

A DNA (meta)barcoding approach to assess changes in seabed ecosystems related to human-induced pressures

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I. Aim

Development of a DNA metabarcoding pipeline to assess marine **benthic biodiversity** in the North Sea.

Evaluation of DNA metabarcoding to assess effects of sand extraction on **bacterial species composition** in the Belgian part of the North Sea.

II. Methodology

Macrobenthos communities

- Amplicon sequencing of the V4 fragment of the 18S rDNA using Illumina technology
→ DNA extracts of individual species and artificial mixtures of various species
- Sanger sequencing of 4 DNA barcode amplicons: COI (313-319 bp), COI (655-661 bp), V4 18S rDNA (370-582 bp), V7-V8 18S rDNA (281-592 bp)

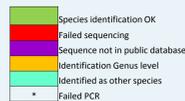
Bacterial communities

- Amplicon sequencing of the V3-V4 fragment of the 16S rDNA using Illumina technology
(De Tender et al., 2015)
→ DNA extracts of sediment samples (Buiten Ratel sand bank)

IV. Macrobenthos

Sanger sequencing

Taxonomic resolution of the barcoding primers:



Phylum	Class	Species	COI		18S			
			large	small	V4	V7-V8		
Mollusca	Bivalvia	<i>Abra alba</i>	OK	OK	OK	OK		
		<i>Donax vittatus</i>	OK	OK	OK	OK		
		<i>Ensis directus</i>	OK	OK	OK	OK		
		<i>Limecola balthica</i>	OK	OK	OK	OK		
		<i>Kurtella bidentata</i>	OK	OK	OK	OK		
		<i>Fabulina fabula</i>	OK	OK	OK	OK		
		<i>Asbjornsenia pygmaea</i>	OK	OK	OK	OK		
		<i>Mya truncata</i>	OK	OK	OK	OK		
		<i>Spisula solidula</i>	OK	OK	OK	OK		
		Gastropoda	Gastropoda	<i>Buccinum undatum</i>	OK	OK	OK	OK
				<i>Crepidula fornicata</i>	OK	OK	OK	OK
				<i>Nassarius reticulatus</i>	OK	OK	OK	OK
				<i>Nomides oxycephalus</i>	OK	OK	OK	OK
				<i>Aphrodita aculeata</i>	OK	OK	OK	OK
				<i>Capitella (C. capitata)</i>	OK	OK	OK	OK
Annelida	Polychaeta	<i>Chaetozoa setosa</i>	OK	OK	OK	OK		
		<i>Cirratulidae (Cirratulus)</i>	OK	OK	OK	OK		
		<i>Eteone longa</i>	OK	OK	OK	OK		
		<i>Eunereis longissima</i>	OK	OK	OK	OK		
		<i>Glycera alba</i>	OK	OK	OK	OK		
		<i>Glycera tridactyla</i>	OK	OK	OK	OK		
		<i>Lanice conchilegia</i>	OK	OK	OK	OK		
		<i>Lagis koreni</i>	OK	OK	OK	OK		
		<i>Lumbrineris futilis</i>	OK	OK	OK	OK		
		<i>Mediomastus fragilis</i>	OK	OK	OK	OK		
		<i>Magelana johnstoni</i>	OK	OK	OK	OK		
		<i>Nephtys assimilis</i>	OK	OK	OK	OK		
		<i>Nephtys caeca</i>	OK	OK	OK	OK		
		<i>Nephtys cirrosa</i>	OK	OK	OK	OK		
		<i>Nephtys hombergii</i>	OK	OK	OK	OK		
		<i>Nephtys longosetosa</i>	OK	OK	OK	OK		
		<i>Notomastus latericeus</i>	OK	OK	OK	OK		
		<i>Ophelia borealis</i>	OK	OK	OK	OK		
		<i>Owenia fusiformis</i>	OK	OK	OK	OK		
		<i>Phaloe baltica</i>	OK	OK	OK	OK		
		<i>Phyllodoce mucosa</i>	OK	OK	OK	OK		
		<i>Poecilochaetus serpens</i>	OK	OK	OK	OK		
		<i>Malmgrenia lunulata</i>	OK	OK	OK	OK		
		<i>Scoletopis bonnieri</i>	OK	OK	OK	OK		
		<i>Scoloplos armiger</i>	OK	OK	OK	OK		
<i>Spiofananes bombyx</i>	OK	OK	OK	OK				
<i>Spio</i>	OK	OK	OK	OK				
<i>Sthenelais boa</i>	OK	OK	OK	OK				
Echinodermata	Ophiuroidea	<i>Acrocnida brachiata</i>	OK	OK	OK	OK		
		<i>Ophiura ophiura</i>	OK	OK	OK	OK		
		<i>Ophiura albida</i>	OK	OK	OK	OK		
Echinoidea	Echinoidea	<i>Echinocardium cordatum</i>	OK	OK	OK	OK		
		<i>Echinocardium pusillum</i>	OK	OK	OK	OK		
Arthropoda	Malacostraca	<i>Paramecium millaris</i>	OK	OK	OK	OK		
		<i>Abiodromella obtusata</i>	OK	OK	OK	OK		
		<i>Bathyporeia elegans</i>	OK	OK	OK	OK		
		<i>Bathyporeia guilliamsoniana</i>	OK	OK	OK	OK		
		<i>Leucothoe incisa</i>	OK	OK	OK	OK		
		<i>Megalurupus agilis</i>	OK	OK	OK	OK		
		<i>Microprotopus maculatus</i>	OK	OK	OK	OK		
		<i>Nototropis falcatus</i>	OK	OK	OK	OK		
		<i>Nototropis swammerdami</i>	OK	OK	OK	OK		
		<i>Pariambus typicus</i>	OK	OK	OK	OK		
		<i>Pontocrates arenarius</i>	OK	OK	OK	OK		
		<i>Siphonocetes kroyeranus</i>	OK	OK	OK	OK		
		<i>Tryphosa nana</i>	OK	OK	OK	OK		
		<i>Urothoe brevicornis</i>	OK	OK	OK	OK		
		<i>Urothoe poseidonis</i>	OK	OK	OK	OK		
<i>Diaethys bradyi</i>	OK	OK	OK	OK				
<i>Iphinoe trispinosa</i>	OK	OK	OK	OK				
Cnidaria	Anthozoa	<i>Sagartia troglodytes</i>	OK	OK	OK	OK		
Arthropoda	Malacostraca	<i>Gastrosaccus spinifer</i>	OK	OK	OK	OK		
		<i>Palaeomon serratus</i>	OK	OK	OK	OK		
		<i>Processa modica</i>	OK	OK	OK	OK		
		<i>Thia scutellata</i>	OK	OK	OK	OK		
Chordata	Leptocardii	<i>Pestarella tyrrhena</i>	OK	OK	OK	OK		
		<i>Branchiostoma lanceolatum</i>	OK	OK	OK	OK		

