêche de mer, boudin de mer noir	Mavro angouri tiiss thalassass	Siyah denizhıyarı	C	spp 4	1

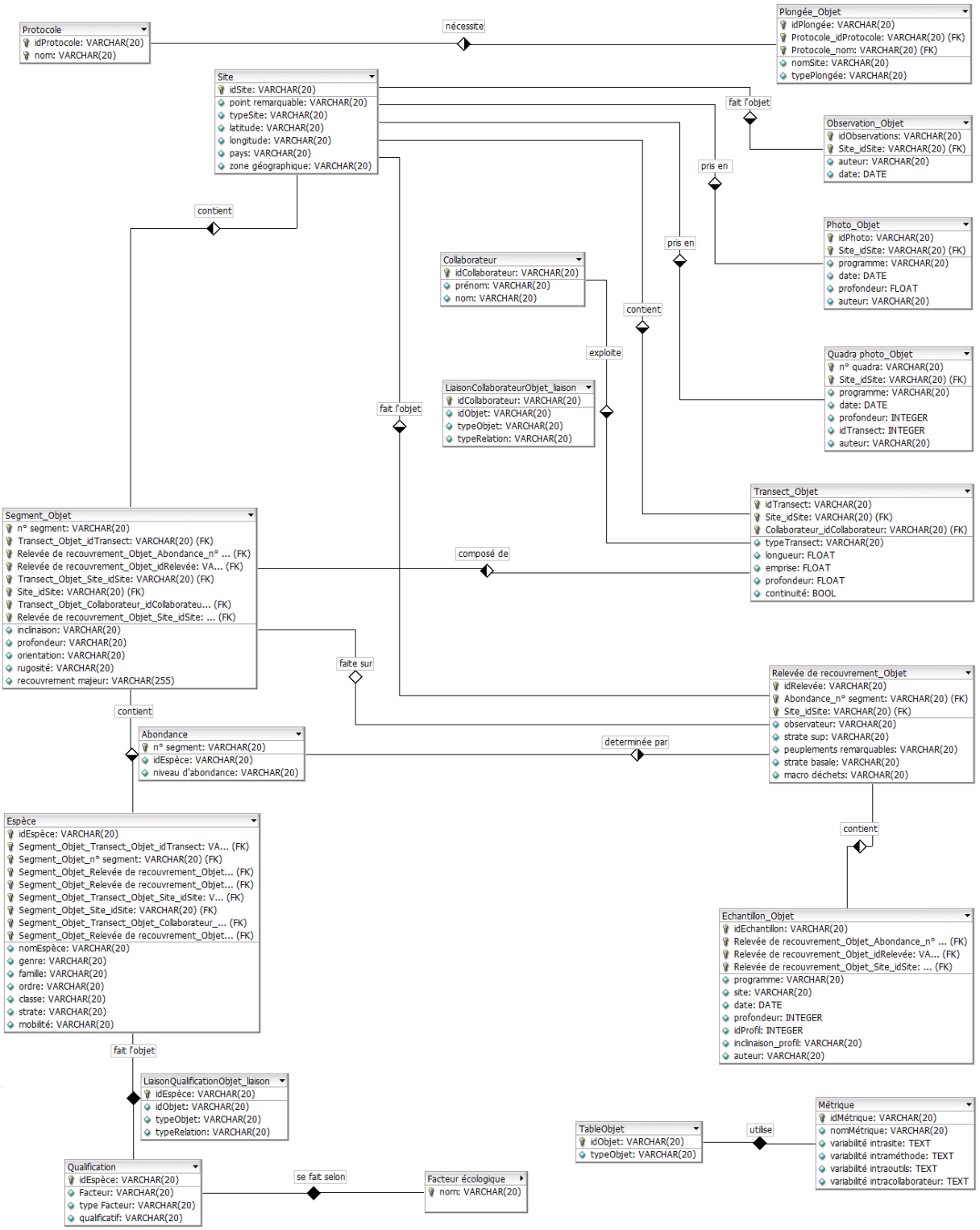
Ocnus planci	Brandt, 1835	Lèche-doigts de Plancus, holothurie brune, concombre de mer, cornichon de mer	Angouraki tiiss thalassass	Denizhıyarı	C	NA	2
Centrostephanus longispinus	Philippi, 1845	Oursin-diadème méditerranéen	Kentrostéohanoss	Uzun dikenli denizkestanesi	B	NA	1
Cidaris cidaris	Linnaeus, 1758	Oursin lance gris,	Molivakhinoss	Mızraklı denizkestanesi	A	NA	1
Sphaerechinus granularis	Lamarck, 1816	Oursin granuleux, oursin violet à pointes blanches, oursin émoussé, oursin lampion	Violétiss akhinoss, akhinoss balla	Beyaz dikenli mor denizkestanesi	C	NA	1
Echinus melo	Lamarck, 1816	Oursin melon	Péponakhinoss	Kavun denizkestanesi	B	NA	1
Arbacia lixula	Linnaeus, 1758	Oursin noir,oursin mâle	Akhinoss évréoss, mavros akhinoss	Siyah denizkestanesi	C	NA	2
Paracentrotus lividus	Lamarck, 1816	Oursin violet, oursin femelle, châtaigne de mer, oursin pierre	Vrosimoss akhinoss, kinoss akhinoss	Mor denizkestanesi	C	NA	2
Spatangus purpureus	O.F. Müller, 1776	Spatangue pourpre, oursin de vase violet	Spatangoss	Mor çamur denizkestanesi	A	NA	1
Stylocidaris affinis	Philippi, 1845	Oursin lance rouge, oursin crayon, oursin baguette, oursin porte-lance	Kokkinoss molicakhinoss	Kırmızı mızraklı denizkestanesi	A	NA	2
Holothuria (Roweothuria) polii	Delle Chiaje, 1824	Concombre de mer ensablé, holothurie à pointes blanches, holothurie de Poli			A	spp 4	1
Coscinasterias tenuispina	Lamarck, 1816	Etoile de mer épineuse, étoile de mer bleue, étoile de mer irrégulière, étoile à fins piquants	Agathothoss astériass	Mavi denizyıldızı	B	NA	1
ARTHROPODA							
Galathea strigosa	Linnaeus, 1761	Galathée bicolore, galathée multicolore, galathée striée, écrevisse de mer	Polykhromi galatia	Dikenli bodur yengeç	B	NA	1

