MARINE BIODIVERSITY RELATED TO ESTABLISHMENT OF OFFSHORE WIND TURBINES

A case study from Kriegers Flak in the western Baltic Sea

Scientific Report from DCE – Danish Centre for Environment and Energy No. 643 2025

AARHUS UNIVERSITY
DCE – DANISH CENTRE FOR ENVIRONMENT AND ENERGY

AAGE V. JENSEN NATURFOND

VELUX FONDEN

MARINE BIODIVERSITY RELATED TO ESTABLISHMENT OF OFFSHORE WIND TURBINES

A case study from Kriegers Flak in the western Baltic Sea

Scientific Report from DCE – Danish Centre for Environment and Energy No. 643 2025

Karsten Dahl
Helle Buur
Karolina R. Andersen
Cordula Göke
Peter A.U. Stæhr
Adam Koziol
Rumakanta Sapkota
Anne Winding

Aarhus University, Department of Ecoscience

AARHUS UNIVERSITY
DCE – DANISH CENTRE FOR ENVIRONMENT AND ENERGY

AAGE V. JENSEN NATURFOND

VELUX FONDEN

Data sheet

Series title and no.:	Scientific Report from DCE – Danish Centre for Environment and Energy No. 643
Category:	Research contribution
Title:	Marine biodiversity related to establishment of offshore wind turbines
Subtitle:	A case study from Kriegers Flak in the western Baltic Sea
Author(s):	Karsten Dahl, Helle Buur, Karolina R. Andersen, Cordula Göke, Peter A.U. Stæhr, Adam Koziol, Rumakanta Sapkota and Anne Winding
Institution(s):	Aarhus University, Department of Ecoscience
Publisher:	Aarhus University, DCE – Danish Centre for Environment and Energy ©
URL:	http://dce.au.dk/en
Year of publication:	2025
Editing completed:	January 2025
Referee(s):	Annette Bruhn
Quality assurance, DCE:	Anja Skjoldborg Hansen
Linguistic QA:	Charlotte Elisabeth Kler
External comments:	No external comments
Financial support:	VELUX FONDEN and Aage V. Jensen Naturfond
Please cite as:	Dahl, K., Buur, H., Andersen, K.R., Göke, C., Stæhr, P.A.U., Koziol, A., Sapkota, R. ad A. Winding (2025). Marine biodiversity related to establishment of offshore wind turbines. A case study from Kriegers Flak in the western Baltic Sea. Aarhus University, DCE – Danish Centre for Environment and Energy, 40 pp. Scientific Report No. 643

Abstract:	This report describes the biodiversity within the Danish Kriegers Flak wind farm using several different techniques and compares the results with pelagic reference stations outside the wind farm area, and reef sites investigated as part of the national monitoring program, NOVANA.

Keywords:	Offshore wind turbines, Marine biodiversity, Methods, Kriegers Flak, eDNA
Front page photo:	Karsten Dahl
ISBN:	978-87-7156-927-8
ISSN (electronic):	2244-9981
Number of pages:	40
Supplementary notes:	Version 2: New cover photo, and photo deleted from report

Contents

Preface

This project, WIND ENERGY AND NATURE-BASED SOLUTIONS INTEGRATED AT SEA (WIN@sea), investigates the potential to combine multiple activities, such as the production of fossil-free energy and cultivation of seaweed and mussels for food and feed, at a test site within the Danish Kriegers Flak wind farm. The aim is to optimize the space usage, manpower, and resources through close collaboration between the wind industry, low trophic aquaculture companies, and scientific institutions responsible for research, marine monitoring, and public sector consultancy.

Between the wind turbines, sugar kelp and blue mussels are cultivated for food and feed in a way that ensures that the wind turbines remain fully operational. The project involves modeling the nutrient uptake by seaweed and mussels and evaluating potential benefits to the marine environment. Additionally, it investigates biodiversity within the area and on the turbine towers.

The project also facilitates mutual experience and knowledge exchange between the offshore industry and public institutions, focusing on the needs, uses, and challenges of collecting scientific data to support the sustainable development of the offshore wind industry while expanding knowledge about marine environments surrounding offshore wind farms (OWFs).

The WIN@sea concept, activities, results, and perspectives will be widely disseminated to the public, decision-makers, authorities, researchers, and the business community.

The project is funded by the VELUX FOUNDATION and Aage V. Jensen Naturfond and works in close collaboration with the EU project OLAMUR. The project is led by Aarhus University (AU), with participation from Danish Technical University (DTU), University of Copenhagen (UCPH), Kerteminde SeaFarm, the Kattegatcentre, and Vattenfall.

Further information on the project and activities can be found on www.winatsea.com.

This report focuses on biodiversity and the methods used to assess the biodiversity, associated with the new artificial substrate introduced by wind turbine towers and their scour protection structures.

Sammenfatning

Vi har undersøgt biodiversiteten i Kriegers Flak havmøllepark ved hjælp af eDNA-metoder for henholdsvis skrabeprøver taget lige under havoverfladen på tre vind turbinetårne samt eDNA prøver taget i den øvre og nedre del af vandsøjlen tæt på de samme tårne og uden for havmølleparken. Skrabeprøverne er tillige oparbejdet i et laboratorium af en taksonom til sammenligning. Endelig er de biologiske samfund på turbinetårnene, den tilhørende erosionsbeskyttelse, den omkringliggende sandbund og på tre lokaliteter på et naturligt rev beskrevet ud fra en undervandsdrone (ROV)-undersøgelse med efterfølgende visuel bedømmelse af artsforekomster og deres dækningsgrad. ROV og skrabning blev gennemført som erstatning for en planlagt dykkerundersøgelse, som ikke kunne gennemføres, da havmølleparker er omfattet af en særlig offshore-dykkerbekendtgørelse, hvis krav ikke kunne imødekommes ved normale videnskabelige dykkerundersøgelser.

Undersøgelsen viste, at de forskellige metoder identificerede meget forskellige arter. eDNA undersøgelsen fandt flest arter, men der var også overraskende mangler. Østersø blåmuslingen, *Mytilus trossolus,* som uden sammenhæng var den mest dominerende organisme, kunne kun identificeres til *Mytilus*, og observerede rødalger blev ikke registreret ved eDNA-metoden. eDNA-analyserne havde deres styrke i at identificere fiskearter og mange mindre arter af fx krebsdyr og havbørsteorme. Derudover blev en større mængde planktonarter også fundet.

Undersøgelsen viste også, at der var et lille sammenfald mellem arter fundet på nærliggende stationer, der undersøges ved dykning som led i det nationale overvågningsprogram NOVANA, og arter fundet ved brug af henholdsvis ROV, skrabning samt eDNA analyse på Kriegers Flak. Generelt blev hverken makroalge arter eller taxa identificeret i Kriegers Flak undersøgelsen. Til gengæld identificeredes der arter ved Kriegers Flak, som ikke er en målgruppe i det nationale overvågningsprogram og mangler fra de to overvågningslokaliteter, som fx pelagiske arter og fritlevende mindre arter af fx krebsdyr, muslinger og børsteorme.

Det var kun muligt at bestemme dækningsgraden af relativt få organismer ud fra ROV- undersøgelsen, som viste, at store blåmuslinger var klart dominerende på mølletårnene. Blåmuslinger var også til stede på erosionsbeskyttelsen på bunden og på naturlige revforekomster i områder, men med mindre dækning og med mindre individstørrelser.

Kortlægning, artsidentifikation og indsamling af prøver vha. en dykker ville have været et væsentligt supplement til eDNA, ROV og skrabeprøverne og ville have givet mulighed for at sammenligne de to habitaters biodiversitet med samme metode. Den nuværende dykkerlovgivning, der i praksis er en hindring for videnskabelig dykning i havmølleparker, udgør derfor en begrænsning for at sikre et fagligt robust kendskab til havmølleparkers biodiversitet.

Summary

The biodiversity at the Danish Kriegers Flak wind farm was investigated using multiple methodologies, including environmental DNA (eDNA) analysis of scraping samples from turbine towers (near the surface), eDNA analysis of water samples collected at two water depth near the towers and outside the wind farm, and laboratory-based taxonomic analysis of scraped specimens. Additionally, biological communities on turbine towers, associated erosion protection structures, surrounding sandy seabed, and three nearby natural reef sites were examined through Remote Operated Vehicle (ROV) surveys and subsequent visual assessments. The ROV and scraping for conventional identification were employed as substitutes for a planned diving survey that could not be conducted due to offshore diving regulations, which impose requirements beyond the scope of standard scientific diving protocols.

The study revealed significant differences in species detection across the methods employed. The eDNA analysis detected the highest number of species, particularly small organisms such as crustaceans, polychaetes, and plankton, and proved effective for identifying fish species. However, it failed to detect some dominant organisms, such as *Mytilus trossulus* (identified only at the genus level as Mytilus), and red algae species were not detected at all, despite two different species being observed during visual assessments.

A comparison between species identified at natural boulder reef sites (through years of diving surveys conducted as part of the national monitoring program) and species detected at Kriegers Flak using ROV surveys and scraping with conventional identification, as well as eDNA analysis on water samples and scrapings, revealed a very little overlap. Macroalgal species and taxa were largely absent from the Kriegers Flak investigation. On the other hand, species were identified at Kriegers Flak, which are not a target group in the national monitoring program and are missing from the two monitoring locations, such as pelagic species and free-living smaller species of crustaceans, mussels and brush worms.

The ROV survey identified the coverage of a limited number of organisms but demonstrated that large blue mussels were overwhelmingly dominant on the turbine towers. Blue mussels were also present on the seabed scour protection structures and at nearby natural reef sites, though they exhibited lower coverage and smaller individual sizes on both of those habitats.

Diver based collection of material and mapping of species would have been a valuable supplement to the investigation at the wind farm and would have allowed for a direct comparison. Current offshore diving legislation, which restricts scientific diving at wind farms, presents a significant obstacle to acquiring robust scientific knowledge on wind farm biodiversity.

1 Introduction

The rapid development of offshore wind farms (OWF), marked by the establishment of thousands of turbines, has seen significant progress over recent years. The spatial zoning of marine ecosystems challenges the way we use the sea today, restricting and potentially limiting its potential use for other purposes in the future.

In addition, this development may also impact the marine life above and below the sea surface. In most OWFs, the establishment of wind turbine towers and associated scour protection with cockle sized stones transforms the seabed habitat from a soft sandy sediment to a hard substrate. These structures introduce an artificial habitat spanning from the photic and highly exposed upper water mass to the less or nonphotic lower water mass, which experiences less physical stress. The structure may also intersect a pycnocline, where salinity increases, and temperature decreases in the bottom layer. Finally, the vertical structure of the tower provides a physical environment unlike natural reef structures in Danish waters, which are typically composed of boulders, cockles and smaller stones laying on the seabed.

The direct effect on the marine environment of introducing artificial substrate through wind turbine constructions, the indirect effect of changing food webs around the structures, and the risk of introducing steppingstone effects for invasive species are poorly investigated.

The collection of epi-biological material was initially planned to be conducted via diving. However, diving was not feasible within the project due to special regulations governing offshore diving in oilfields and wind farms in terms of diving equipment and procedures. Before fieldwork began, it was unclear whether the "normal" or the offshore regulations applied to scientific investigations in established wind farms. As a consequence, our investigations relied on three alternative methods: 1) scrapings used for conventional identification obtained from a boat, 2) video surveys conducted with a Remotely Operated Vehicle (ROV), and 3) environmental DNA (eDNA) metabarcoding using water samples and scraped material.

2 Aim

This report evaluates the effectiveness of various methods in describing the biodiversity associated with OWFs. The results are compared with monitoring data from the National Reef Monitoring Program at the nearest locations.

The report also outlines the differences in biodiversity among the artificial substrates introduced by the turbine towers, the original sandy seabed surrounding the turbines, and a natural reef site within the Danish Kriegers Flak wind farm.

