Studying macrozoobenthos diversity in the Belgian North Sea: comparing Oxford Nanopore with short-read sequencing

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Introduction

Benthic biodiversity monitoring of the North Sea is a vital component of the assessment of environmental status and ecosystem services of the North Sea. Different DNA based monitoring efforts closely validate morphological findings. For metabarcoding, Illumina MiSeq is currently the standard sequencing platform as the method is highly accurate. Oxford Nanopore sequencing has the advantage that it is cost effective on smaller scales, and can be used for real-time in field sequencing. It has been proposed that metagenomics could be the future environmental biodiversity assessments. However, it remains unknown to which extend different DNA based methods can be compared to one another in DNA based biodiversity monitoring of the benthic North Sea.

Q: Is there variation in detected alpha and beta diversity between DNA based methods and morphology?

Sampling Design

Location 120, 330, 840 and ZVL using 3 replicates

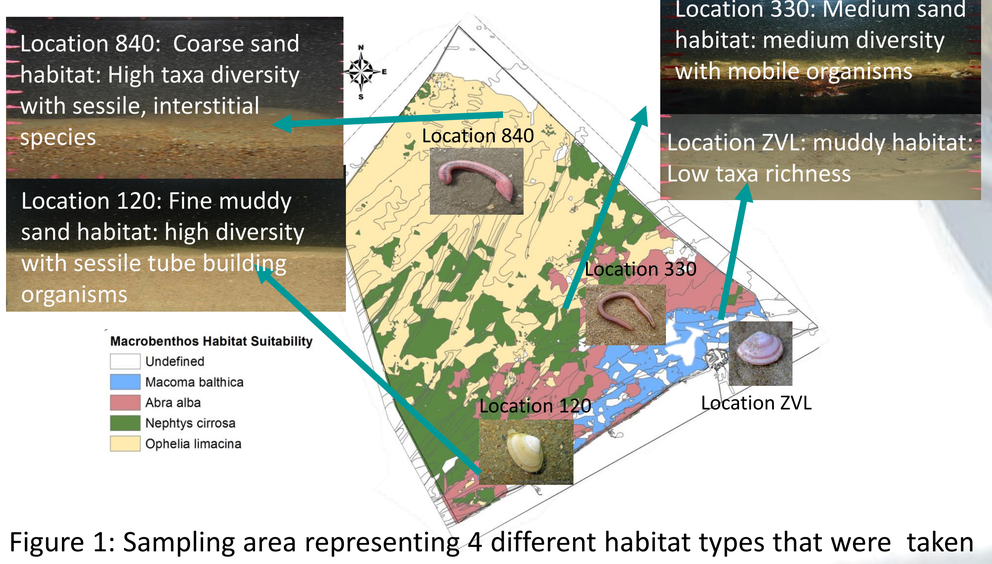


Figure 1: Sampling area representing 4 different habitat types that were taken using a van Veen grab. One replicate of each community was taken for morphological analysis. The samples were sieved using a 1 mm seave and then blended for DNA based analysis

Illumina Miseq and ONT Nanopore results are highly similar, but differ from shotgun metagenomics and morphological data.