Galathea squamifera	Leach, 1814	Galathée noire, galathée verte, galathée écailleuse	Mavri galatia	Siyah bodur yengeç	A	NA	2
Munida rugosa	Fabricius, 1775	Galathée rose			B	NA	2
Homarus gammarus	Linnaeus, 1758	Homard européen	Astakokaravida, astakos	İstakos, stacoz	B	NA	1
Palinurus elephas	Fabricius, 1787	Langouste européenne, langouste commune, langouste rouge	Astakoss	Böcek, beudic	C	NA	1
Scyllarides latus	Latreille, 1803	Grande cigale de mer, cigale noir, cigale courte, grand scyllare	Mégali koolokhtipa, lyra	Büyük ayı istakozu, karavida	A?	NA	1
Scyllarus arctus	Linnaeus, 1758	Petite cigale de mer, cigale blanche, petit scyllare	Mikri koolokhtipa, tzitziki, astakoudaki	Cüce karavida, küçük ayi istakozu	A?	NA	1
Maja squinado	Herbst, 1788	Grande araignée de Méditerranée, esquinade	Megali arakhni tiss thalassass	Büyük örümcek yengeç, ayna	B?	NA	2
Maja crispata	Risso, 1827	Petite araignée de mer			B?	NA	2
Dromia personata	Linnaeus, 1758	Dromie, dromie velue, crabe-béret basque, crabe lanigère, crabe-pierre, crabe dormeur, crabe-éponge, crabe-nounours	Patata, dromiass	Ayi pavuryasi, yengeç	B?	NA	1
Scyllarus pygmaeus	Bate, 1888	Cigale de mer naine, cigale pygmée			A	NA	2
Galathea intermedia	Liljeborg, 1851	Galathée intermédiaire			A	NA	1
PLATHELMINTHE							
Pseudobiceros splendidus	Lang, 1884	Ver plat noir, ver plat splendide, planaire noire	Platihelmintha mavri	Siyah yassi solucan	A	NA	1
Yungia aurantica	Delle Chiaje, 1822	Ver plat orange, planaire orange	Platihelmintha portokali	Turuncu yassi solucan	A	NA	2
Prostheceraeus roseus	Lang, 1884	Ver plat rose de Méditerranée, planaire rose	Platihelmintha roz	Pembe yassi solucan	A	NA	3
Prostheceraeus vittatus	Montagu, 1815	Planaire blanche, grande planaire rayée	Platihelmintha lefki	Beyaz yassi solucan	A	NA	4
Prostheceraeus giesbrechtii	Lang, 1884	Ver plat bleu de Méditerranée, planaire bleu	Platihelmintha ble	Mavi yassi solucan	A	NA	5
Prostheceraeus moseleyi	Lang, 1884	Planaire tachetée, planaire de Moseley	Platihelmintha stikti	Benekli yassi solucan	A	NA	6

