Title: Innovative methods of characterizing benthic community composition for biodiversity monitoring

The **SEAMoBB** European project <https://seamobb.osupytheas.fr/> is a collaborative partnership among 3 academic institutions and 2 enterprises from France, Italy and Spain with the objective of delivering a monitoring protocol for benthic marine biodiversity of rocky habitats. We placed artificial structures (ARMS and ASUs <https://seamobb.osupytheas.fr/?page_id=496>) at 28 hard-bottom habitats (Figure 1) and, upon deployment, performed scraping of benthos on natural substrate. Each year (2 or 3 successive years) samples of marine communities were taken from these artificial (after one year immersion, see Figure 2) or natural substrates.

Describing community composition is central to biodiversity monitoring and thus biodiversity protection. Traditional methods (e.g., taxonomic experts, photo identification) can accomplish this task, however, due to the rich taxonomic diversity of hard bottom environments, additional methods need to be incorporated for monitoring. Metabarcoding provides a high-throughput, automatable and repeatable way of studying community composition, in contrast to taxonomic experts and photo identification that usually suffer from methodological bias.

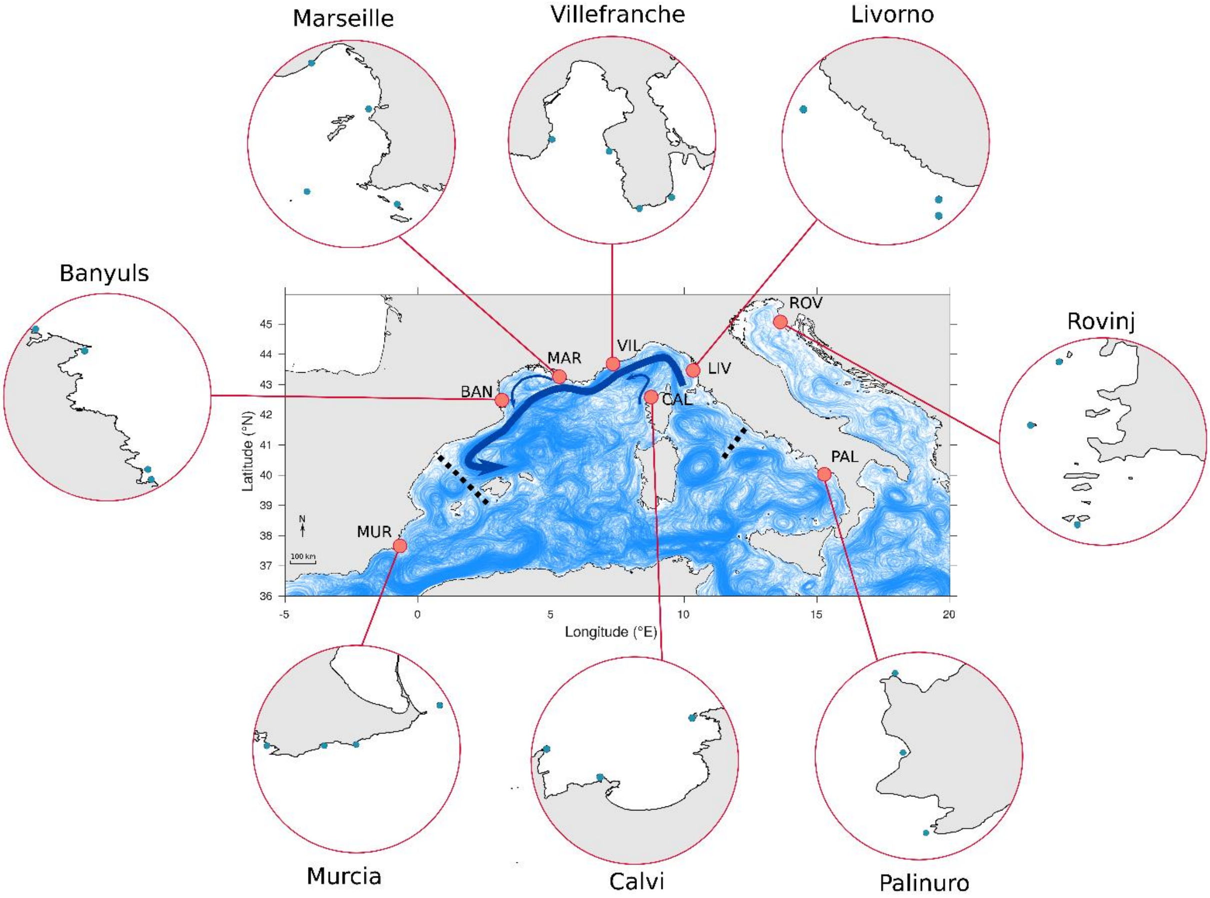

Figure 1: Geographical locations of the 8 locations (red dots) surveyed by SEAMoBB. Each location (shown in the surrounding panels) contains 3 to 4 sites (blue dots), leading to a total of 28 sites scattered in the western Mediterranean. The background blue spaghettis are millions of trajectories of virtual larvae transported by ocean currents. The dark blue thick arrow indicates the dominant

dispersal pathway associated with the Liguro-Provencal current; it acts as a corridor of enhanced connectivity. Blue thin arrows symbolize weaker and more sporadic connections due to eddies and turbulence. Black dotted lines represent hypothesized barriers of connectivity.