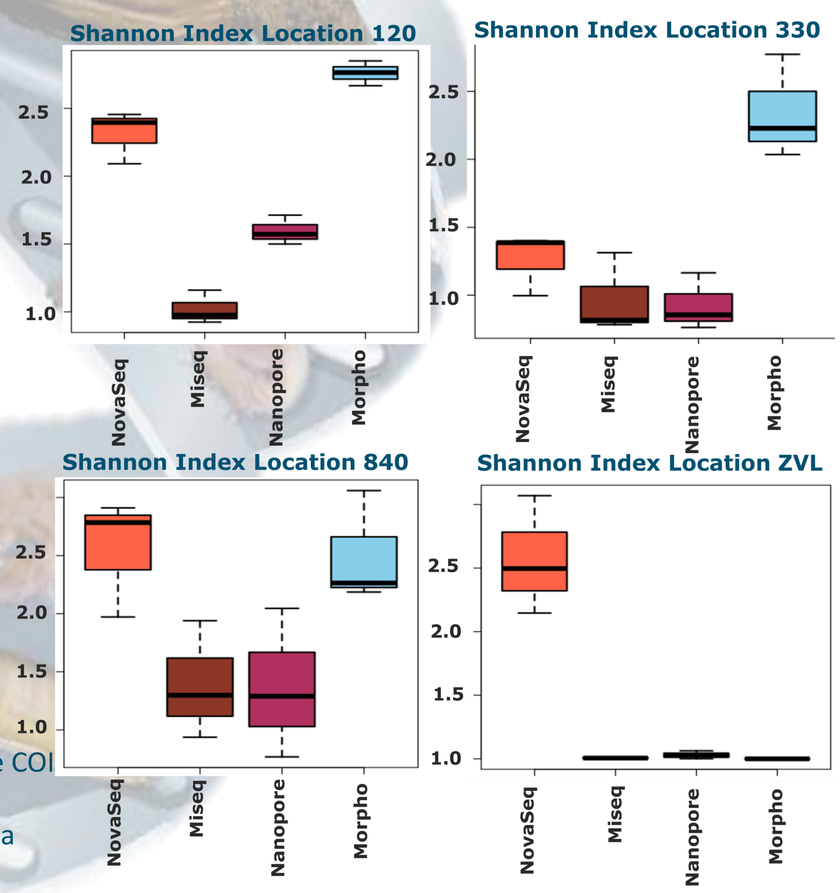
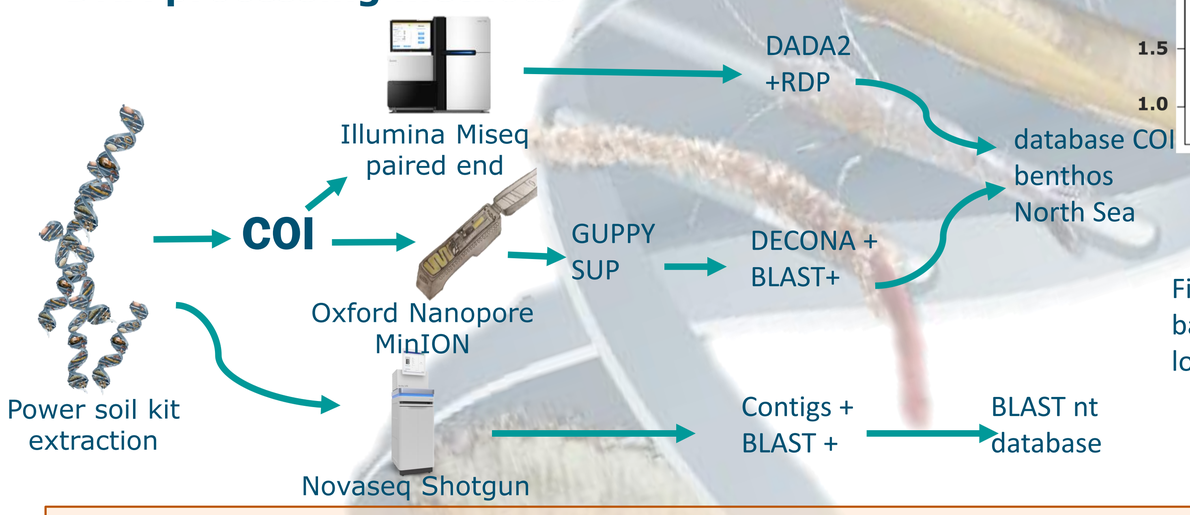


Figure 2: Shannon Diversity Index plots of the different DNA based methods and morphological analysis from location 120 location 330 and 840 and ZVL.

Table 1: Heat map of the 50 most abundant genera of the datasets. Colour and number shows the relative abundance of the sgenera.