Prostheceraeus spp (albinos)	Schmarda, 1859	Ver plat albinos de Méditerranée, planaire albinos	Platihelmintha albino	Albino yassi solucan	A	NA	7
SPONGE							
Aplysina cavernicola	Vacelet, 1959	Eponge cavernicole jaune	Kitrino sfouggari	Altın mağara sungeri	C?	8	1
Axinella damicornis	Esper, 1794	Axinelle plate, axinelle ramure de daim	Axinelli	Axinella	C?	4	2
Axinella polypoides	Schmidt, 1862	Axinelle commune, éponge corne de cerf	Axinelli	Sari geyik boynuzu sünger	C?	8	2
Axinella verrucosa	Esper, 1794	Axinelle verruqueuse			C?	7	2
Chondrosia reniformis	Nardo, 1847	Eponge-rognon	Sfouggari néphro	Böbrek sünger, deri sünger	C?	5	1
Clathrina lacunosa (ex-Guancha lacunosa)	Johnston, 1842	Clathrine pédonculée	Clathria épimiskhia	Beyaz saplı sünger	C?	NA	1
Clathrina clathrus	Schmidt, 1864	Clathrine jaune	Kitrini clathria	Sarı dantel sünger	C?	NA	1
Cliona viridis	Schmidt, 1862	Clione verte, éponge perforante verte	Prasino diatritiko sfouggari	Yeşil dikenli sünger	C?	spp 4	2
Cliona celata	Grant, 1826	Clione jaune, éponge perforante jaune	Kitrini vioa, cliona	Sari delici sunger	B?	spp 4	1
Crambe crambe	Schmidt, 1862	Eponge encroûtante orange-rouge, éponge de spondyle	Kokkino sfouggari	Kırmızı kabuk sünger	C?	NA	2
Spirastrella cunctatrix	Schmidt, 1868	Eponge encroûtante orange	Plati portokali sfouggari	Portakal rengi kabuk sünger	C?	NA	2
Crella (Crella) elegans	Gray, 1867	Crellie élégante			A?	NA	3
Dysidea avara	Schmidt, 1862	Eponge cheminée rose			A?	4	1
Haliclona (Soestella) mucosa	Griessinger, 1971	Eponge baveuse à languettes			A?	9	2
Haliclona fulva	Topsent, 1893	Haliclone orange			B?	9	2
Haliclona mediterranea	Griessinger, 1971	Eponge tubulaire rose, haliclone rose	Aliclona	Pembe tüp sünger	A?	NA	1
Hemimycale columella	Bowerbank, 1874	Eponge rose à cratères, Eponge-cratères	Stylidio	Pembe kraterli sünger	C?	NA	1
Hexadella racovitzai	Topsent, 1896	Eponge fibreuse rose			B?	spp 10	3
Ircinia oros	Schmidt, 1864	Ircinie sombre, ircine grise	Féa isinia	Gri sünger	B?	spp 8	2
Ircinia variabilis	Schmidt, 1862	Ircinie variable, ircinie fibreuse	Agrio sfouggari	Değişkengri sünger	B?	spp 8	2

Oscarella lobularis	Schmidt, 1862	Oscarelle bleu-violet	Oscarella	Mavi sünger, mor sünger	C	12	2
Oscarella tuberculata	Schmidt, 1868	Oscarelle jaune-verdâtre	Oscarella	Mavi sünger, mor sünger	C	10	2
Petrosia ficiformis	Poiret, 1789	Eponge-pierre	Sfouggari pétra	Taş sünger, sert sünger	C	8	1
Phorbas tenacior	Topsent, 1925	Eponge encroûtante bleue	Plati kiano sfouggari	Mavimsi kabuk sünger	C	6	1
Pleraplysilla spinifera	Schulze, 1879	Eponge épineuse blanche	Sfounggari skantzokhiros	Beyaz dikenli sünger	B?	NA	1
Sarcotragus foetidus	Schmidt, 1862	Eponge fétide	Mavrosfouggaro	Siyah deli sünger	B?	NA	2
Sarcotragus spinosulus	Schmidt, 1862	Ircinie noire épineuse	Gaïdourosfouggaro	Siyah deri sungeri, dikenli siyah deri sünger	B?	NA	2
Scalarispongia scalaris	Schmidt, 1862	Eponge cornée noire, mauvaise éponge	Afti éléfanta	Siyah deli sünger, Siyah boynuzlu sunger	B?	NA	2
Spheciospongia papillosa	Ridley & Dendy, 1886				A?	NA	3
Spongia (Spongia) lamella	Schulze, 1879	Eponge oreille d'éléphant de Méditerranée			B?	8	2
Spongia officinalis	Linnaeus, 1759	Éponge de toilette, éponge fine	Sfouggari baniou	Banyo süngeri	B?	NA	2
Tethya aurantium	Pallas, 1766	Orange de mer de Méditerranée			C?	NA	1
Plakina trilopha	Schulze, 1880				A?	NA	3
Myceliospongia araneosa	Vacelet & Perez, 1998				A?	NA	3
Cacospongia spp	Schmidt, 1862	Eponge cornée noire			B?	5	3
Suberites spp	Nardo, 1833	Subérite			B?	NA	3
Aplysilla sulfurea	Schulze, 1878	Aplysille jaune soufre, éponge jaune sulfureuse			B?	NA	2
Hippospongia spp.	Schulze, 1879				B?	NA	3
Crella pulvinar	Schmidt, 1868	Eponge cratère jaune			B?	spp 11	1
Hexadella pruvoti	Topsent, 1896				B?	NA	3
Dictyonella spp.	Schmidt, 1868				B?	NA	3
Raspaciona aculeata	Johnston, 1842	Eponge rouge hérissée			B?	NA	3
Corticium candelabrum	Schmidt, 1862	Eponge-coussinet orange, éponge-chandelier	Sfouggari kiropighio	Yastıkçık sünger	B	NA	2
Hexadella racovitzai	Topsent, 1896	Eponge fibreuse rose			B?	spp 10	1