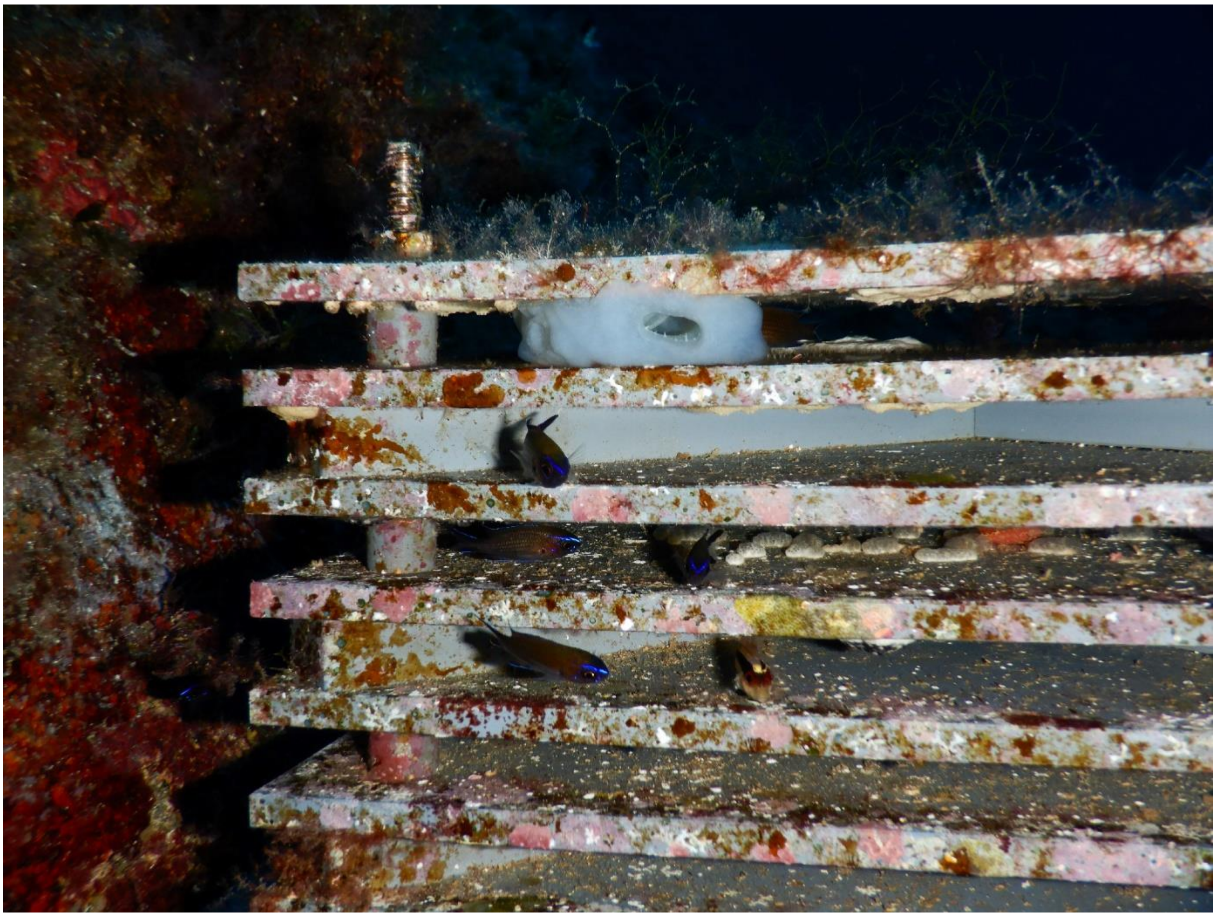

Figure 2: An Autonomous Reef Monitoring Structure (ARMS) deployed for less than a year. Recruitment of algae (red, brown and green), sessile organisms and cryptic fishes are clearly visible.

Among the achievements obtained so far, we established a robust molecular metabarcoding protocol for efficient and reliable analyses of eukaryotic species composition. In particular, we found that the DNA extraction method significantly influences the type of organisms recovered. While we observed similar results when comparing animal taxonomic diversity, one method (optimized for isolating genomic DNA from soil) was more efficient in recovering genomic DNA from algal taxa than the other method (i.e. we observed as many animal taxa, but more non-animal taxa: e.g. algae, diatoms, etc.). We thus selected this DNA extraction kit for subsequent analyses. Furthermore, we determined the optimal polymerase enzyme to maximize PCR yield (i.e. the amount of DNA sequences of appropriate size and quality recovered). Whatever the method employed for metabarcoding, a very important and encouraging result confirmed our expectations: the number of taxa recovered using metabarcoding methods are approximately ten-fold greater than taxonomic estimates revealed by photographic analyses. We are confident that the number of taxa recovered is not an overestimate, as we developed an extremely stringent and rigorous bioinformatic-filtering pipeline to eliminate any non-animal DNA sequences (e.g., contaminants, PCR artifacts, and sequencing errors). For example, the three French locations (Banyuls, Marseille, and Villefranche; 12 sites total), **the photo analyses identified > 100 taxa,**

while the innovative method of metabarcoding, performed on a much smaller range of localities (within Marseille) **identified > 700 taxa**.

