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## Marine benthic metabarcoding

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# SUMMARY



## SUMMARY

The marine ecosystem is one of Earth's most valuable natural resources. Marine benthic communities are a crucial component of the marine ecosystem because of their role in nutrient cycling and primary and secondary production. High benthic biodiversity contributes to higher levels of ecosystem functioning. However, benthic communities are under stress due to anthropogenic influences such as habitat alterations, overexploitation and pollution. Due to these influences, species composition can alter and biodiversity can decrease. To be able to anticipate on such changes, monitoring the benthic diversity in a consistent and reliable way is essential. Traditional methods for measuring biodiversity, using morphological taxonomy of individual specimens, is time-consuming, labour-intensive and requires specialized taxonomic knowledge. These methods are often limited to larger specimens and/or specimens belonging to well-known taxa; thereby impairing biodiversity estimates.

In the past decade, molecular tools and more specific DNA metabarcoding combined with next generation sequencing (NGS) has emerged as an alternative method for species identification. DNA metabarcoding is based on species identification through the concept of DNA barcodes. These barcodes are short variable regions containing taxonomic information e.g. valuable for species identification. Massive parallel sequencing, NGS, can process millions of these barcodes derived from environmental samples, simultaneously. Metabarcoding facilitates consistent and reproducible biodiversity assessment across different ecosystems.

In this thesis I explored the application of metabarcoding methods to assess marine benthic biodiversity. Metabarcoding methods were compared with classic morphological methods in chapters 2-3 to define a 'best-practice'. Both qualitative as quantitative aspects of the metabarcoding methods were reviewed. Subsequently, in chapter 4-6 these metabarcoding methods were applied to assess anthropogenic influences in marine benthic ecosystems, from shore to deep-sea. In this summary I briefly highlight the most important finding of each chapter.

### **Metabarcoding methodology**

Metabarcoding methods have been applied increasingly in biodiversity assessments, including marine benthic studies. The metabarcoding approach most often relies on the extraction of DNA from sediments or water, followed by the amplification of a DNA barcode via polymerase chain reaction (PCR). Subsequently, these barcodes are sequenced, and the reads are taxonomically assigned after comparison against a reference database. Each step in the approach is subjected to bias the outcome of the biodiversity assessment. In the first part of this thesis I explored different methods within the DNA metabarcoding approach and compared the outcomes with the traditional morphological approach.

In **Chapter 2** different DNA extraction protocols and bioinformatic pipelines were compared. A direct extraction protocol – the extraction of DNA directly from the sediment matrix – was best suited for assessing macrofauna and meiofauna simultaneously. Also, taxonomic assignment using the RDP-classifier was preferred over a BLAST-algorithm. Including a mock community in the metabarcoding approach was useful to detect methodological biases caused by primer specificity or bioinformatics pipelines.

The best performing metabarcoding approach was further tested in **Chapter 3** for its quantitative performance with respect to abundance and biomass estimates compared to a traditional morphological approach. The results from this chapter showed that quantitative measurements are hampered by ecological features of DNA. The ecological features include differences in DNA release by the benthic specimens (the source), due to morphological characteristics and seasonality and distribution patterns of the benthic taxa. The only taxon which showed a positive relationship for both abundance and biomass was *Pygospio*, a small and widespread annelid.

#### **Application of metabarcoding for anthropogenic impact assessments**

In the second part of this thesis I applied a metabarcoding approach to assess anthropogenic influences on marine benthic ecosystems and to evaluate the suitability of the methods for this purpose. The metabarcoding approach was applied in three areas with increasing complexity of the benthic community compositions. Firstly, a relatively simple ecosystem; the intertidal mudflats of the Wadden sea, followed by the continental shelf; the North Sea, and finally a complex deep-sea ecosystem; the Rainbow hydrothermal vent in the Atlantic Ocean.

**Chapter 4** focussed on the intertidal and examined the influence of bottom dredging for *Arenicola spp.* on the benthic biodiversity using a metabarcoding approach along a traditional morphological approach. The study followed a BACI-design; a before and after impact control design. Because this study involved temporal aspects (recolonization in time), sediment samples were pre-rinsed with a phosphate-buffer prior to DNA extraction to remove extracellular DNA from the sediments. Significant differences between the dredged and control transects were found with both approaches, morphological and molecular. Small-sized opportunistic taxa recovered fast and became more dominant in the dredged transects compared to the control transects. In contrast, the presence of long-lived species recovered slowly and remained lower in the dredged transects compared to the control transects over the entire experimental period. The outcomes of the traditional morphological approach and the metabarcoding approach were comparable. However, the metabarcoding approach included more taxa in the assessment which resulted in a more powerful analysis.

In **Chapter 5**, the long-term effects of a gas-platform in the Southern North Sea on the surrounding benthic community were studied. Artificial structures, such as gas-platforms, are known to influence the surrounding environment either due to alteration of currents or due to facilitating the presence of an artificial reef. Biodiversity was assessed along four transects diverging from the gas platform using a traditional morphological approach and a metabarcoding approach. The metabarcoding approach recovered up to three times the number of families compared to the morphological approach. Both approaches showed small differences in species compositions among the four transects suggesting that the presence of the platform evoked changes in the surrounding benthic communities.

**Chapter 6** dealt with the deep-sea system and assessed the influence of a hydrothermal vent plume on benthic communities in the surrounding environment. These communities are poorly known and possibly endangered due to future deep-sea mining activities during which toxic plumes with high amounts of suspended material and high metal concentrations are created. Studying these benthic communities under the influence of a natural plume might provide valuable information towards the mining related plumes. Results showed that the benthic communities differed among locations and were significantly related to the plume's fall-out. Biodiversity was lowest closer to the vent, the communities were dominated by arthropod taxa. As communities were significantly influenced by the plume's fall-out, deep-sea mining and the resulting artificial plumes will possibly affect the local benthic communities. Also, as the diversity among communities might be lost due to deep-sea mining activities.

## CONCLUSION

The work I presented in this thesis showed that metabarcoding is a valuable approach to assess anthropogenic effects on marine benthic communities. Compared to traditional morphological taxonomy, the metabarcoding approach gave comparable outcomes regarding the impact of anthropogenic influences. Moreover, the metabarcoding approach was able to include more taxa in the assessment and hence increase the power of the analysis. Also, metabarcoding methods are less hampered by a lack of specialized taxonomic knowledge and long sample processing times. The metabarcoding approach outcompeted the traditional approach because of the inclusion of meiofauna and the ability to faster assess complex, relatively unknown communities. However, the metabarcoding approach also has limitations compared to the traditional approach. For instance, quantitative analyses are limited and gaps in the reference library can lead to misidentifications of taxa. A thorough knowledge about the exact features of an approach makes the approach more powerful. Whereas traditional taxonomy has been applied for centuries, the metabarcoding approach, including the knowledge of its limitations is relatively new.

Nonetheless, rapid developments are still made for the metabarcoding approach and molecular approaches in general. These developments may soon overcome many of the limitations and will probably increase the value of this approach even further.