Conceptual Data Model of CIGESMED protocols



List of softwares used and/or recommended

Software's name	Type	Object	Version	Licence
Apache	Server application	Online web page via http	2.2.6	Open source
Gimp	Image processing	Image processing or maps /figures construction	2.8.10	Open source
ACDsee Pro	Photo manager	Management, organization, sorting and sharing of pictures	7	Commercial
Avast	Logiciel antivirus	Automatic protection and/or antivirus scan	9.0.2013	Open source / commercial
GoogleEarth	Virtual globe	Handling globe and input of information	7.1.2	Open source
Grass Gis	Geographic Information System		6.4.2	Open source
Inskape	Creation of vector drawings		0.48.2	Open source
Latex	Text editor	Writing, compiling texts	0.53	Open source
MySQL	Management system database		5.6.16	Open source
Mendeley desktop	Bibliography management	Insert references in a text, format bibliography	1.10.3	Open source
Notepad++	Source editor	Syntax highlighting and formatting	6.5.4	Open source
OpenOffice	Office suite	Word processing, spreadsheet, database presentation	4.0.1	Open source
Paint	Image processing	Modification, creation of image and drawing	Depends on the operating system	original
Php	HTML scripts editor		5.5.8	Open source

PhotoQuad	Photo processing	Photo-quadrat processing	1.0	Open source
Powerpoint	From Microsoft office professional plus	Creating and formatting a slideshow	2013	Commercial
QGis	Geographic Information System	Management of raster and vector maps, and database	1.2	Open source
R	Statistics	Linear and nonlinear modelling, classical statistical tests, classification, clustering, graphics	3.0.2	Open source
RDF Ticker	Search and display information from RSS/RDF		1.2	Open source
Spip	Publishing system for the internet	Layout of items, modification of website structure	3.0.14	Open source
Spybot	Anti spyware	Detection and removal of spyware	2.1.21	Open source
GoPro Studio	Underwater video editor		2.0.1.319	Commercial
XML				

List of photo and video devices used and/or recommended

LEICA DFC420C de LEICA Microsystems Ltd.

Ajouter des éléments du rapport de Romain BRICOUT

Lists of useful websites

Institutions and organizations:

- AZTI-tecnalia : http://www.azti.es/
- CEAB (Centre d'Estudis Avançats) : http://www.ceab.csic.es/web/
- CESCO : http://www2.mnhn.fr/cersp/
- CIESM (The Mediterranean Science Commission) : http://www.ciesm.org/
- CNRS : www.cnrs.fr
- Ege University : http://egefish.ege.edu.tr
- ESPACE (UMR 7300) : http://www.umrespace.org/
- HCMR : http://www.hcmr.gr
- ICM (Institut de Ciènces del Mar) : http://www.icm.cat/en
- IFREMER : http://www.ifremer.fr
- INRA : http://www.ecologiemicrobiennelyon.fr/?lang=en/
- LIGAMEN : http://ligamen.fr
- MedRecover : http://medrecover.org/
- MNHN (National Museum of Natural History) : http://www.mnhn.fr/fr
- NMPZ : www.nmp-zak.org
- OHM littoral méditerranéen : http://www.ohm-littoral-mediterraneen.fr/

Mailing lists

-

Tools

- Photoquad (photo quadrat analysis software) : http://www.mar.aegean.gr/sonarlab/photoquad

Programmes

- ANR ADACNI http://adacni.imbe.fr/
- CARTHAM http://cartographie.aires-marines.fr/?q=node/43
- BIODIVERSA : http://www.biodiversa.org/
- CIGESMED http://www.cigesmed.eu/
- DEVOTES http://www.devotes-project.eu/
- GDR MARCO http://wwz.ifremer.fr/gdrmarco
- PROTEKER http://www.proteker.net
  To complete by greek and Turkish

Social networks (dissemination vector)

- LinkedIn : https://www.linkedin.com/
- Mendeley : http://www.mendeley.com/
- Researchgate : https://www.researchgate.net
- Twitter : https://twitter.com/

- Viadeo **:** http://fr.viadeo.com/fr/

Species database

- Algaebase (base de données sur les « algues ») : http://www.algaebase.org
- Biodiversité méditerannéenne : http://meddiversa.medrecover.org/
- Coralligène : http://www.coralligene.fr
- Coralligenous species : http://corspecies.medrecover.org/
- Corallinales Identification Integrated System a project of Guido BRESSAN - Lorenza BABBINI - Furio POROPAT : http://www2.units.it/biologia/Corallinales
- Doris (Données d'Observations pour la reconnaissance et l'identification de la faune et de la flore subaquatique) : http://doris.ffessm.fr
- Emodnet seabed habitats : http://www.emodnet.eu/seabed-habitats
- Eol (Encyclopedia of life) : http://eol.org
- ILTER (Ecological Information Management System) : http://data.lter-europe.net/deims/search/dataset
- INPN : http://inpn.mnhn.fr/
- Le monde des échinodermes : http://www.echinodermes.org/
- Medrecover : http://corspecies.medrecover.org/detail.php
- Mediterranean slug site : http://medslugs.de/
- Phoronida : http://paleopolis.rediris.es/Phoronida/
- Photographe David Luquet : http://www.davidluquet.com/mediterrannee.php?ch=communs
- Recent and Fossil Bryozoa : http://www.bryozoa.net/
- Sea Slug Forum : http://www.seaslugforum.net/
- World Hydrozoa Database : http://www.marinespecies.org/hydrozoa/
- WoRMS (World Register of Marine Species) : http://www.marinespecies.org