3 Materials and Methods

The Danish Kriegers Flak is located in the western Baltic Sea off the island of Møn and close to German and Swedish waters. The wind farm comprises two spatially separated sections established between 2020 and 2021. This study focused on the western section.

The seawater is brackish with an average salinity at the surface around 8 ppm, increasing to 17 ppm at 20m water depth at the nearest national monitoring station 954 south east of Gedser (Dahl et al, 2003).

The field study was conducted in June 2023 during two separate cruises, one aimed at collecting data on epibenthic organisms (13 June), and another focused on environmental DNA (eDNA) sampling (20 June).

3.1 Epibenthic survey

The field study was conducted using Aarhus University's motorboat, Niisa.

Selection of sampling sites

Three wind turbine towers (no. 10, 14 and 17) were selected as study sites (Figure 3.1) due to their central location within the wind farm. The water depth was approximately 24 m at the southern turbine (17), 20 m at turbine 14, and 21 m at turbine 10. A bathymetry map with locations is provided in Appendix 1.

Table 3.1. Information on sampling station locations.

Station name	Latitude	Longitude
Turbine 10	55°01.1981' N	12°49.1600' E
Turbine 14	54°59.9408' N	12°48.9690' E
Turbine 17	54°59.0741' N	12°48,6420' E
Reef site 1	55°00.4257' N	12°86.3051' E
Reef site 2	55°00.5100' N	12°51.2752' E
Reef site 3	55°00.8492' N	12°51.3430' E

In addition, three study sites on a natural reef location were identified. The reef sites were identified by analyzing variations in seabed slope using multibeam data and were verified with the sediment map provided by Vattenfall. The slope was calculated from a bathymetry, with a spatial resolution of 0.5*0.5 m provided by Vattenfall (Figure 3.1). The depth varied from 20.6 to 22 m at the three natural reef transects.

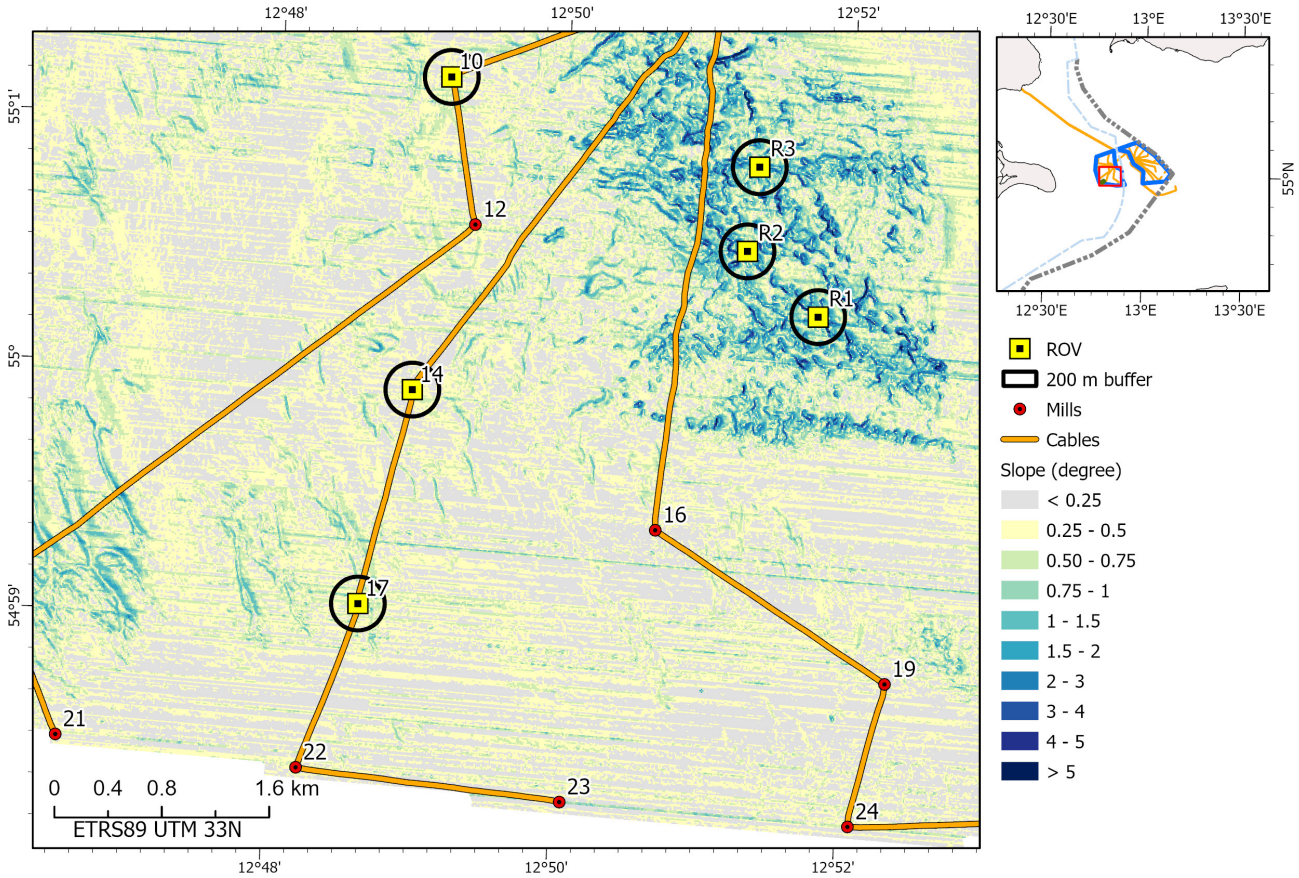


Figure 3.1. Map of the south -west corner of the Danish Kriegers Flak wind farm. The map shows an overview of the sampling area showing the bottom slope using colour codes, turbine positions, and cable routes. Remotely Operated Vehicles (ROV) start positions are also shown with a 200 m buffer zone.

Scraping samples

Three samples of epibenthos were collected on each selected turbine tower using a scraper mounted on a 4 m-long pole. The scraping of biological material was restricted to the uppermost part of the tower (app. 2 m depth) (Figure 3.2).

The samples were preserved in 70% ethanol, and the species composition of macroalgae and epibenthic fauna was investigated in the laboratory.

Following species identification, the samples were homogenized using a robust immersion blender for further analysis using eDNA techniques (see Chapter 3.2).

Figure 3.2. Scraping samples at a turbine tower. Photo Karsten Dahl.

Collection of species cover data

The collection of epibenthic species coverage was conducted using Aarhus University's underwater drone (SRV-8 ROV from Oceanbotics), see Figure 3.3). An initial interpretation of species coverage was conducted on site during the survey.

Figure 3.3. The underwater drone used for observations.

Three transects were planned at each selected wind turbine tower, oriented in the north, east and west directions. Each transect was ideally extended from the surface down the tower to the seabed, continuing across the scour protection and then approximately 100 m beyond the towers over the original seabed (Figure 3.4).

Figure 3.4. Overview of the wind turbine and ROV survey transects. The figure illustrates the layout of the wind farm, including the scour protection and the transect investigated by the ROV.

Large lifting points, connection joints on towers, power cables, and abandoned lifting straps from the construction phase made the retrieving of information challenging and risky, as the ROV became trapped several times.

During the ROV operation, the added value of conducting additional transects in terms of identifying new species and variations was weighted against the risk of losing the equipment. Since the species composition on the first 5 transects was highly restricted and uniform, the number of transects was reduced from 9 to 6. Three transects were conducted at wind turbine no. 17, two on no. 14, and only one on turbine no. 10.

All three planned transects were conducted at the natural reef sites.

Back in the office, all recorded videos were analyzed for species composition by two taxonomic specialists experienced in data from diving and ROV investigations.

The transects conducted on the turbine tower were subdivided into sub-transects following a preliminary analysis. The division was based on depth intervals, where major changes in the biological community were observed.

The tower was divided into an upper zone of 0-2 m, 2-10 m 10-22 m, and 22-22.5 m. The scour protection and the sandy seabed beyond the scour protection were described separately.

Average species cover was assigned to the observed species for each sub-transect. In addition, the number of observed crabs and fish was recorded.

The three transects on the natural reef sites were described by average species covers for the hard substrate and the sandy seabed as two distinct categories, along with the overall abundance of crabs for the entire transect.

3.2 Environmental DNA analysis

Sampling program

The eDNA sampling was carried out at the Danish Kriegers Flak wind farm using Aarhus University's research vessel, AURORA. Water samples were collected using a water sampler app. 50 m downstream from turbines no. 10, 14 and 17 (see Table 3.1 and Figure 3.5), as previously surveyed by the motorboat Niisa using ROV and scraping methods (see Chapter 3.1). Initially, our sampling program aimed to cover increasing distances from the wind farm in upstream and downstream directions, guided by prevailing currents. However, at the onset of the sampling period, only a very weak northward current of 0.1 knots was detectable, dissipating rapidly and hindering our planned sampling within the wind farm. Consequently, we redirected our sampling efforts to three locations app. 200 m north of the farm (OUT1, OUT2, and OUT3; see Table 3.2 and Figure 3.5).

Table 3.2. Information on sampling station locations. OUT station refers to the reference station for water samples outside the wind park.

Station name	Latitude	Longitude
OUT1	55°04,245' N	012°47,822' E
OUT2	55°04,429' N	012°48,081' E
OUT3	55°04,607' N	012°48,460' E

Our sampling strategy initially aimed to collect samples from both the surface and bottom water layers to account for potential stratification in the water column. However, upon analysis we found no evidence of a halocline; instead, only a thermocline was observed at 7 m. Accordingly, water samples were obtained from above and below this thermocline, specifically at depths of 5 and 15 m, across all six stations. Onboard the research vessel, these water samples were filtered using a pressure pump with a sterivex filter attached. Our water sampling procedures generally followed the technical guidelines outlined by Knudsen et al. (2020). To ensure the reliability of subsequent analyses, three replicates (sample replicates) were obtained from each water sample. This involved filtering three water subsamples of 1000 ml from each original sample. The 36 filters were then stored at -18°C and subsequently at -20°C upon arrival at the laboratory until DNA extraction.

