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Metabarcoding in the abyss: uncovering deep-sea biodiversity through environmental DNA

Soutenance de thèse

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Abstract

The abyssal seafloor covers more than half of planet Earth. It can host a large number of, mostly small and still undescribed, organisms (~50,000-5 million individuals/m^2), contributing to key ecosystem functions such as nutrient cycling, sediment stabilisation and transport, or secondary production. Technological developments in the past 30 years have allowed remarkable advances, yet due to the vastness and remoteness of deep-sea habitats, ecological studies have been limited to local or regional scales. Indeed, we have so far explored less than 1% of the deep seafloor, although the latter is under increased threat from a variety of anthropogenic pressures.

This PhD aimed at bringing new perspectives for the study of biodiversity and biogeography in the deep-sea, to bridge this large knowledge gap, and advance toward the development of efficient biomonitoring protocols to preserve this vast and elusive backyard. We investigated the potential of multi-marker environmental DNA (eDNA) metabarcoding to assess the extent and distribution patterns of biodiversity in this remote ecosystem. Using mitochondrial and nuclear marker genes, this PhD aimed at producing and testing an optimized eDNA metabarcoding workflow for deep-sea sediments, on a bioinformatic, molecular, and sample processing level, applicable to multiple life compartments including bacteria, protists, and metazoans.

Biodiversity assessment with eDNA is confronted with the difficulty in defining accurate "species-level" molecular Operational Taxonomic Units (OTUs), as nu- merous sources of error induce frequent overestimations. The first part of this thesis describes how newly developed bioinformatic tools can be combined in order to get more conservative and reliable biodiversity inventories, approaching a 1:1 species-OTU correspondence, and underline the advantages of clustering and LULU-curation for producing more reliable metazoan biodiversity inventories. Moreover, the accuracy of protocols based on eDNA in deep sea sediments still needs to be assessed, as results may be biased by ancient DNA, resulting in biodiversity assessments not targeting live organisms. This thesis assessed the potential bias of ancient DNA by 1) evaluating the effect of removing short DNA fragments, and 2) comparing communities revealed by co-extracted DNA and RNA in five deep-sea sites. Results indicated that short DNA fragments do not affect alpha and beta diversity, but that DNA obtained from 10g of sediment should be favoured over RNA for logistically realistic, repeatable, and reliable surveys. Results also confirm that increasing the number of biological rather than technical replicates is important to infer robust ecological patterns. Sieving sediment to separate benthic size classes increased the number of detected metazoan meiofauna OTUs, but was not essential for achieving comprehensive biodiversity estimates, and should be avoided if unicellular taxonomic compartments are also of interest. Finally, this thesis applied the optimized eDNA metabarcoding protocols to investigate the influence of biotic and abiotic factors on the extent and distribution of deep-sea metazoan biodiversity on an East-West transect ranging from the Central Mediterranean to the Mid-Atlantic Ridge. Results, consistent to morphology-based studies, confirm that small-scale biotic and abiotic factors lead to significant vertical changes in metazoan richness and community structure within the sediment, and highlight that regional beta-diversity patterns result from a combined influence of past biogeography and present day processes. This thesis opens the way to large-scale eDNA-based studies in the deep-sea realm, thus contributing to a better understanding of biodiversity, biogeography, and ecosystem function in this vast and still poorly known biome.

Key-words

Environmental DNA, Multigene metabarcoding, Deep-sea, Benthos, Biodiversity, Eukaryotes (18S, COI), Prokaryotes (16S)

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