Marine citizen science networks

- Biolitt : http://www.biolit.fr/ and http://www.aires-marines.fr/Connaitre/Sciences-participatives/Observations-de-l-estran
- Maldives Whale Shark Research Programme http://maldiveswhalesharkresearch.org/bigfishnetwork/
- Observateurs en plongée : http://www.observateurs-plongee.fr/
- Quizz vie marine : http://puteauxplongee.com/bio/quizz.php?nb=20
- Scratchpad : http://scratchpads.eu/
- Vigie mer : http://vigienature.mnhn.fr/

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Wirtz P., Debelius H., 2003, Mediterranean and atlantic, invertebrate guide, ConchBooks, Hackenheim, Germany. 305 p.

(Ouvrage collectif), 2000, L'inventaire znieff-mer dans les DOM : bilan methodologique et mise en place, Coll. Patrimoines naturels, Publications scientifiques du M.N.H.N., Paris, Vol. 42, 228p.

(Ouvrage collectif), 2004, Cahiers d'habitats natura 2000 : connaissance et gestion des habitats et des espèces d'intérêt communautaire, Tome 2 Habitats côtiers, ed. La documentation française, 399p. (avec CD-ROM).

List and map of marine station and laboratory of oceanology

French coastline (Mediterranean only)

- Laboratoire océanologique de Perpignan
- Station marine d'Endoume (SME), Marseille
- Station méditerranéenne de l'environnement littoral (SMEL), Sète
- Station océanologique de Monaco
- Observatoire océanologique de Banyuls-sur-Mer
- Observatoire océanologique de Villefranche- sur-Mer
-
- Station marine de La Trinité-sur-Mer
- Station marine de Bouin
- Station marine de Palavas-les-Flots
- Toulon ?
- Perpignan
- Nice (ecomer)
- Montpellier

Greek coastline (Mediterranean only)

To Complete

Turkish coastline (Mediterranean only)

To Complete

Determination key for coralline algae (from M. VERLAQUE)

ENCRUSTING CORALLINALES

1. Cell fusions + secondary pit connections, isolate sporangia (no conceptacles) …….. ***Sporolithon ptychoides***

1. Secondary pit connections, conceptacles uniporate…….. 2

2. Two opposite palisade layers…….. ***Tenarea undulosa***

2. One basal palisade layer, erect filaments (Y/N) coaxial…… ***Titanoderma* spp.**

2. One to several basal, not palisad layers, erect filaments with cells ± co-axial……. . ***Lithophyllum* spp.**

L. cabiochae, L. stictaeforme (= L. frondosum), Lithophyllum spp.

1. Cell fusions, conceptacles …….. 3

3. Tetra/bisporangial conceptacles uniporate..... 4

4. Thallus thin, epibiontic, basal layer unistratose……..... 5

5. Trichocytes terminal..... ***Hydrolithon* spp.**

5. Trichocytes intercalary..... ***Pneophyllum* spp.**

4. Thallus thick, free-living or saxicolous, basal layer multistratose..... 6

6. Basal layers co-axial, Trichocytes terminal..... ***Neogoniolithon* spp.**

N. brassica-florida, N. mamillosum

6. Basal layers not co-axial, No trichocytes..... ..... ***Spongites fruticulosus***

3. Tetra/bisporangial conceptacles multiporate…..... 7

7. Thallus thin, epibiontic, …….. ***Melobesia membranacae***

7. Thallus thick, free-living or saxicolous..... 8

8. Basal layer multistratose, coaxial, epithallial cells domed (not flared)…….. ***Mesophyllum* spp.**

M. alternans, M. expansum, M. lichenoides (?), [M. macedonis], M. macroblastum

8. Basal layer multistratose no-coaxial ……..... 9

9. Epithallial cells flared…….. ***Lithothamnion*** spp.

L. crispatum, L. minervae, L. philippii, L. sonderi

9. Epithallial cells not flared……..... ***Phymatolithon* spp.**

P. tenuissimum, P. lamii, P. lenormandii

Example of request for authorization

***Objet** : demande d'autorisation pour effectuer des observations et des récoltes non destructives dans le cadre de deux programmes européens de recherche CIGESMED et DESVOTES*

Mr F. Bland,
Directeur du Parc National des Calanques
Bât A4 - Parc Valad
Impasse Paradou
13 009 MARSEILLE
Marseille, le 29 Janvier 2014,

Monsieur le directeur

Suite à la mise en place des règlementations du Parc National des Calanques, nous vous demandons l'autorisation de procéder à plusieurs suivis et prélèvements sous-marins durant l'année 2014 dans le cadre de la poursuite de programmes en cours.