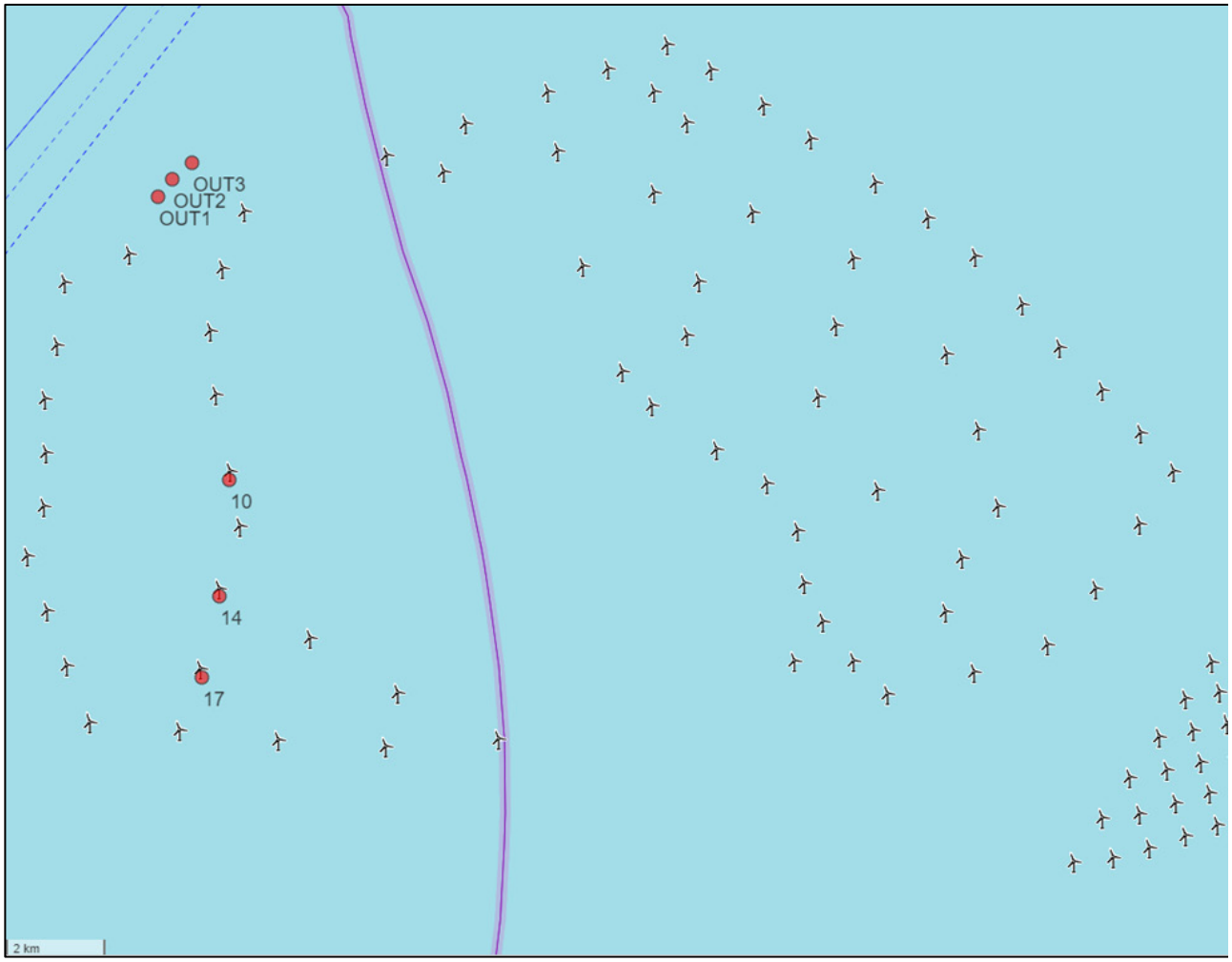


Figure 3.5. Map visualizing the distribution of wind turbines at Kriegers Flak and the sample station locations inside the windfarm (10, 14 and 17) and outside (OUT) the windfarm. Sample locations are marked with red dots.

DNA extraction

Sterivex filters: DNA extraction from the 36 filters followed the procedures outlined in Sapkota et al. (2023). The extraction was conducted using the DNeasy Blood & Tissue kit (QIAGEN), with 'spin-columns,' following the manufacturer's protocol with a minor modification that included proteinase K treatment. The filters were opened and processed under sterile conditions in a flow hood. Instead of the standard 720 µL ATL buffer, a mixture of 720 µL ATL buffer and 80 µL proteinase K (600 U/ml) was used. Subsequently,

the filters were incubated on a rotor in a heating cabinet at 55 °C (± 1 °C) for 4 to 24 hours to ensure complete lysis of the filtrates. The subsequent steps in the extraction procedure followed the manufacturer's protocol. The extracted DNA was divided into multiple Eppendorf tubes and stored at -20 °C until metabarcoding analysis.

Scraping samples: The scraping samples collected from the same three wind turbine foundations, as previously described in Chapter 3.1, were also analyzed for eDNA. The three scraping samples preserved in ethanol were homogenized into a uniform solution using a robust immersion blender (MDH2000, Dynamic). Between samples, the blender was rinsed with tap water to remove all visible material. Subsequently, the blender surface was sterilized sequentially with a chlorine solution (0.05-0.50%) and then with 96% ethanol. Lastly, the blender was exposed to UV radiation for 1 hour to eliminate any residual DNA particles. The homogenized samples were left overnight at room temperature for sedimentation.

From this sedimented homogeneous mixture, three smaller subsamples were extracted from each original scraping sample using a 10 ml pipette and distributed into 50 ml centrifuge tubes. All 9 subsamples were centrifuged (3000 rpm for 5 min) to remove supernatant ethanol. This step was repeated 3 times to ensure thorough removal of the supernatant (see Figure 3.6). Samples were then air-dried for 2 hours at room temperature to remove traces of ethanol and subsequently stored at -20 °C until DNA extraction. Samples were lyophilized for 72 hours and ground using a bead beater. A total of 15 metal beads of 2.4 mm diameter were used for grinding three times for 30 s at a speed of 4 m/s in a bead mill homogenizer (Bead Ruptor Elite, Omni International). Following grinding, DNA extraction was performed using the DNeasy PowerLyser PowerSoil kit (QIAGEN) according to the kit's protocol with minor modifications. Half of the recommended sample quantity (125 mg instead of 250 mg) was used for DNA extraction due to the "powder-like" consistency of the ground sample, which absorbed excessive amounts of PowerBead and C1 solutions, hindering homogenization and vortexing. This adjustment was considered appropriate, given that scraping samples mainly consist of organic material, which typically contains much higher concentrations of DNA compared to the soil samples that the protocol was initially intended for. DNA concentrations were quantified using a Qubit 4.0 fluorometer. DNA from the scraping samples was divided into several Eppendorf tubes and stored at -20 °C until metabarcoding analysis.

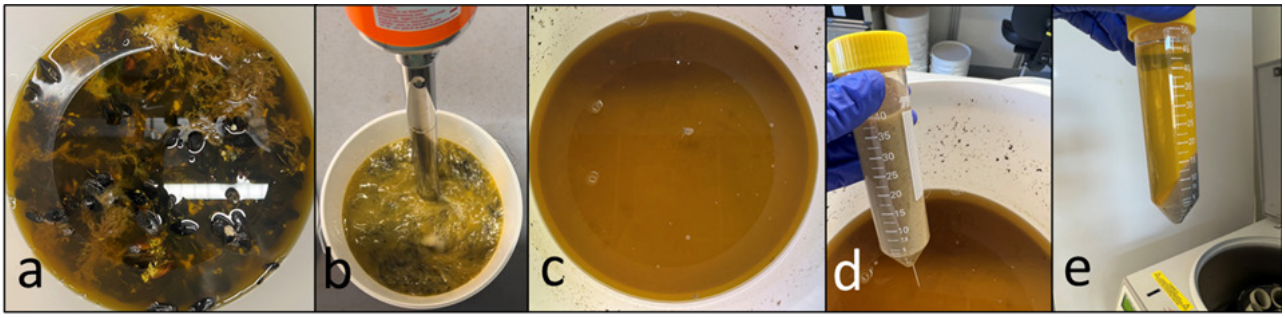


Figure 3.6. Pictures showing a scraping sample stored in an 11.4 L plastic bucket (a), with the preserved sample in ethanol occupying roughly 2/5 of the bucket's volume. The sequence includes blending (b-c), distribution into 50 ml tubes (d), and centrifugation (e).

Metabarcoding

A total of 36 water filter samples and nine scraping samples were subjected to DNA metabarcoding analysis. Our approach generally followed the methodology outlined by Sapkota et al. (2023), with minor modifications to the PCR2 protocol. To study eukaryote, fish, and invertebrate communities, we employed a two-step dual indexing strategy for Illumina MiSeq sequencing, generating sequencing libraries for each. Three different primers targeting the 18S rDNA, 12S rDNA, and COI region of mitochondrial DNA were utilized for this purpose (Table 3.3).

PCR amplification was conducted in a 25 µl reaction mixture containing KaPa HiFi HotStart ReadyMix 2x (Roche), forward and reverse primers, bovine serum albumin, PCR DNA/RNA free water, and DNA template. For 18S rDNA (eukayotes), the PCR cycling conditions consisted of an initial denaturation at 98 °C for 2 min, followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 10 min. Similar thermal cycling conditions were applied for the amplification of invertebrates and fish, with the exception of annealing temperatures set at 48 °C for COI and 65 °C for 12S rDNA.

Table 3.3. Target genomic region, primer sets, and their references used in this study

Locus/Target community	Primers	Sequence	References
12S rDNA /Fish	MiFish-F	GTCGGTAAAACTCGTGCCAGC	Miya et al. 2015
	MiFish-R	CATAGTGGGGTATCTAATCCCAGTTTG	
18S rDNA /Eukaryote	SSU F04	GCTTGTCTCAAAGATTAAGCC	Fonseca et al. 2010
	SSU R22	GCCTGCTGCCTTCCTTGGA	
COI / Invertebrates	mlCOIintF	GGWACWGGWTGAACWGTWTAYCCYCC	Leray et al. 2013
	jgHCO2198	TANACYTCNGGRTGNCCRAARAAYCA	

Subsequently, a 15-cycle indexing PCR (PCR2) was performed to add unique index combinations (i7 and i5) and adaptors. The thermocycler conditions for PCR2 included an initial denaturation at 98 °C for 1 minute, followed by 13 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 20 s, extension at 68 °C for 40 s, and a final extension at 68 °C for 5 min. The size of PCR products was confirmed by visualization on a 1.5% agarose gel stained with SYBR Green.

Following gel visualization, the amplicon products were purified using HighPrep™ magnetic beads according to the manufacturer's instructions. The concentration of the purified amplicons was then determined using a Qubit 4.0 fluorometer. Amplicons were equimolarly pooled to ensure balanced representation in the sequencing library. The size distribution and concentration of the pooled amplicons were assessed using the TapeStation 4200 with the D1000 ScreenTape assay (Agilent Technologies), confirming the expected amplicon sizes and the absence of significant primer dimer formation.

The 12S rDNA PCR amplification resulted in primer dimers detected during the TapeStation analysis. This was resolved by a new purification of the pooled samples and an additional purification step of excision of the desired DNA fragment from a 1.5% agarose gel under EPI BLUE light, and DNA was extracted using the QIAquick Gel Extraction Kit (QIAGEN) following the manufacturer's protocol.

The final purified amplicons were sequenced on the Illumina MiSeq platform using the V2 500 cycle chemistry (Illumina, United States) at DCE, Aarhus University

Bioinformatics and data analysis

Sequencing data from Illumina MiSeq reads were analyzed using QIIME2 ver. 2020.10.0 (Bolyen et al., 2019). Before downstream analysis, reads were truncated for primer sequences and after 230 base pairs for both forward and reverse reads to remove the low-quality bases. Reads were filtered, denoised, merged, chimera checked, and dereplicated using the DADA2 plugin in QIIME2 with default parameters (Callahan et al., 2016). Taxonomic classification of 18S rDNA ASVs was performed using the SILVA rRNA database (v. 138) (Quast et al., 2013). COI and 12S rDNA ASVs were blasted against the BOLD public database and MitoFish v 3.97 using a sequence-id tool (www.gbif.org).

Taxonomic assignments were curated to increase confidence in species/genus level assignments. In all cases, unassigned ASVs were omitted. Next, putative ASVs were required to have at minimum 10 reads across all samples to reduce the likelihood of occurrence from PCR or sequencing errors. Following this, for the COI invertebrate and 12S fish primers, species-level assignments were made if the ASV yielded an identity match of ≥ 99% across 100% of the amplicon, while genus-level assignments were made if the identity match was ≥ 95%. Comparatively, for the 18S rDNA eukaryote primer, species assignments were truncated to the genus level as this gene region is highly conserved across eukaryote species and cannot easily assign species-level annotations. Due to the conservative nature of the 18S rDNA gene (Tang *et al*. 2012), genus-level assignments were considered where the identity match was ≥ 99% across the entire amplicon length. Species identified below the genus level were omitted from further analysis. Lastly, we removed 13 taxa from the detected species list due to contamination of off-target taxa (e.g., *Homo neanderthalensis* and *Mus musculus*), due to the matched hits from species unlikely to be present in the study area, but misidentified with a related native species (e.g., *Pleuronectes quadrituberculatus* and *Clupea pallasii*), or distributions unlikely to be true (e.g., *Limanda aspera, Liopsetta glacialis, Lutjanus argentimaculatus, Lycodes, Micromesistius poutassou, Pleuronectes quadrituberculatus,* and *Stiliger ornatus)*. Upon generation of the final ASV table, the matrix was converted to presence/absence, as metabarcoding data has not yet been demonstrated to be reliably quantitative.

