

## Towards a DNA (meta)barcoding approach to assess changes in seabed ecosystems related to sand extraction activities

Devriese Lisa<sup>1</sup>, De Backer Annelies<sup>1</sup>, Maes Sara<sup>1</sup>, Van Hoey Gert<sup>1</sup>, Haegeman Annelies<sup>2</sup>, Ruttink Tom<sup>2</sup>, Wittoeck Jan<sup>1</sup>, Hillewaert Hans<sup>1</sup>, De Tender Caroline<sup>1</sup>, Hostens Kris<sup>1</sup>

<sup>1</sup> ILVO (Flanders research institute for agriculture, fisheries and food), Animal Sciences Unit, Aquatic environment and quality, Ankerstraat 1, 8400 Oostende, Belgium

<sup>2</sup> ILVO (Flanders research institute for agriculture, fisheries and food), Plant Sciences Unit, Growth and Development, Caritasstraat 39, 9090 Melle, Belgium

Biodiversity is influenced by a wide range of environmental and human-induced pressures. Physical disturbance of the seabed is probably the best known pressure to affect benthic ecosystems. The aim of this work is to evaluate the potential of DNA (meta)barcoding to assess human-induced changes in the seabed ecosystem. We focus on two benthic ecosystem components: bacteria and macrobenthos, in relation to sand extraction in the Belgian part of the North Sea.

The bacterial community composition in the sediment was assessed by amplicon sequencing of the V3-V4 fragment of the 16S rRNA gene using Illumina technology. Disturbance of the seabed due to sand extraction activities on the Buiten Ratel sand bank revealed a significant impact on the bacterial communities.

Macrobenthos is recognized as a good biological indicator to measure changes in marine ecosystems. Excellent morphological identification tools exist, but these are time-consuming. DNA barcoding (species) and DNA metabarcoding (communities) may provide a fast alternative. However, accurate DNA-based species identification is lacking.

Therefore, COI and 18S fragments of 70 macrobenthos species were amplified using several barcoding primers and Sanger sequenced to evaluate which amplicon provides the best taxonomic resolution. The 18S and COI barcode sequences were added to our DNA reference library. We developed a DNA metabarcoding method using Illumina MiSeq technology and amplicon sequencing was executed using the 18S target regions and DNA extracts both on individual species, and on pooled samples in which tissues or DNA extracts of different species were mixed. This setup allowed us 1) to check the effectiveness of the primers to detect species in both single and pooled samples; and 2) to investigate the relationship between read counts per species and the relative proportion of species in mixed samples.

This work is ongoing and will be used to establish a complete North Sea DNA barcode reference database for bacteria and macrobenthos, in close cooperation with other institutes along the North Sea.

**Keywords:** DNA (meta)barcoding; macrobenthos; bacteria; amplicon sequencing; biodiversity