Les chercheurs de l'IMBE (Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale) sont impliqués depuis plusieurs années dans divers projets de recherche sur le coralligène sur le site des calanques. Les programmes actuellement en cours impliquent des prélèvements en cœur de parc sur les stations suivantes : Riou Moyade, Riou Riou Sud, Proximité de la grotte de Figuier, Phare de Cassidaigne - La bigue. Une autre station prélevée se trouve en zone adjacente (Ile Du Frioul).

Ces sites sont désignés par les scientifiques de ce programme européen sur le coralligène comme des sites typiques pour la région marseillaise. Des spécificités génétiques, d'acquisition de données préliminaires et de configurations en ilots sont uniques dans ces sites, et ne peuvent être reproduites ailleurs aux alentours de Marseille. Notre principal projet (CIGESMED http://www.cigesmed.eu/) est un projet SeasEra, financé par l'Agence Nationale de la Recherche, et se fait en partenariat avec une dizaine d'autres laboratoires et parcs de différents pays européens en France, Grèce et Turquie. Il a débuté en 2013, et se terminera en 2016. Durant toute cette période, nous aurions besoin de pouvoir continuer nos travaux sous-marins impliquant des prélèvements de *Myriapora truncata*, de *Lithophylum cabiochae* (morceaux de colonies de 1 à 3cm), de quadriplicats de petites surfaces de benthos (0.01 m2), ainsi que des cartographies et transects photos des profils de coralligène à deux profondeurs (30m et 45m, sur les sites précédemment cités).

D'autre part, certains de ces sites sont également des sites de suivi des dynamiques de recrutement (Programme DESVOTES http://www.devotes-project.eu/). Travaillant sur l'impact des activités humaines et la prise en compte de ces milieux fragilisés dans le cadre de la DCSMM, ces données nous sont indispensables et nous souhaiterions pouvoir continuer ce suivi (prévu jusqu'à fin 2014).

Vous trouverez dans une fiche de déclaration de travaux ci joint la description plus détaillée des différents échantillonnages que nous souhaitons effectuer. Le protocole produit par CIGESMED doit ensuite aider les gestionnaires à mieux surveiller l'état du milieu coralligène, et une copie des jeux de données produits vous sera fournie. Je me tiens, ainsi que le chercheur responsable de ce chantier R. DAVID, à votre entière disposition pour vous rencontrer ou pour présenter au Conseil Scientifique et aux agents du Parc concernés les programmes que nous poursuivons sur le site.
Si vous en êtes d'accord et nous accordez cette autorisation, nous aurions besoin d'un document à présenter lors d'éventuels contrôles.

Veuillez agréer, Monsieur le Directeur, l'expression de mes salutations distinguées.

Jean-Pierre Féral

Example of work declaration

Fiche de déclaration de travaux

Dans le périmètre du Parc National des Calanques

Programmes scientifiques concernés : **CIGESMED et DEVOTES**

A) Responsables des chantiers : Romain DAVID et Frédéric ZUBERER

Tous nos travaux sous-marins sont réalisés avec le Service Plongée de l'Institut Pytheas, dont le responsable est Frédéric Zuberer.

Bateaux utilisés

- Armandia : MA 595732
- Antédon : MA 914216
- Pytheas MAD84785 (semi rigide)

Noms des opérateurs : Romain Bricout, Sandrine Chenesseau, Romain David, Zinovia Erga, Jean-Pierre Féral, Dorian Guillemain, Anne Haguenauer, Christian Marschal, Sandrine Ruiton, Stephane Sartoretto, Marc Verlaque Frédéric Zuberer.

Tous les plongeurs sont Classe 1B - 2B, 2A ou 3B. Pour chaque plongée, la plongée sera effectuée par 2 équipes de 2 plongeurs parmi les intervenants cités précédemment.

B) Objectifs des programmes :

1) CIGESMED (http://www.cigesmed.eu)
   Responsables du programme : Jean-Pierre FERAL

CIGESMED (2012 – 2016 Coralligenous based Indicators to evaluate and monitor the "Good Environmental Status" of the MEDiterranean coastal waters) est un programme européen « ERANET » de recherche à visée d'aide à la gestion sur l'habitat coralligène, habitat patrimonial de l'écosystème marin méditerranéen. Dans ce projet européen, une approche écologique (développement et test d'indicateurs, prélèvements en plongée sous-marine et transects et techniques « visual sensus », analyses de photographies) est couplée au développement d'approches génétiques de nouvelle génération (barcoding, méta-barcoding, phylogéographie, génétique des populations). Ces approches permettront en premier lieu de mieux décrire la composition en espèces d'algues rouges bio-constructrices de ce milieu. L'approche génétique vise également à fournir des outils innovants pour la bio-indication, basés sur la diversité génétique intra-spécifique d'un panel d'espèces. Utilisant les technologies de séquençage nouvelle génération (Next-Generation Sequencing, NGS), ce projet caractérisera, par méta-barcoding, la composition en divers organismes (plusieurs phylums d'animaux et algues) de différents profils écologiques de coralligène.