3.3 Additional biological data

Data from the two nearest reef monitoring stations, one site off Møns Klint and the other at Adler Grund (Figure 3.7), were extracted from the NOVANA monitoring database. These data were collected using the technical guideline T14 for the National Reef Monitoring Program (Dahl og Lundsteen, 2018). Data collection involved divers describing the coverage of hard bottom species relative to the suitable stable substrate. Data collection and handling followed two different strategies at the two sites. The site off Møns Klint is an intensive monitoring site with yearly sampling, and the collected material is carefully analyzed for a complete species list. The Adler Grund site is an extensive sampling location, so far only visited twice. At this site, collected material was used solely to verify species identified by divers, not for a complete species list.

Data from off Møns Klint represent the depth interval from 4 to 21 m, collected almost yearly from 2014 to 2020 at six depth stations (app. 4, 6, 8, 11, 14, 18 and 21 m). Data from Adler Grund were limited to two years, 2013 and 2016, and were collected at four depths between 14 and 23 m.

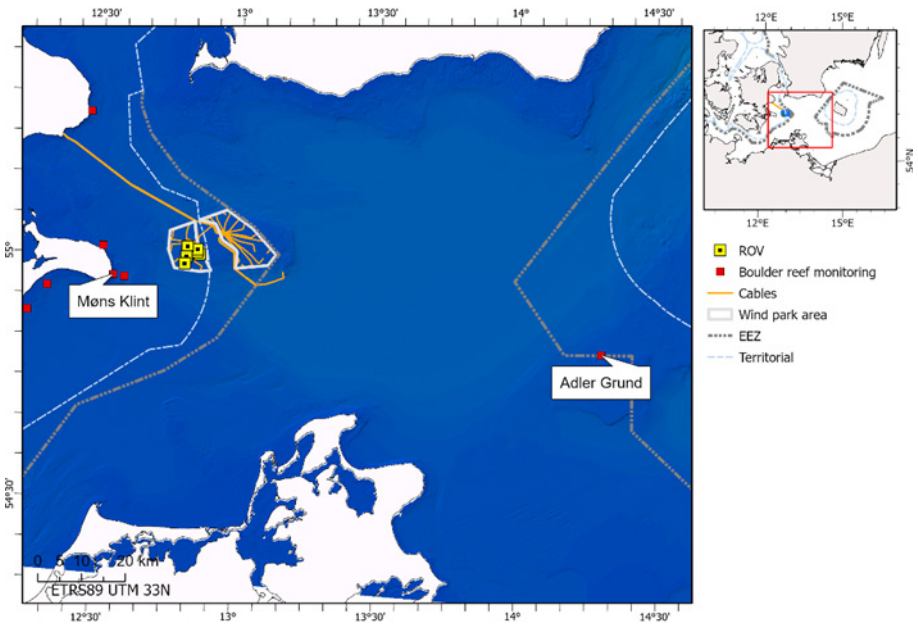


Figure 3.7. Location of the nearest NOVANA reef monitoring stations off Møns Klint and Adler Grund. The Danish Kriegers Flak windfarm is marked with the white polygon.

4 Results

4.1 Species visually identified from the scraping samples

Using this method, a total of 10 distinct species were identified in the upper approximately two meters of the tower under the water surface (Table 4.1).

The crustaceans, *Gammarus zaddachi* and *Amphibalanus improvises,* the blue mussel *Mytilus trossulus,* and the brown algae species *Ectocarpus fasciculatus* and *Ectocarpus penicillatus* were identified in samples from all three wind towers.

The larvae of a mosquito species (Chironomidae) were only found on tower no. 14, the bryozoan *Einhornia crustulenta* was only found on tower no. 17, whereas the brown algae *Scytosiphon lomentaria,* the green algae *Cladophora glomerata* and *Prasiola* sp. were identified on towers no. 17 and 10.

Table 4.1. Species visually identified from scraping samples from 0-2 meters depth on three wind turbine towers at Kriegers Flak.

Scientific Name	Phylum	Class	Station 14	Station 17	Station 10
Gammarus zaddachi	Arthropoda	Malacostraca	x	x	x
Amphibalanus improvisus	Arthropoda	Thecostraca	x	x	x
Chironomidae sp.	Arthropoda	Hexapoda	x		
Einhornia crustulenta	Bryozoa	Gymnolaemata		x	
Mytilus trossulus	Mollusca	Bivalvia	x	x	x
Ectocarpus fasciculatus	Ochrophyta	Phaeophyceae	x	x	x
Ectocarpus penicillatus	Ochrophyta	Phaeophyceae	x	x	x
Scytosiphon lomentaria	Ochrophyta	Phaeophyceae		x	x
Cladophora glomerata	Chlorophyta	Ulvophyceae		x	x
Prasiola sp.	Chlorophyta	Trebouxiophyceae		x	x

4.2 Species and substrate description from ROV videos

In total, 12 distinct species or higher taxonomic taxa were identified. Seven fish, two algae taxa, two invertebrates and one unknown organism.

The upper part of the towers (0-2 m) and the very lower part (app. 0.5 m) just above the seabed had less epibenthic cover. *Mytilus* were generally dominant (Table 4.2), but on turbine no. 10 there was also a relatively high coverage of filamentous algal vegetation (Figure 4.1).

Apart from the tower sections near the surface and very close to the bottom, *Mytilus* covered almost 100% of the tower surface (Figure 4.2). The mussels here were larger than those observed on the boulders at the natural reef site (see below).

Table 4.2.

			INVERTEBRATES					FISH					MACROPHYTES		OTHER
		Depth interval	Mytilus trossulus	Bryozoa	Carcinus maenas	Myoxocephalus scorpius	Gadus morhua	Pholis gunnellus	Finfish	Platichthys flesus	Ctenolabrus rupestris	Filamentous algae	Crustforming algae	Unidentified organisme
TURBINE TOWER 17														
ROV 1	North	0 - 2½m	50											
		2½-10m	100	1		0.1 (1)								
		10-23,5	98	1										0.1
		23,5 - 24,0	5											
		Scour	90			0.1 (2)		0.1 (2)	0.1 (1)					
		Sandy bottom	0.1							0.1 (1)				
ROV 2	West	0 - 2½m	70											
		2½-10m	97	0.1		0.1 (1)								
		10-23,5	95	0.1							0.1 (1)			0.1
		23,5 - 24,0	5											
		Scour	90			0.1 (1)	0.1 (2)	0.1 (1)						
		Sandy bottom	1							0.1 (1)				
ROV 3	South	0 - 2½m	5											
		2½-10m	90	0.1								0.1		
		10-23,5	99	1										0.1
		23,5 - 24,0	5											
		Scour	95		0.1 (1)	0.1 (2)					0.1 (3)			
		Sandy bottom												
TURBINE TOWER 14														
ROV 4	North	0 - 2½m	40											
		2½-10m	95	0.1								0.1		
		10-19m	95	1										0.1
		19-19,5m	lidt											
		Scour	100							0.1 (1)		0.1		
		Sandy bottom	2											
ROV5	East	0 - 2½m												
		2½-10m	99	1		0.1 (1)						0.1		
		10-19m	95	1										
		19-19,5m												
		Scour	100			0.1 (1)	0.1 (1)			0.1 (1)				
		Sandy bottom	1						0.1 (1)	0.1 (1)				

Continues

			INVERTEBRATES				FISH		MACROPHYTES			OTHER
		Depth interval	Mytilus trossulus	Bryozoa	Carcinus maenas	Myoxocephalus scor-pius	Depth inter-val	Mytilus trossulus	Bryozoa	Carcinus maenas	Myoxocephalus scor-pius	
TURBINE TOWER 10												
ROV 6	West	0 - 2½m	25							50		
		2½-10m	100	2						1		1
		10-20,0m	99									1
		20,0-20,5m	Not investigated									
		Scour	Not investigated									
		Sandy bottom	5					0.1 (2)				
REEF SITES												
R-1		Boulders Sandy bottom	70	2			0.1 (1)	0.1 (1)		15	0.1	
R-2		Boulders Sandy bottom	60	1						2	0.1	
R-3		Boulders Sandy bottom	50	1			0.1 (1)			15		

Small spots of bryozoans were present on some of the mussel shells. One organism was observed scattered across most transects on the turbine tower, but it could not be identified (Figure 4.2). It may have been an algae species or the hydroid colony, *Bougainvillia muscus*, a species found off Møns Klint.

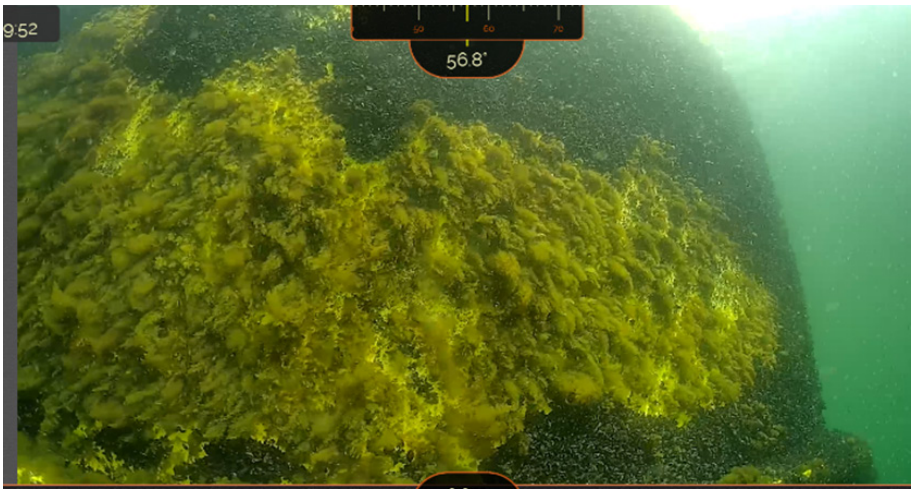


Figure 4.1. Unidentified filamentous algae species in-between mussels at the upper part of the turbine tower.

Figure 4.2. Unidentified species and dense cover of *Mytilus* on the tower.

Common ulcer (*Myoxocephalus scorpius*) was the most common fish observed. They were distributed both on the towers on top of the mussels and at the scour protection, but not observed on the sandy bottom (Figure 4.3).

Figure 4.3. Common ulcer sitting on the turbine tower.

A few goldsinny wrasses (*Ctenolabrus rupestris)* were observed swimming around the towers, and a few butterfish (*Pholis gunnellus)* were hiding between the cockle sized stone making up the scour protection. Only two small cods and one unidentified finfish were seen.

Five flounders (*Platichthys flesus*) were observed on the sandy seabed, and two more were seen on the scour protection (Figure 4.4).

Figure 4.4. Natural reef with boulders covered with *Mytilus* and filamentous red algae species and a flounder on top.

A long linear line of *Mytilus*, likely associated with a power cable laying in level with the sandy seabed, was observed. The round structure of the cable could not be recognized.

Scattered mussels (small patches) were located on the sandy seabed close to the scour protection. Except for flounders and the scattered mussels, no other epibenthic organisms were observed.

All three selected transect lines used for investigating epibenthic organisms on the natural reef site were dominated by hard substrate. Transect 1 and 3, in particular, had a high coverage of very large boulders. Transect 2 was characterized by smaller and more scattered stones, though some areas had denser aggregations of stones and a few large boulders.