Obviously, photo analyses cannot reliably identify small organisms and those hidden under ones on the surface, and cannot distinguish closely related species, which metabarcoding (using DNA sequences) can differentiate. Nevertheless, we are developing an interesting and innovative method for rapid and efficient photographic analyses by utilizing computer vision algorithms implemented through the NOAA web platform CORALNET UCSD < https://coralnet.ucsd.edu/>. Developments of such automated methods are valuable because they can save time and provide very reliable results. For instance, the photographs from the first location took our taxonomic expert 3 weeks to analyze 200 photos. After training the CORALNET platform, however, it took him only 4 days for the third region, due to the help of artificial intelligence (deep learning) algorithms. It automatically proposed taxon identifications which the expert had to confirm or to correct manually. The more images analyzed, the faster the identification, and the confidence of the taxonomic assignment. Thus, we expect that the speed and accuracy of photo analyses will further increase as the SEAMoBB project continues.

Whatever the technique employed for monitoring, we are also trying to understand what factors control the spatial patterns of benthic biodiversity. One factor that could explain the similarity in community composition between distant regions, while others largely differ, is marine connectivity. Marine connectivity results from the heterogeneous dispersion and transport of larvae by ocean currents across the seascape. Depending on dispersal routes imposed by ocean circulation, some sites may be receiving similar larval supply, possibly explaining why their community composition may look analogous. For instance, the community compositions of Livorno, Villefranche/Mer and Marseille are expected to be similar due to the Liguro-Provencal current (Figure 1). Weaker connections could result in some degrees of similarity between Banyuls and Marseille or Calvi, while Villefranche/Mer. Connectivity barriers would instead result in large differences of community compositions; this is the case for instance between Murcia and Banyuls or Palinuro and Livorno.

Our ongoing analyses of the variability of benthic biodiversity among the 28 sites as measured by complementary state-of-the-art and standardized protocols, promise further exciting discoveries to be announced by the SEAMoBB consortium soon.