2) DEVOTES (http://www.devotes-project.eu/)
Responsables du programme : Anne CHENUIL

DEVOTES (2012 - 2016 DEVelopment Of innovative Tools for understanding marine biodiversity and assessing good Environmental Status.) vise à améliorer la compréhension des impacts des activités humaines (cumulés, synergiques, antagonistes) et des variations dues au changement climatique sur la biodiversité marine via l'utilisation de séries de données à long terme (pélagiques et benthiques).

Un des objectifs majeurs de DEVOTES est de tester les indicateurs proposés par la Communauté Européenne et d'en développer de nouveaux permettant l'évaluation du bon état écologique des peuplements, des habitats et des écosystèmes. Cette évaluation doit permettre de donner un statut aux masses d'eaux marines, intégrant les indicateurs dans une évaluation unifiée de la biodiversité et la mise en œuvre rentable de ces indicateurs (c'est-à-dire en définissant le contrôle et des stratégies d'évaluation).

C) Objet des travaux :

Espèces concernées : *Myriapora truncata*, *Lithophylum cabioche, espèces sessiles*

Les protocoles mis en œuvre dans le cadre du programme « DEVOTES » n'impliquent aucun prélèvement dans l'habitat, ils nécessitent la pose puis le retrait de pièges pour le recrutement qui seront ensuite collectés et analysés avec de nouveaux outils d'étude génétiques (méta-barcoding).

Les protocoles testés dans le cadre du programme « CIGESMED » doivent apporter des informations sur le fonctionnement de sa composante biogène, en terme d'organisation, de connectivité des individus et des peuplements des espèces « matrices » et structurantes. Afin d'affiner au mieux la connaissance des conditions liées aux agencements des différents peuplements structurants, quatre approches biologiques doivent être superposées pour caractériser les sites d'étude :

- Une **cartographie des peuplements majoritaires** (et des profils associés) le long de deux **isobathes**[1] sur 3 sites minimum par localité (Pour la région de Marseille, 28 m ±1m et 45 m ± 1 m).

- Le **prélèvement de 32 morceaux d'individus de 2 espèces (*Lithophylum cabioche* et *Myriapora truncata*)** par niveau bathymétrique répartis sur des profils tranchés et deux côtés opposés de chacun des sites permettra de chercher les espèces cryptiques et d'étudier la connectivité et la structure génétique entre population, de l'échelle locale à l'échelle méditerranéenne.

[1] Ligne imaginaire détourant un relief sous marin à profondeur égale.

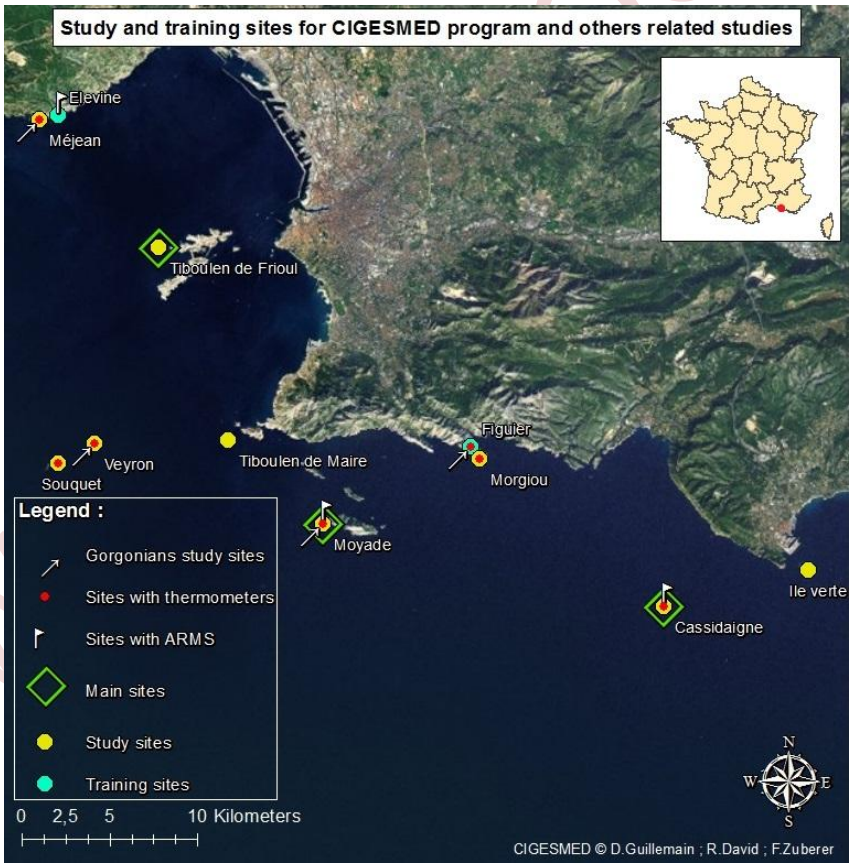
- Le **prélèvement de la biomasse totale sur 4 petites surfaces (0.01 m^2)** pour les deux profils en question, ceci sur chaque côté du site et aux deux profondeurs (soit 32 prélèvements) permettra de déterminer les divers organismes et les différences relatives de niveau de la biodiversité pour chacun des phylums considérés grâce à une approche visuelle (tri et photo) complétée par des méthodes de méta-barcoding.