The coverage of *Mytilus* on the natural reef boulders was lower compared to the turbine towers, but still above 50% (Table 4.1). The mussels on the natural reefs also appeared smaller in size compared to the mussels established on the towers. Another difference was the presence of filamentous red algae species and crust forming algae species, which were only observed at the transect lines on natural reef sites. The filamentous algae covered up to 15% at the two transects 1 and 3, where the highest density of boulders was observed. The crust forming algae were only observed twice with very low cover. A few cod were observed on the scour protection and on the natural reef site.

4.3 Species identified by environmental DNA

Environmental DNA metabarcoding completed the visual surveys performed with ROV and taxonomic identification of scaped material. After filtering and quality control, we detected a total of 21 taxa at the species level and 28 taxa at the genus level across all three assays, resulting in 49 different unique taxa. A full list of species detected at the genus and species level by eDNA can be found in Appendix 2.

Comparison between environmental DNA sampling methods

In total, eDNA extracted from the seawater detected the most diverse and comprehensive list of taxa, ranging from 6 to 17 taxa per sampling effort. For comparison, the species detected from scrapings ranged from 4 to 7 taxa per sampling effort. Using pairwise Wilcoxon tests, we tested for significant differences in species richness between sampling sites and between sampling methods (eDNA extracted from water vs scrapings). This yielded no significant differences in the richness between water samples at each depth across either the reference site or at Kriegers Flak (see Fig. 4.5). However, species richness was significantly higher at all depths and locations from water samples when compared to scrapings.

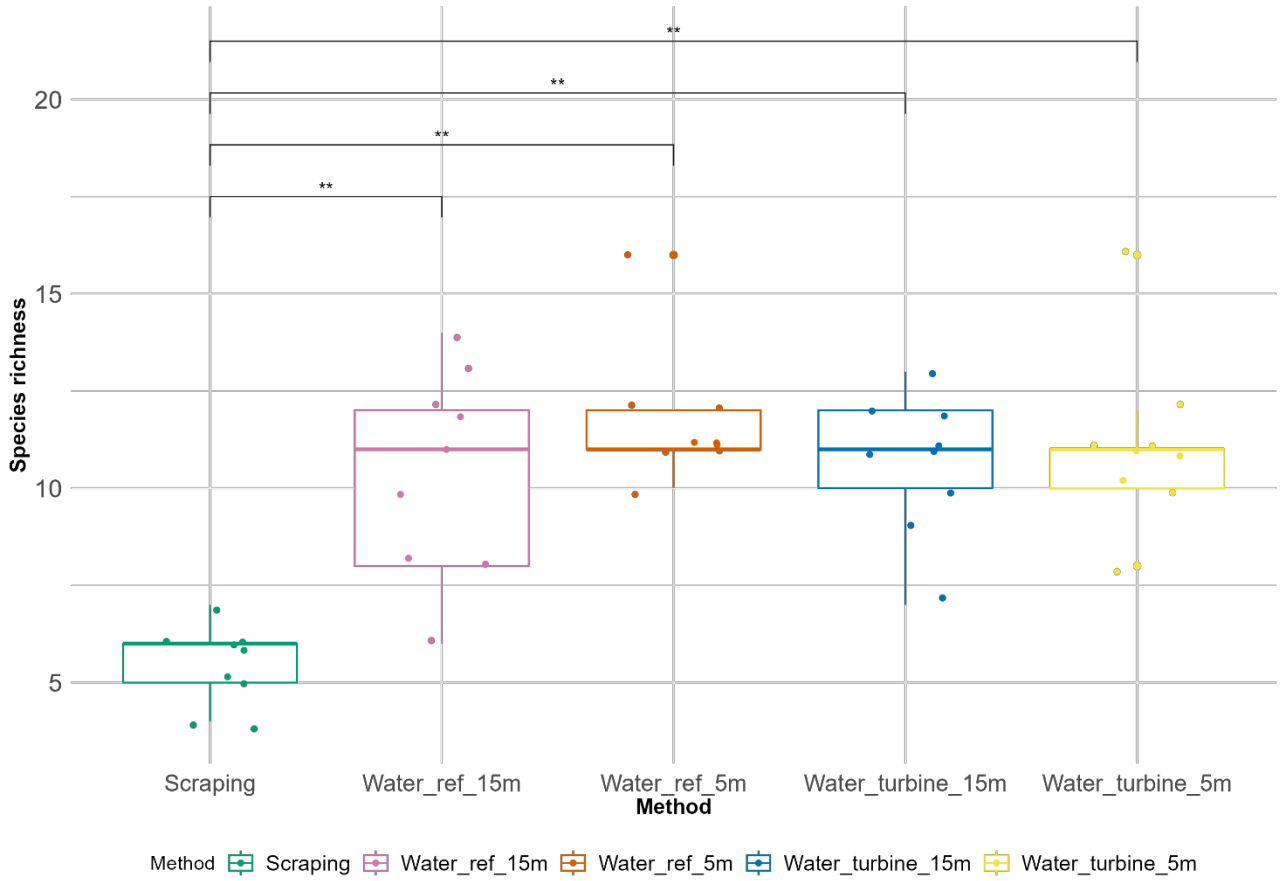


Figure 4.5. Species richness plots created from eDNA samples collected from water samples at 5- and 15-meters depth at both the reference site (orange and pink) and the wind turbine towers at Kriegers Flak (yellow and blue) as well as eDNA extracted from scrapings at the Kriegers Flak (green). Pairwise Wilcoxon tests were used to assess the significance across species richness between methods and locations. Significant differences were found between species richness detected from scrapings when compared to water samples at all locations and depths. No significant differences were found between water samples within or between sites. The significant differences are indicated by the presence of asterisks ** indicating a significant difference between alpha diversities at $p<0.01$.

When comparing eDNA-based community compositions across sampling methods, we found that scrapings exhibited low species diversity, yet unique compositions compared to water samples (see Figure 4.6 and Figure 4.7). Scrapings were more effective at detecting species living at the tower's surfaces, with some of these detections supported by the visual identification (ROV). Most notably, *Mytilus*, which dominated both the towers and the natural reef site, was only detected in eDNA analysis from the scraping samples and not from the water samples. Additionally, species such as *Gammarus zaddachi* and several Arthropoda species were also exclusively recorded in the scraping samples (see Appendix 2). These species likely inhabit the turbine towers directly, suggesting a more localized signal that is not adequately dispersed into the water column (Koziol *et al*. 2019; Holman *et al*. 2019).

The water samples, in contrast, detected a wide range of zoo- and phytoplankton species, as well as fish species not detected in the scraping samples (Appendix 2).

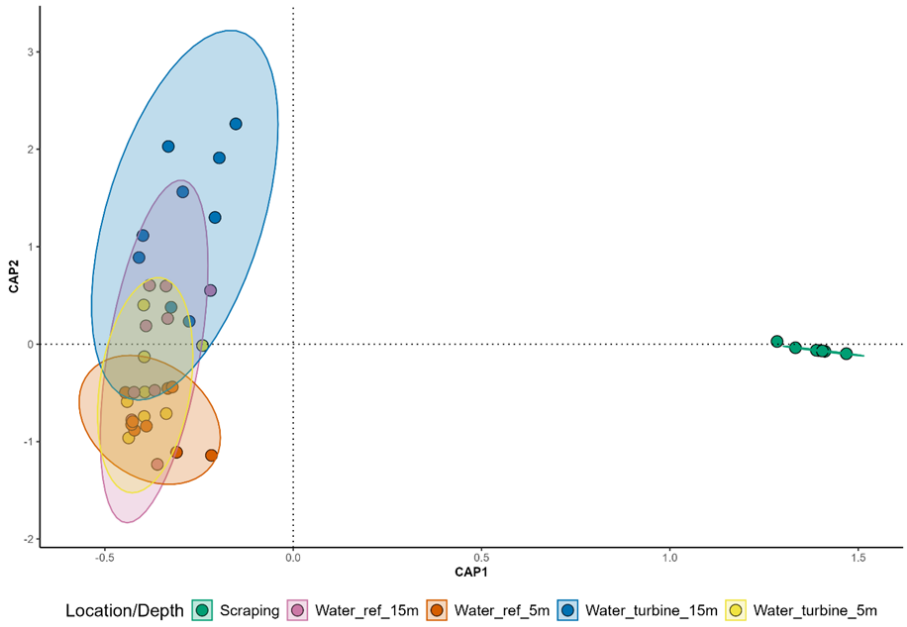


Figure 4.6. Canonical analysis of principal (CAP) coordinates between eDNA metabarcoding community assemblages detected across location and methods from species and genera level detections from water samples at the reference site at 5 meters (N = 9, orange) and 15 meters (N = 9, pink), water samples from Kriegers Flak at 5 meters (N = 9, yellow) and 15 meters (N = 9 , blue), and metabarcoding from scrapings at Kriegers Flak (N = 9, green). Each point represents community assemblage of a biological sample analyzed through metabarcoding. Ellipses represent 95% confidence intervals across groups. Community assemblages detected with eDNA from scraping demonstrate high separation from water samples.

Comparison between Kriegers Flak and reference site

Species compositions were highly similar when comparing the eDNA from water samples taken outside the windfarm (reference location) and inside the Danish Kriegers Flak windfarm area. Of the 39 species detected from water samples, 30 were detected at both locations (Figure 4.7).

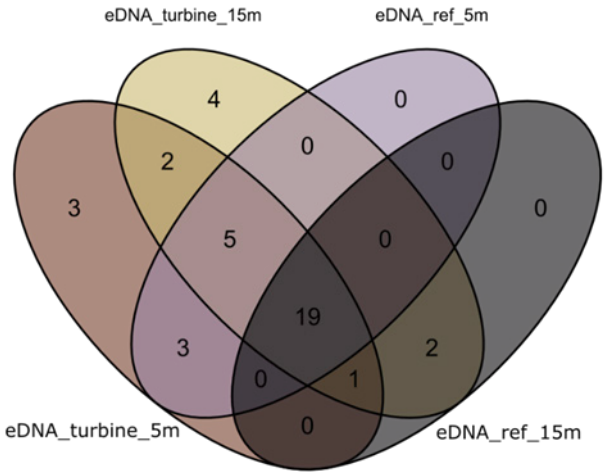


Figure 4.7. Venn diagram of detected species from eDNA water samples at Kriegers Flak (turbine towers) and the reference site at both 5 and 15 meters depth.

There were, however, 9 unique species only detected at the turbine towers at Danish Kriegers Flak (Figure 4.7). The nine species include three fish species: *Zoarces viviparus* (Eelpout), *Gadus morhua* (Atlantic cod) and *Hyperoplus lanceolatus* (Great sand eel). There was one species of barnacle (*Amphibalanus improvisus*), which is dependent on hard structures as substrate, and the remaining 5 species (*Eurytemora affinis, Urotricha sp., Pelagodinium sp., Paraphysomonas sp., Thalassiosira sp.*) are all plankton species. Because we used the 18S and COI markers instead of plankton specific markers, taxonomic resolution varied, with four species identified only at the genus level.

Figure 4.8. Species/genus detections from eDNA water samples differing from the reference site and wind towers at Kriegers Flak. Nine unique taxa (at species or genus level) were detected at the turbines which were not detected at the reference location.

4.4 Method comparison

Species were detected using 4 different methodologies: Visual identification from scrapings, and ROV and eDNA extracted from water and from scrapings. The species lists differed markedly between methods, suggesting a number of advantages and disadvantages when compared. Comparing the cumulated number of species detected, eDNA metabarcoding demonstrated overall higher total numbers of detections when compared to traditional taxonomic techniques from the ROV/visual identifications from scrapings (Figure 4.9).

