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## **COMMUNITY ECOLOGY OF CORALLIGENOUS ASSEMBLAGES USING A METABARCODING APPROACH**

### **Abstract**

*Coralligenous habitats are bioconstructed, emblematic habitats of the Mediterranean Sea, which display a remarkably complex tridimensional structure and are considered as one of the most important biodiversity hotspots of the Mediterranean Sea. In order to assess the specific diversity of these habitats we sampled small surfaces (10 cm<sup>2</sup>) of these habitats using a suction sampler in different environmental conditions on 19 sites in the Marseilles area (France). These samples were crushed and total DNA was extracted. A small portion of the COI gene was amplified using universal primers targeting metazoans. A total of 3029 Molecular Operational Taxonomic Units (mOTUs) were found among all samples. Most of the mOTUs belong to three taxonomic groups: Rhodophyta, Arthropoda and Annelida but many other phyla were detected. Environmental factors such as depth, slope and locality influence the community composition of coralligenous habitats. This first assessment of the specific diversity of these habitats using a metabarcoding approach confirms the status of biodiversity hotspot of these habitats and underlines the importance of abiotic variables to structure the community. Methodological developments can be used to design new monitoring protocols of these habitats.*

**Key-words:** coralligenous, metabarcoding, community ecology, COI

### **Introduction**

Coralligenous habitats are bioconstructed, emblematic habitats of the Mediterranean Sea, which present a remarkably complex tridimensional structure resulting of the permanent dynamics between bioconstruction and bioerosion. The main builders of these habitats are coralline red algae but other marine invertebrates such as bryozoans directly contribute to the frameworks of the habitats as they build their own calcareous skeletons. This highly complex framework represents a habitat for around 1600 species (Ballesteros 2006) and is thus considered as one of the most important biodiversity hotspots of the Mediterranean Sea. It provides many ecosystem services such as: (i) recreational (coralligenous habitats are the favorite spot of divers in the Mediterranean Sea), (ii) food provision (several species of high commercial values live or feed on these habitats), (iii) jewelry, as it is the habitat where the red coral is harvested. These habitats are threatened by human activities, in particular fishing activities and global change. Mechanical degradation due to anchoring, divers' fins or nets of fisheries are responsible for a direct destructing of the habitats. Many organisms of these habitats are affected by global warming especially gorgonians, which undergo mass mortality events correlated with positive thermal anomalies of the seawater. Combination of acidification and warming has been shown to have a deleterious impact on calcareous organisms of the habitats such calcareous red algae or bryozoans. The increasing amount of organic matter due to human activities, invasive species and overfishing are also impacting the composition of

coralligenous communities. Moreover, as coralligenous habitats are found quite deep in the sea, they are poorly studied and our lack of knowledge is probably the major constraint to the setup of efficient protection strategies. Until now the assessment of species richness and monitoring of coralligenous habitats has been mainly conducted using conventional approaches especially direct assessment by scuba diving or based on photographic surveys. These methods can be inefficient in detecting particular taxonomic groups or very small organisms in particular in the very complex framework of coralligenous habitats. Moreover, they rely on a very good taxonomic expertise, which make them difficult to apply for monitoring purposes (Weeler *et al.*, 2004). Metabarcoding is a fast, powerful, potentially cost effective molecular approach to study species diversity (Dejean *et al.*, 2012), and has not yet been used to study coralligenous habitats.

### **Materials and methods**

240 samples from 19 localities of the bay of Marseilles were collected by scuba diving using a suction sampler and a hammer to extract a 10 cm<sup>2</sup> of coralligenous habitats. Depth of sampling and topographic data such as slope and exposition were recorded during sampling. Samples were collected at 20, 30 and 40 meters depth, on vertical walls as well as flat bottoms. A site is defined by its location, depth, slope and exposition. On each site 4 samples placed at a 1 meter radius circle were collected. The samples were weighted, examined and a quick counting of the different easily recognizable taxa (Figure 2) was done before crushing them with a blender. DNA extractions, PCR amplification of a 313 bp fragment of the COI and of the 28S gene were conducted. Sequencing was completed on an Illumina Miseq sequencer (in a 2x250 bp Paired End run). After bioinformatic processing and Molecular Operational Taxonomic Units (mOTUs) clustering with the SWARM algorithm (Mahé *et al.*, 2014; 2015), a mOTU abundance table was obtained and filtered according to sequence quality, length, chimera detection, taxonomic affiliation and comparison of abundances with negative control abundances (for each mOTU). Community ecology analyses (e.g. PERMANOVA) were realized with the statistical software PRIMER (Anderson *et al.*, 2008; Clarke *et al.*, 2014; 2015) using the Sorensen distance.

### **Results**

We found a total of 3029 mOTUs in 59 sampling sites on Marseilles area (Tab. 1). Among those mOTUs 13,4 % very unidentified, 63 % were assigned to animal organisms and 23 % to algae. Most mOTUS belonged to three taxonomic groups: Arthropoda (19,5%), Rhodophyta (18,7%), Annelida (14,5%). Other taxonomic groups such as Bryozoans, Cnidarians, Mollusca and Porifera represented a fair proportion of the mOTUS. The total number of mOTUs on each site varied from 151 to 678 (Fig. 1). Environmental factors influenced the community composition of our samples (Tab. 2): the locality, the depth and the slope had a significant effect on assemblage composition.

**Tab 1: Number of mOTUs assigned to the different taxonomic groups**

<b>Taxonomic group</b>	<b>Number of mOTUs</b>
<b>Annelida</b>	438
<b>Arthropoda</b>	591
<b>Bryozoa</b>	171
<b>Chloroplastida</b>	3
<b>Chordata</b>	32
<b>Cnidaria</b>	172
<b>Echinodermata</b>	75
<b>Fungi</b>	14
<b>Mollusca</b>	150
<b>Other animals</b>	52
<b>Other eukaryotes</b>	6
<b>Porifera</b>	233
<b>Rhodophyta</b>	567
<b>Stramenopiles</b>	120
<b>Unidentified</b>	405
<b>All</b>	3029

**Tab. 2: PERMANOVA table for the model including the environmental factors: locality, slope, depth and exposition.**

	<b>Df</b>	<b>SS</b>	<b>MS</b>	<b>Pseudo F</b>	<b>P(perm)</b>	<b>Unique Perms</b>
<b>Locality</b>	17	55292	3252	1,52	0,0001	9503
<b>Slope</b>	1	4033	4033	1,89	0,0015	9858
<b>Depth</b>	2	7146	3572	1,67	0,0011	9867
<b>Exposition</b>	2	4848	2424	1,13	0,1991	9828
<b>Res</b>	29	61972	2137			
<b>Total</b>	51	1.42E05				



increased resolution obtained by using this approach allowed us to detect the effect of environmental factors such as depth that was not detected in a study using a photographic approach in the same area (Doxa *et al.*, 2016). Improvements of the first protocol, completion of sequences databases and the constant decrease of sequencing costs make this approach a very promising method to monitor coralligenous habitats.

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