	Miseq	Nanopore	Shotgun	Morpho
Nephtys	20.48	23.43	22.06	2.31
Limecola	26.37	25.25	18.49	0.83
Cylista	14.86	13.3	0	0
Scolelepis	9.91	8.58	0	0.05
Processa	8.24	6.07	1.6	0.36
Urothoe	2.41	2.19	0.98	7.74
Tubificoides	0.03	0.08	0.03	12.59
Echinocardium	5.26	3.86	0.98	0.42
Abra	0.06	0.01	5.95	4.21
Unclassified	0	0	0	10.02
Ensis	0	0.1	8.68	0.47
Bathyporeia	5.41	0.68	0.64	1.95
Ophiura	0.46	2.03	0.32	4.99
Spisula	0.02	1.22	5.81	0.68
Glycera	0	0	6.74	0.57
Owenia	0.52	2.86	1.67	1.25
Fabulina	0	0	0	6.02
Spirobranchus	0	0	0	4.93
Kurtiella	0	0	0	4.8
Gastrosaccus	2.51	0.79	1.19	0.21
Ophelia	0.11	3.32	0.76	0.39
Eucheilota	0	0.02	3.56	0
Cirriformia	0	0	0.06	3.32
Tellimya	0.01	0.04	0.06	3.22
Asterias	0	0	3.27	0
Notomastus	0	0.01	1.53	1.19
Anapagurus	0.41	1.96	0.01	0
Spiophanes	0.07	0.55	0.44	1.09
Arctica	0	0	2.03	0
Magelona	0.04	0.13	0.14	1.71
Eumida	0	0.03	0.45	1.48
Spio	1.23	0	0.31	0.34
Hesionura	0.02	0	0.37	1.45
Other	0	1.7	0	0
Aonides	0.01	0.01	0.03	1.61
Mediomastus	0	0	0.01	1.58
Donax	0	0	1.52	0.05
Venerupis	0	0	1.24	0.21
Crepidula	0.16	0.02	1.01	0.21
Mytilus	0	0	0.6	0.68
Acrocnida	0.22	0.98	0.01	0.05
Monopseudocuma	0	0	0	1.25
Polygordius	0	0	0.87	0.29
Goodallia	0	0	0	1.14
Pariambus	0	0.01	0	1.09
Liocarcinus	0.68	0.02	0.07	0.26
Phoronis	0	0	0	0.99
Microphthalmus	0	0	0	0.83
Sagartiogeton	0.03	0	0.71	0

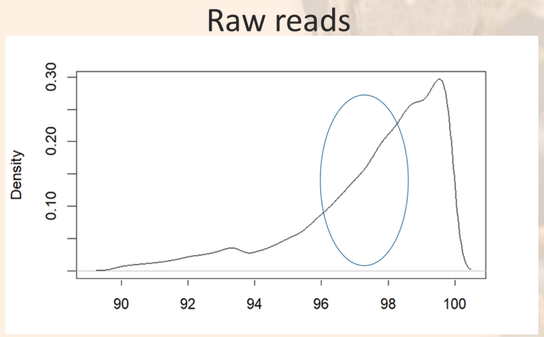
DNA processing methods



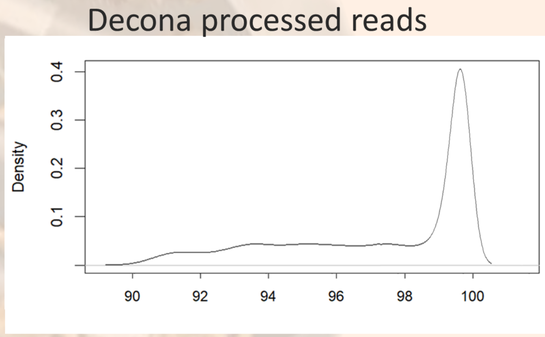
R10.4.1 flowcell + LSK114 Q20 intermezzo: **To polish or not to polish for Species Level ID?**

Species identification accuracy of raw reads

Raw reads



Decona processed reads



New approach: use Decona + the singletons that are not clustered

Outcomes

- There is a high similarity in α-diversity as well as β- diversity between Illumina and Nanopore metabarcoding. There is also high similarity between the 50 most abundant genera.
- Shotgun metagenomics shows deviating biodiversity patterns as considerably more taxa where found. However, shotgun metagenomics appear to be more comparable to the morphological data in terms of genus level overlap as well as relative abundance.

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