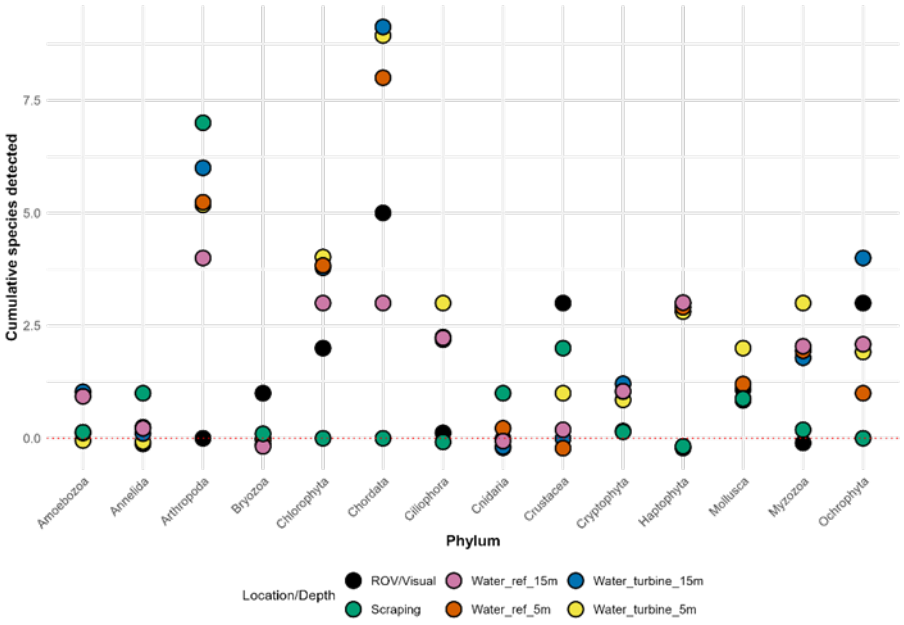


Figure 4.9. Species richness ordered by Phylum across all different methodologies and depths for both eDNA from water and scrapings. Water samples were collected from 5 and 15 meters from Kriegers Flak (yellow and blue respectively) and the reference site at 5 and 15 meters (orange and pinkrespectively. Metabarcoding from scrapings (yellow) and visual based methods using ROV and morphological identification (black) are included.

However, this difference was not concordant across all phyla. Taxa known to have mobile stages demonstrated the highest differences between visual observations and eDNA (e.g. fish detections; Chordata and planktonic species; Ochrophyta, Ciliophora, Myozoa, Chlorophyta and some zooplanktonic species, such as Arthropoda). However, there were notable gaps in the eDNA detections, with some highly abundant species observed through visual methods being missed (e.g. Rhodophyta detected through the ROV transects) or

identified at lower taxonomic ranks. For example, *Mytilus trossulus* was reduced to *Mytilus* in the eDNA extracted from scrapings and was not identified in the eDNA water samples at all. This highlights the importance of substrate selection and how primer selection and biases can influence species detection probabilities and resolution (Van der Loos *et al*. 2020). Despite this, each method demonstrated a high degree of complementarity, with no single method outperforming all methods across all domains, demonstrating an additive benefit of combining multiple methods and sample types.

4.5 Comparison between Kriegers Flak species observations and NOVANA reef monitoring sites

Appendix 2 shows the various fauna and macroalgae species found at Krieger Flak and the nearest boulder reef locations off Møns Klint and Adler Grund southwest of Bornholm.

At the upper end of the Kriegers Flak turbine towers, we identified 10 different species. Additionally, the ROV investigation revealed five fish species and a shore crab, resulting in a total of 14 different species (Figure 4.10). At the low-salinity Adler Grund, a total of 23 species were recorded during the extensive monitoring program in 2013 and 2016. Off Møns Klint, the long time series data showed a total of 97 species.

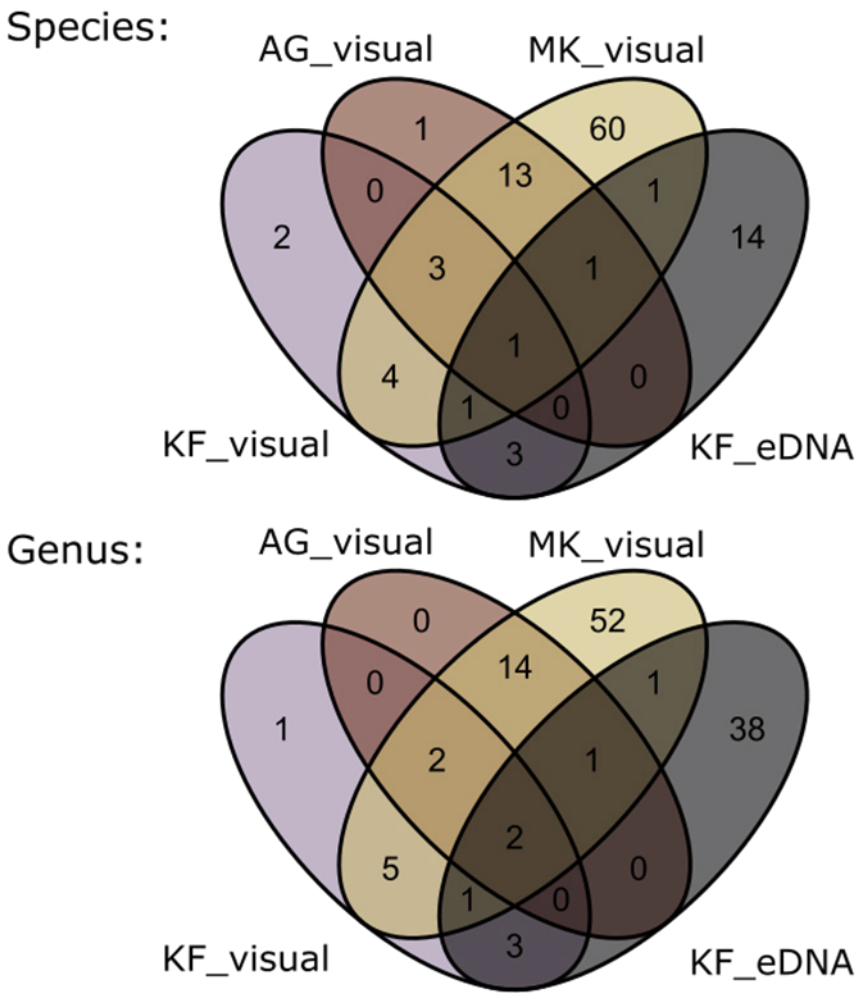


Figure 4.10. Venn diagram of detected species (top) and genus (bottom) from eDNA water samples at Kriegers Flak (close to the Towers) KF_eDNA, ROV investigation within the wind farm (KF_visual), and diver investigations at the two NOVANA stations: Adler Grund (AG_visual) and off Møns Klint (MK_visual).

Comparing the boulder reef sites provides insight into the species potential in the wind park area, which appears to be quite high despite the relatively low salinity. Although the diver-based monitoring program off Møns Klint over a long period recorded the highest number of species, the monitoring deliberately does not focus on small mobile species like Arthropoda, which were identified by the eDNA method. Including small mobile species would increase the species number off Møns Klint even further.

Scraping the upper part of the submerged tower is useful for detecting species located in the upper water column. However, this method has limitations, as it is not possible to collect material from most of the tower or from the scour protection at the seabed.

The species list from the station off Møns Klint exhibits the highest biodiversity, including various fauna species and primarily smaller filamentous macroalgae species. The list also demonstrates many species across a wide depth interval (4-20 m), which is not unusual given the variations in abiotic conditions such as light, salinity, and temperature, which vary with depth.

Both the Adler Grund and Møns Klint species lists were compiled based on diving and material collection, following the guidelines outlined in the technical guideline for reef monitoring (Dahl og Lundsteen, 2018). The methods provide a solid foundation for investigating hard structures, like boulder reefs in Danish sea areas.

The mussel cover on hard substrate on the two monitoring locations, Møns Klint and Adler Grund (Figure 4.11), is comparable to the cover found on the three natural reef sites investigated at Kriegers Flak (50, 60 and 70%) using the ROV. The mussel size on the two monitoring sites is relatively small, typically less the 20 mm.

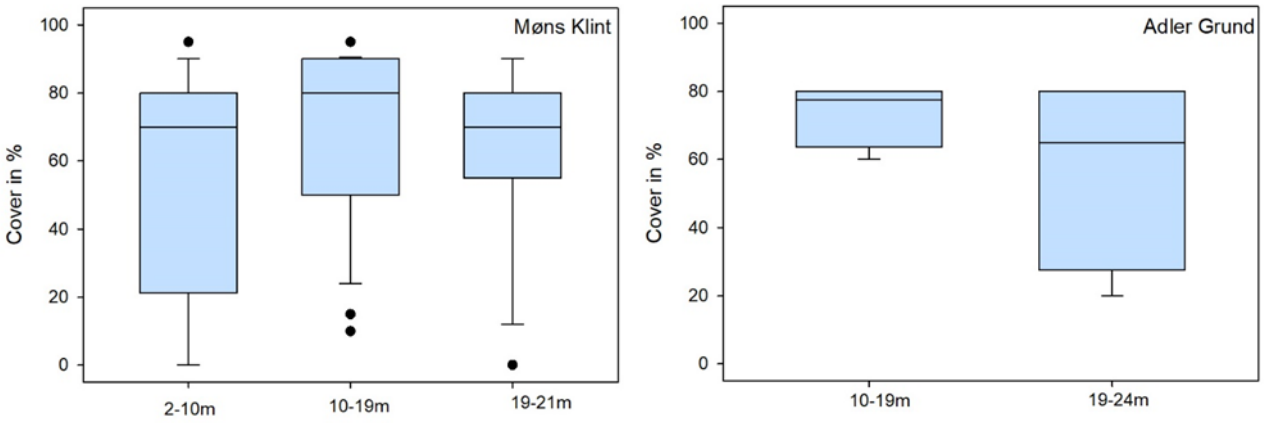


Figure 4.11. Boxplot showing distribution of *Mytilus* cover at different depth intervals in the area off Møns Klint and at Adler Grund.

The cumulative cover of erect green, red and brown macrophyte species at different depths off Møns Klint and Adler Grund is shown in Figure 4.12. The cover is considerably higher at Møns Klint compared to the less saline Adler Grund. The cover observed in Kriegers Flak through ROV is in-between with 2-15%.

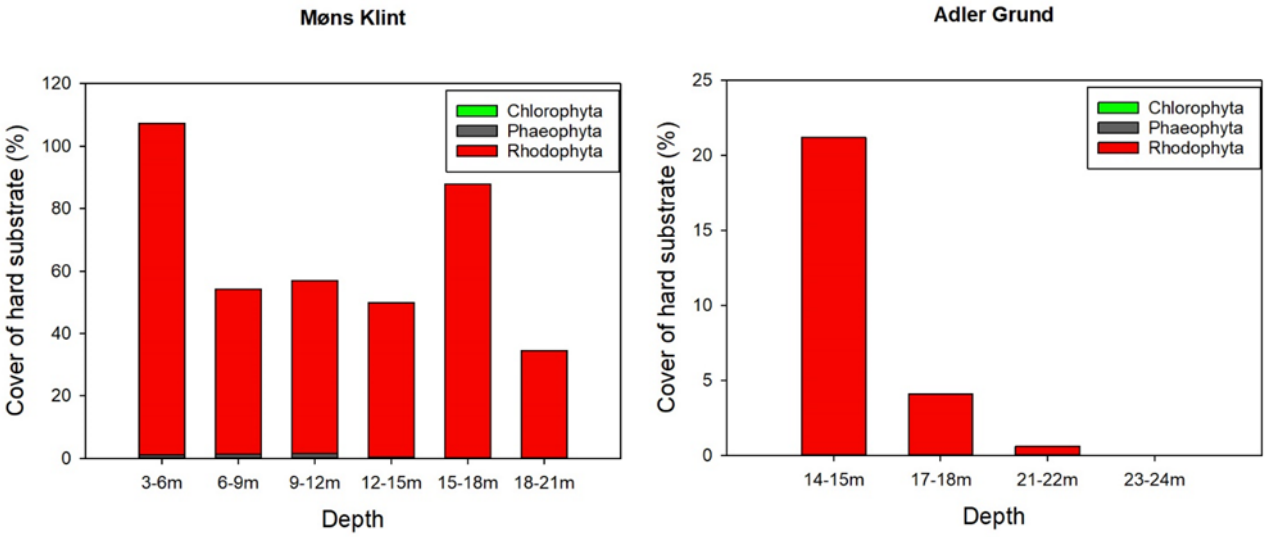


Figure 4.12. Cumulative cover of erect green (Chlorophyta), brown (Phaeophyta) and red (Rhodophyta) macroalgae species aggregated in at different depth intervals from the monitoring stations off Møns Klint and at Adler Grund. The cover is given for hard stable substrate.