- **L'analyse des peuplements par transects et quadras photos** sur les profils majoritaires. Ces transects photos permettront d'exploiter des métriques supplémentaires en terme de recouvrement relatif des espèces / taxons. Certains transects permanents permettront d'inter calibrer les différentes méthodes et matériels utilisés par les différents partis prenants.

Le nombre de prélèvement tient compte des besoins de robustesse statistique mais a été minimisé afin de préserver le milieu. Les interventions se feront avec le maximum de précautions afin de ne pas altérer les stations de l'habitat concerné.

D) Sites concernés :

Les projets de recherche sur le coralligène qui impliquent des travaux sur les stations suivantes :



Pour les prélèvements

Sites en cœur de parc :

- Riou Moyade RMO 43° 10.600'N 5° 22.240'E (zone de non pêche)
- Riou Riou sud RRS 43° 10.370'N 5° 23.420'E (zone de non pêche)
- Phare de Cassidaigne, La bigue 43°08.740’N 5°32.742’E

Sites en zone adjacente :

- Ile Du Frioul, Tiboulen du Frioul 43,2805°N 5,2859° E

Les autres stations envisagées pour les transects, selon les résultats des premières observations, pourront être :

Grand congloué Tombant est Grand Conglue GCE 43° 10.530'N 5° 24.090'E,
Grand congloué Pierre de Cassis GCP 43° 10.580'N 5° 24.170'E,
Grand congloué Tombant nord Grand Conglue GCN 43° 10.600'N 5° 24.100'E,
Jare Pierre de briançon JPB 43° 11.820'N 5° 21.700'E,
Jare Grotte arc en ciel JAC 43° 11.810'N 5° 21.820'E,
Jare Grotte passelaigue JGP 43° 11.770'N 5° 21.930'E,
Jare Grotte mystérieuse JGM 43° 11.725'N 5° 21.980'E,
Jaron Passe jare/Jaron JJJ 43° 11.980'N 5° 21.510'E,
Jaron Cap de jare JCJ 43° 12.080'N 5° 21.290'E,
Maire Pierre du Matelot MPM 43° 12.318'N 5° 20.619'E,
Marseilleveyre Plateau des Chévres MPC 43° 12.200'N 5° 22.000'E,
Morgiou Morgiou MOR 43° 12.060'N 5° 27.100'E,
Petit congloué Petit congloué PCO 43° 10.755'N 5° 23.730'E,
Plane Les arches PLA 43° 11.140'N 5° 23.400'E,
Plane Grotte à Peres PGP 43° 11.190'N 5° 23.470'E,
Plane Pointe Ouest Plane PPO 43° 11.430'N 5° 22.930'E,
Plane Ecueil de Miet PEM, Plane Pierre à Joseph PPJ 43° 11'134N 5°23'462
Plane Calanque de Pouard PCP 43° 11.300'N 5° 23.100'E,
Riou Moyadons RMN 43° 10.680'N 5° 22.120'E,
Riou Boulegeade RBO 43° 10.650'N 5° 22.370'E,
Riou Contrebandier RCO 43° 10.430'N 5° 23.140'E,
Riou Impérial de terre RIT 43° 10.370'N 5° 23.580'E,
Riou Impérial du milieu RIM 43° 10.300'N 5° 23.620'E,
Riou Impérial du large RIL 43° 10.200'N 5° 23.650'E,
Riou Cone impérial du large RCI 43° 10.210' N 5°23.750' E,
Riou Caramassane RCA 43° 10.410'N 5° 23.920'E,
Riou Ecueil du milieu REM 43° 10.913'N 5° 23.290'E,
Riou Pierres tombées RPT 43° 10.510'N 5° 22.880'E,
Sormiou Grottes aux capelans SGC 43° 12.260'N 5° 25.620'E,
Sormiou Figuier SFI 43° 12.330'N 5° 26.790'E.

Un site d’entrainement en condition à faible profondeur est aussi utilisé pour les apprentissages de relevés de quadras photos :

- proximité de la grotte de Figuier 43° 12.330'N, 5° 26.790'E

Calendrier des plongées :

- Janvier à mars 2014 : plongées de cartographie et de prélèvement
- Février / Mars / Avril / Mai / Juin / Juillet : plongée mensuelle sur chaque site – photographie des dispositifs de suivi du recrutement et transects photos
  Automne 2014: plongées prévues pour retirer les dispositifs en place

Gestion des données :
Après étude, l'ensemble des données (tableaux, photos, publications) seront bien entendu mis à la disposition du Parc National des Calanques pour archivage. Cet ensemble sera conservé sur les sites des programmes et alimentera en données les systèmes d'information régionaux, nationaux et internationaux.