→ Barcode sequences for 70 most abundant macrobenthos species were added to ILVO DNA barcode reference database.

III. Macrobenthos

Amplicon sequencing, e.g. V4 18S rDNA



Phylum	Pure	Mix I	Mix II		
Mollusca	<i>Abra alba</i>	OK	OK		
	<i>Limecola balthica</i>	OK	OK		
	<i>Kurtella bidentata</i>	OK	OK		
	<i>Mya truncata</i>	OK	OK		
	<i>Spisula solidula</i>	OK	OK		
	Annelida	<i>Chaetozoa setosa</i>	OK	OK	
		<i>Lanice conchilegia</i>	OK	OK	
		<i>Notomastus latericeus</i>	OK	OK	
		<i>Owenia fusiformis</i>	OK	OK	
		<i>Poecilochaetus serpens</i>	OK	OK	
		<i>Scoloplos armiger</i>	OK	OK	
		<i>Spiofananes bombyx</i>	OK	OK	
		<i>Spio</i>	OK	OK	
		<i>Sthenelais boa</i>	OK	OK	
		Echinodermata	<i>Echinocardium cordatum</i>	OK	OK
<i>Echinocardium pusillum</i>			OK	OK	
Arthropoda			<i>Paramecium millaris</i>	OK	OK
			<i>Abiodromella obtusata</i>	OK	OK
			<i>Bathyporeia elegans</i>	OK	OK
			<i>Bathyporeia guilliamsoniana</i>	OK	OK
	<i>Leucothoe incisa</i>		OK	OK	
	<i>Megalurupus agilis</i>		OK	OK	
	<i>Microprotopus maculatus</i>		OK	OK	
	<i>Nototropis falcatus</i>		OK	OK	
	<i>Nototropis swammerdami</i>		OK	OK	
	<i>Pariambus typicus</i>		OK	OK	
	<i>Pontocrates arenarius</i>		OK	OK	
	<i>Siphonocetes kroyeranus</i>		OK	OK	
	<i>Tryphosa nana</i>		OK	OK	
	<i>Urothoe brevicornis</i>	OK	OK		
	<i>Urothoe poseidonis</i>	OK	OK		
<i>Diaethys bradyi</i>	OK	OK			
<i>Iphinoe trispinosa</i>	OK	OK			

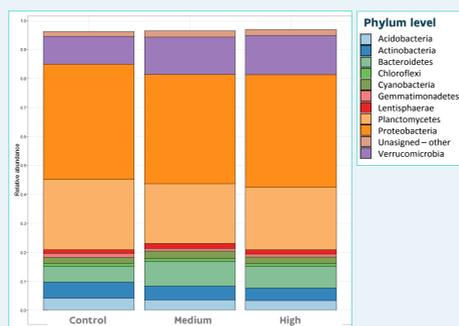
Evaluation of the effectiveness of the barcoding primers (V4 18S) on individual species and artificial mix samples.

Good taxonomic resolution, except for species of the order Amphipoda.

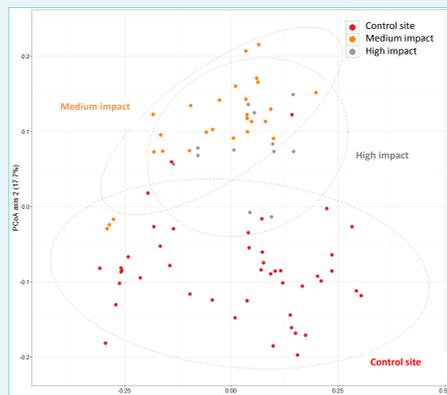
V. Bacteria

Amplicon sequencing: V3-V4 16S rDNA

Bacterial communities on sediment in relation to the impact of sand extraction activities:



Proteobacteria are the dominant classes of bacterial communities at Buiten Ratel.



Dredging intensity:
High impact: >10000 m³/ha
Medium impact: 1000-5000 m³/ha

Sand extraction activities lead to shifts in the bacterial community composition.

VI. Conclusion

DNA barcode reference database
Our aim is to establish a complete North Sea DNA barcode reference database for bacteria and macrobenthos, in close cooperation with other institutes along the North Sea

Macrobenthos communities
Taxonomic resolution can be increased by combining multiple barcodes

Next step:
Evaluation of DNA metabarcoding pipeline for real (sieved) sediment samples

Bacterial communities
DNA metabarcoding revealed that disturbance of the seabed affects bacterial communities

Bacterial assemblage composition could be an interesting tool to measure changes in marine ecosystems

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