5 Discussion and conclusion

The ROV video documentation provided an overview of the turbine towers and nearby reef structures, offering valuable insights into the biological communities and hard structures in the area. In this study of approximately two-year-old turbine structures, we observed a sessile community completely dominated by blue mussels from close to the sea surface to the seabed. The mussel coverage was higher, and the size of mussels seemed considerably bigger, on the towers as compared to the natural reef structures in the area. The size of mussels at Møns Klint at all investigated depths was less than 20 mm. The location on the tower away from the seabed, higher in the water column, might supply more food (plankton) and, hence, stimulate growth, but a reduced predation pressure from shore crabs (*Carcinus maenas*), which was not observed on the towers, may also play a role. Blue mussels (*Mytilus trossulus)* and in the western part also *Mytilus edulis* are generally abundant on hard substrate in large parts of the Baltic Sea. The mussels benefit from very reduced predation pressure, as the starfish, *Asteria rubens,* cannot survive in the low saline water in the Baltic Sea.

Filamentous and crust-forming algae vegetation was observed on the natural reef locations within the farm area. The algae were absent on the scour protection and only a few individuals were observed on the turbine towers between 1 and 10 m water depth.

However, ROV monitoring has limitations in detecting species, as it can only identify larger macroalgae and fauna species and cannot detect species in the lower layers of multilayered community structures. On the natural monitoring reef locations off Møns Klint and at Adler Grund, dwarf forms of the macrophytes *Ahnfeltia plicata* and, more prominently, *Furcellaria lumbricalis* grow entangled with layers of *Mytilus*. Such populations can hardly be observed using a ROV, but will require samples on deck or careful investigations by divers.

For this reason, the cover of erect macroalgae may be underestimated at Kriegers Flak, where only larger algae overgrowing the Mytilus was observed.

The striking absence of visible algae vegetation on the towers, except at the very top, raises some questions. It is possible that algae lost the initial competition for space to blue mussels, or that living on a vertical structure reduces their ability to thrive due to limited light caused by selfshading and shading of the structure itself. Similar observations were made at the Anholt wind farm, where vegetation on the towers was scarce and restricted to the upper part of the towers (Dahl et al, 2025). Anholt wind farm, located in Kattegat, has a higher salinity, and here we found that most of the towers were dominated by fauna, notably the sea anemone *Metridium senile* (Dahl *et al.* 2025). No differences were observed in algae cover and species distribution between southwestern, southeastern and northern transects in Anholt wind farm

eDNA metabarcoding was utilized to complement biodiversity assessments at the wind turbine towers and the reference site. The combination of water and scrapings yielded varying communities, with significantly more species detected in the water column compared to those found in the scrapings. Many

of these additional species were planktonic and not the focus of this study, However, combining both scrapings and water samples revealed unique communities, highlighting the importance of substrate selection for eDNA analyses. This was particularly exemplified in the small overlap between species identified visually from the scraping samples and those detected using metabarcoding from the same samples. For instance, *Mytilus trossulus*, abundant in visual assessments, could not be resolved to species level in the metabarcoding analyses.

Notably, there was little congruence between eDNA metabarcoding and conventional methods in the detected biodiversity at Kriegers Flak.

In some cases, species were observed on the turbine tower and the natural reef site, e.g. unidentified red algae *Rhodophyta* sp. and the bryozoan *Einhornia crustulenta,* but they were not detected with eDNA metabarcoding despite their abundance. This highlights some of the limitations of the applied eDNA methods. Firstly, amplification bias could have preferentially amplified amplicons from other species with higher affinity, effectively drowning out the signal with our chosen primers. Secondly, primer selection may have impacted the detection of these species, as our chosen primers may not have matched well to these species, inhibiting species detection. In a study on boulder reefs biodiversity using both diving and eDNA, the eDNA method, relying on COI and 18S rDNA makers, only detected 16 out of 94 (13%) species of macroalgae observed through diver-based sampling (Staehr et al, 2022). This suggests that either the absence of macroalgae-specific primers or low levels of DNA shedding rates of species within other algal species (e.g., *Caulerpa prolifera,* Waters *et al*. 2023) may lead to under-detection of several algae species.

The comparison between wind towers at Kriegers flak and the reference location outside the farm area demonstrates that wind turbine constructions provide an additional hard substrate that supports the growth of new species. More species were detected exclusively at the wind farm, including the invasive barnacle *Amphibalanus improvisus*. Moreover, the detection of four fish species within the farm areas is also notable, as the more heterogeneous habitat created by the natural reef sites together with the construction appears to attract higher fish diversity.

When compared to the communities at the nearest natural reef sites, the combined results from visual identification of scraping samples, ROV and eDNA investigations highlight the need to complement these methods with diving and material collection for further taxonomic analyses in the laboratory. This approach will enhance our understanding of species composition and biodiversity at wind turbine structures across different depths and the surrounding area. Smaller species, such as bryozoans, cnidarians, and crustaceans, which are often firmly attached to substrates or entangled within macroalgae or *Mytilus* are not visible with ROVs, and scraping samples fail to adequately represent different depths. Additionally, eDNA analyses showed several false negatives, such as the absence of the abundant *Einhornia crustulenta*, and *Mytilus* detection only in scraping samples, demonstrating the importance of substrate selection for molecular studies. Moreover, our sampling design captured a snapshot of the biodiversity detected over approximately two years of the establishment of the turbines and may not reflect the future biodiversity development in the surrounding areas. Ongoing monitoring will be required to document and catalogue changes in species composition through time following the succession of the artificial habitats.

Unfortunately, scientific diving at wind farms is subject to offshore diving legislation. In practice, these regulations hinder the collection of highly valuable data on biodiversity and community structure at wind farms, limiting our ability to gain comprehensive insights into the ecological impacts of these structures.

6 References

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger et al., (2019). Reproducible, interactive, scalable and ex-tensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.

Dahl, K. og Lundsteen, S. (2018). Makroalger og hårdbundsfauna på sten- og boblerev Teknisk Anvisning (TA) nr. 14, DCE, Aarhus Universitet https://ecos.au.dk/fileadmin/ecos/Fagdatacentre/Marin/TA_M14__Makroalger_og_bundfauna_paa_sten-_og_boblerev_ver1.pdf.
http://dce2.au.dk/pub/SR344.pdf

Dahl, K., S. Lundsteen, and S. Helmig (2003). Stenrev, Havbundens oaser. Gads Forlag. https://www.stenrev.dk/media/69249/stenrev-havets-oaser.pdf

Karsten Dahl, Ben J. O. Robinson. Helle Buur, Karoline Reducha Andersen, Nikolaj Reducha Andersen, Cordula Göke, Jørgen L. S. Hansen and Anne Winding (2025). Anholt wind farm's impact on benthic biodiversity at turbine and wind farm level Aarhus University, DCE – Danish Centre for Environment and Energy, 47 pp. Technical Report No. 330.

Fonseca, V.G., Carvalho, G.R., Sung, W., Johnson, H.F., Power, D.M., Neill, S.P., Packer, M., Blaxter, M.L., Lambshead, P.J.D., Thomas, W.K., Creer, S., (2010). Second-generation environmental sequencing unmasks marine metazoan biodiversity. Nat. Commun. 2010 11 1, 1–8.

Holman, L.E., de Bruyn, M., Creer, S., Carvalho, G., Rodibart, J., Rius, M. Jl. (2019). Detection of introduced and resident marine species using environmental DNA metabarcoding of sediment and water. *Sci Rep* **9**, 11559 https://doi.org/10.1038/s41598-019-47899-7

Knudsen, S.W., Andersen, J.H., Bekkevold, D., Hesselsøe, M., Jensen, S.K.S, Møller, P.R., (2020). Tekniske anvisninger for eDNA-baseret overvågning af ikke-hjemmehørende marine arter. Tekniske anvisninger for eDNA-baseret overvågning (mst.dk)

Koziol A, Stat M, Simpson T, Jarman, S., DiBattista, J., Harvey, E., Marnane, M., McDonald, J., Bunce, M. (2019) Environmental DNA metabarcoding studies are critically affected by substrate selection. Mol Ecol Resour. 19: 366–376. https://doi.org/10.1111/1755-0998.12971

Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., Ranwez, V., Boehm, J.T., Machida, R.J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. Front. Zool. 10, 1–14.

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J.Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M. and Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. R. Soc. Open Sci. 2.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590-6.

Sapkota, R., Winding, A., Stæhr, P.A.U., Andersen, N.R., Buur, H., Hablutzel, P. (2023). Use of metabarcoding to detect non-indigenous species in Danish harbours: Methods comparison. Aarhus University, DCE – Danish Centre for Environment and Energy, 30 pp. Technical Report No. 267 https://dce2.au.dk/pub/TR267.pdf

Staehr, P. A. U., K. Dahl, H. Buur, C. G+¦ke, R. Sapkota, A. Winding, M. Panova, M. Obst, and P. Sundberg (2022). Environmental DNA Monitoring of Biodiversity Hotspots in Danish Marine Waters. Frontiers in Marine Science **8**. https://www.frontiersin.org/journals/marine-science/articles/10.3389/fmars.2021.800474/full

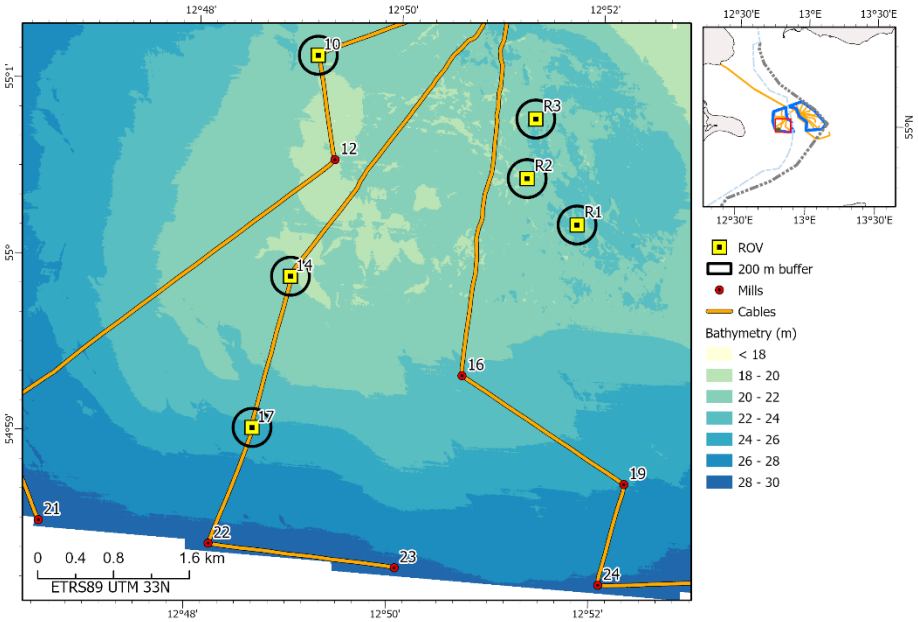
van der Loos LM, Nijland R. (2021). Biases in bulk: DNA metabarcoding of marine communities and the methodology involved. *Mol Ecol*. ; 30: 3270–3288. https://doi.org/10.1111/mec.15592

Tang,C.Q., Leasi, F., Obertegger, U., Kieneke, A., Barraclough, T.G. and Fontaneto, D. (2012). The widely used small subunit 18S rDNA molecule greatly underestimates true diversity in biodiversity surveys of the meiofauna, *Proc. Natl. Acad. Sci. U.S.A.* 109 (40) 16208-16212, https://doi.org/10.1073/pnas.1209160109 (2012).

Waters, T., Langlois, K., Gold, Z., Theroux, S., Eagle, R. (2023). Hidden in plain sight: The invasive macroalga *Caulerpa prolifera* evades detection by environmental DNA methods. *Environmental DNA*. E496. https://doi.org/10.1002/edn3.496

7 Appendixes

7.1 Appendix 1



Figur 7.1. ROV start positions with a circle indication 200 m distance with bathymetry, turbine positions and cable routes.

7.2 Appendix 2: Summary of species/genus detections across sampling methods and locations

Phylum	Scientific name	Krigers Flak					Reference station		NOVANA stations		Planktonic
		ROV	Scraping		eDNA water		eDNA water		Adler Grund	Møns Klint	
		0-2m	Lab. 0-2m	eDNA 0-2m	5m	15m	5m	15m	14-23m	4-20 m	
Amoebozoa	*Vannella sp.*				X			X			
Annelida	*Arenicola marina*									X	
Annelida	*Spirobranchus triqueter*									X	
Annelida	*Spirorbinae indet.*									X	
Annelida	*Alitta succinea*			X							
Arthropoda	*Amphibalanus improvisus*	X	X	X		X			X	X	
Arthropoda	*Amphiblestrum auritum*								X		
Arthropoda	*Bosmina sp.*				X	X	X	X			Zooplankton
Arthropoda	*Carcinus maenas*	X								X	
Arthropoda	*Crangon crangon*									X	
Arthropoda	*Nobia sp.*			X							
Arthropoda	*Amphibalanus sp.*			X							
Arthropoda	*Centropages sp.*				X	X	X				Zooplankton
Arthropoda	*Centropages hamatus*				X	X	X	X			Zooplankton
Arthropoda	*Clunio marinus*			X							
Arthropoda	*Eurytemora affinis*				X						Zooplankton
Arthropoda	*Evadne sp.*				X	X	X	X			Zooplankton
Arthropoda	*Gammarus zaddachi*	X	X	X							
Arthropoda	*Gammarus salinus*			X							
Arthropoda	*Halocladius varians*			X							
Arthropoda	*Jaera albifrons*			X							
Arthropoda	*Mysidae indet.*								X	X	
Arthropoda	*Palaemon varians*									X	
Arthropoda	*Temora longicornis*			X	X	X	X	X			Zooplankton
Bryozoa	*Alcyonidioides mytili*									X	
Bryozoa	*Alcyonidium gelatinosum*									X	
Bryozoa	*Alcyonidium hirsutum*									X	
Bryozoa	*Amathia imbricata*									X	
Bryozoa	*Amphiblestrum auritum*									X	
Bryozoa	*Einhornia crustulenta*	X	X						X	X	
Bryozoa	*Electra pilosa*									X	
Bryozoa	*Eucratea loricata*									X	
Bryozoa	*Walkeria uva*									X	
Chlorophyta	*Bryopsis sp.*									X	
Chlorophyta	*Chaetomorpha melagonium*									X	
Chlorophyta	*Cladophora glomerata*	X	X								

Continues....

Phylum	Scientific name	Krigers Flak					Reference station		NOVANA stations		
		ROV	Scraping		eDNA water		eDNA water		Adler Grund	Møns Klint	
		0-2m	Lab. 0-2m	eDNA 0-2m	5m	15m	5m	15m	14-23m	4-20 m	Planktonic
Chlorophyta	*Cladophora rupestris*									X	
Chlorophyta	*Cladophora sericea*									X	
Chlorophyta	*Epicladia phillipsii*									X	
Chlorophyta	*Prasiola sp.*	X	X								
Chlorophyta	*Ulvella scutata*									X	
Chlorophyta	*Chlamydomonas sp*				X	X	X				Phytoplankton
Chlorophyta	*Choricystis sp.*				X	X	X	X			Phytoplankton
Chlorophyta	*Cymbomonas sp.*				X	X	X	X			Phytoplankton
Chlorophyta	*Pyramimonas sp*				X	X	X	X			Phytoplankton
Chordata	*Ctenolabrus rupestris*	X			X	X	X			X	
Chordata	*Gadus morhua*	X			X						
Chordata	*Gobius niger*								X	X	
Chordata	*Gobiusculus flavescens*				X	X	X	X	X	X	
Chordata	*Myoxocephalus scorpius*	X								X	
Chordata	*Neogobius melanostomus*								X	X	
Chordata	*Nerophis ophidion*									X	
Chordata	*Pholis gunnellus*	X			X	X	X				
Chordata	*Platichthys flesus*	X							X	X	
Chordata	*Pollachius virens*									X	
Chordata	*Pomatoschistus minutus*								X	X	
Chordata	*Scophthalmus maximus*					X	X			X	
Chordata	*Syngnathus typhle*									X	
Chordata	*Thorogobius ephippiatus*									X	
Chordata	*Clupea harengus*				X	X	X	X			
Chordata	*Hyperoplus lanceolatus*				X	X					
Chordata	*Melanogrammus aeglefinus*					X	X				
Chordata	*Salmo salar*				X	X	X	X			
Chordata	*Scomber scombrus*				X	X	X				
Chordata	*Zoarces sp.*				X						
Ciliophora	*Askenasia sp.*				X	X	X	X			Plankton (cilliata)
Ciliophora	*Strombidium sp.*				X	X	X	X			Plankton (cilliata)
Ciliophora	*Urotricha sp.*					X					Plankton (cilliata)
Cnidaria	*Bougainvillia muscus*									X	
Cnidaria	*Campanulariidae indet.*									X	
Cnidaria	*Clava multicornis*									X	
Cnidaria	*Dynamena pumila*									X	
Cnidaria	*Gonothyraea loveni*								X	X	
Cnidaria	*Opercularella lacerata*									X	

Continues....

Phylum	Scientific name	Krigers Flak					Reference station		NOVANA stations		
		ROV	Scraping		eDNA water		eDNA water		Adler Grund	Møns Klint	
		0-2m	Lab. 0-2m	eDNA 0-2m	5m	15m	5m	15m	14-23m	4-20 m	Planktonic
Cnidaria	*Aurelia sp.*			X							makrozooplank-ton
Cryptophyta	*Telonema sp.*				X	X	X	X			Phytoplankton
Echinoder-mata	*Asterias rubens*									X	
Haptophyta	*Chrysochromulina sp.*				X	X	X	X			Phytoplankton
Haptophyta	*Haptolina sp.*				X	X	X	X			Phytoplankton
Haptophyta	*Prymnesium sp.*				X	X	X	X			Phytoplankton
Mollusca	*Hydrobiidae indet.*									X	
Mollusca	*Littorina littorea*									X	
Mollusca	*Mytilus trossulus*		X						X	X	
Mollusca	*Theodoxus fluviatilis*									X	
Mollusca	*Tonicella marmorea*									X	
Mollusca	*Cerastoderma sp.*				X	X		X			
Mollusca	*Macoma balthica*					X	X				
Mollusca	*Mytilus sp.*	X		X							
Myzozoa	*Gymnodinium sp.*				X	X	X	X			Zooplankton
Myzozoa	*Pelagodinium sp.*					X					Zooplankton
Myzozoa	*Azadinium sp.*				X	X	X	X			Zooplankton
Ochrophyta	*Battersia arctica*									X	
Ochrophyta	*Desmarestia viridis*									X	
Ochrophyta	*Ectocarpus fasciculatus*		X								
Ochrophyta	*Ectocarpus penicillatus/ siliculosus*		X							X	
Ochrophyta	*Laminaria digitata*									X	
Ochrophyta	*Pseudolithoderma extensum*									X	
Ochrophyta	*Pylaiella littoralis*								X	X	
Ochrophyta	*Saccharina latissima*									X	
Ochrophyta	*Scytosiphon lomentaria*		X							X	
Ochrophyta	*Sphacelaria sp.*									X	
Ochrophyta	*Sphaceloderma caespitulum*									X	
Ochrophyta	*Hydrurus sp.*				X	X	X	X			
Ochrophyta	*Ochromonas sp.*				X			X			Phytoplankton
Ochrophyta	*Paraphysomonas sp.*				X	X					Phytoplankton
Ochrophyta	*Thalassiosira sp.*				X						Phytoplankton
Porifera	*Halichondria pa-nicea*									X	
Rhodophyta	*Acrochaetium sp.*									X	
Rhodophyta	*Aglaothamnion hookeri*									X	
Rhodophyta	*Aglaothamnion tenuissimum*								X	X	
Rhodophyta	*Ahnfeltia plicata*									X	
Rhodophyta	*Ahnfeltia plicata crust*									X	

Continues....

Phylum	Scientific name	Krigers Flak					Reference station		NOVANA stations		Planktonic
		ROV	Scraping		eDNA water		eDNA water		Adler Grund	Møns Klint	
		0-2m	Lab. 0-2m	eDNA 0-2m	5m	15m	5m	15m	14-23m	4-20 m	
Rhodophyta	*Bonnemaisonia hamifera*									X	
Rhodophyta	*Callithamnion corymbosum*									X	
Rhodophyta	*Carradoriella elongata*								X	X	
Rhodophyta	*Ceramium deslongchampsii*									X	
Rhodophyta	*Ceramium tenu-icorne*								X	X	
Rhodophyta	*Ceramium virgatum*									X	
Rhodophyta	*Coccotylus brodiei*								X	X	
Rhodophyta	*Coccotylus hartzii*									X	
Rhodophyta	*Coccotylus truncatus*									X	
Rhodophyta	*Colaconema strictum*									X	
Rhodophyta	*Cruoria pellita*									X	
Rhodophyta	*Delesseria sanguinea*								X	X	
Rhodophyta	*Furcellaria lumbricalis*								X	X	
Rhodophyta	*Haemescharia hennedyi*									X	
Rhodophyta	*Hildenbrandia crouaniorum*									X	
Rhodophyta	*Hildenbrandia rubra*									X	
Rhodophyta	*Leptosiphonia fibrillosa*									X	
Rhodophyta	*Lithothamnion glaciale*									X	
Rhodophyta	*Melobesia membranacea*									X	
Rhodophyta	*Membranoptera alata*									X	
Rhodophyta	*Phycodrys rubens*									X	
Rhodophyta	*Phyllophora pseu-doceranoides*									X	
Rhodophyta	*Polysiphonia stricta*									X	
Rhodophyta	*Rhodochorton purpureum*									X	
Rhodophyta	*Rhodomela confervoides*								X	X	
Rhodophyta	*Scagelothamnion pusillum*									X	
Rhodophyta	*Spermothamnion repens*									X	
Rhodophyta	*Vertebrata fucoides*								X	X	
Rhodophyta	"red filamentous" algae	X									
Rhodophyta	"red Crustforming algae"	X									

MARINE BIODIVERSITY RELATED TO ESTABLISHMENT OF OFFSHORE WIND TURBINES

A case study from Kriegers Flak in the western Baltic Sea

This report describes the biodiversity within the Danish Kriegers Flak wind farm using several different techniques and compares the results with pelagic reference stations outside the wind farm area, and reef sites investigated as part of the national monitoring program, NOVANA.

ISBN: 978-87-7156-927-8
ISSN: 2244-9981