CHAPTER 3

INCORPORATING HYPERBENTHOS SAMPLING IN OWF MONITORING SURVEYS: CURRENT KNOWLEDGE, CHALLENGES AND OPPORTUNITIES

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Abstract

In this study, findings are reported from the first large-scale hyperbenthic monitoring survey conducted in two Belgian offshore windfarms (OWFs) that differ in terms of local habitat conditions, turbine foundation type and timing of construction. Three replicates (tracks) were collected inside and outside each OWF and the applied sampling design was used to describe the general hyperbenthic communities within these areas and to assess potential OWF-related impacts through a spatial analysis (inside vs outside tracks) as it is proposed that the cumulative effects of turbine presence (“artificial reef effect”) and cease of fishery activities (“fisheries exclusion effect”) might result in enriched hyperbenthic communities within the OWFs. Hyperbenthic distributions at the C-Power study site can be defined as a “mixture community”, receiving influences from offshore and transitional communities. Communities at the Norther study site corresponded with transitional communities, but also harboured species that are found in more nearshore areas and exhibited considerable variation within the concession zone. Total densities, diversity and community composition differed between the inside and outside areas at C-Power and while these trends are aligned with the enrichment hypothesis, it remains unclear whether these spatial differences can be attributed to the presence of the OWF. Results at the Norther study site did not indicate similar trends and it is suggested that the lack of OWF-related effects may be attributed to the “young” lifespan of the Norther site (operational since 2019–2020) and the habitat heterogeneity that characterizes this OWF. Despite the valuable insights gained within this study, it also revealed that increased sampling efforts are needed to enhance the ability to fully characterize the hyperbenthic communities and strengthen the statistical power to detect OWF-related impacts, especially for the Norther OWF. It is also recommended to include relevant abiotic variables such as the near-bottom water pigment concentration, seabed granulometry and organic matter content within the sampling design.

1. Introduction

Research on hyperbenthos is a relatively “young” discipline and this group of animals in the water column, living on or associated with the seabed aroused general interest among researchers only from the late 1950s (Mees & Jones 1997). Over time, hyperbenthos has been known by a variety of names such as "suprabenthos", "demersal zooplankton" or "benthopelagic plankton", leading to a lack of synthesis across studies (Mees & Jones 1997). Mees & Jones (1997) were the first to introduce a general classification of this group into mero- and holohyperbenthos, based on their time spent within the hyperbenthic zone. Holohyperbenthos are animals that spend variable periods of their adult life within this zone and comprise groups such as peracarids, copepods, chaetognaths and hydromedusae (Mees & Jones 1997; Dewicke *et al*. 2003). In contrast, merohyperbenthos species are only found within the hyperbenthal zone during early life stages and include larval decapods, polychaetes and fishes (Mees & Jones 1997; Dewicke *et al*. 2003). Organisms of this ecosystem component are also found in the dynamic, lowest layers of the water column, comprise a broad assemblage of species with diverse traits (*e.g*. morphology, vertical position and mobility) and are known to exhibit a variable distribution, both in space and time. All of these aspects complicate efficient sampling, and specialized sampling devices such as the hyperbenthic sledge have only been developed and used since a few decades (Mees & Jones 1997; Dewicke *et al*. 2003). This type of equipment and its success also strongly depend on local conditions such as ship capacities, weather conditions, depth and seafloor topography, where a relatively flat and even seabed is required for a successful deployment (Mees & Jones 1997; Lefaible *et al*. 2019a). This was confirmed during a feasibility study to sample hyperbenthos in two Belgian offshore wind farms (OWFs) constructed within sandbank systems (Lefaible *et al*. 2019b), which revealed that samples taken along previously used epibenthic tracks (1 km) resulted in several non-representative samples due to high accumulation of sediment in the hyperbenthic sledge collectors because of the relative position of the tracks to the sand ridges (Lefaible *et al*. 2019b).

The issues associated with disparate terminology, the intrinsic dynamic characteristics of these organisms and the difficulties to perform a representative quantitative sampling have resulted in a low number of descriptive baseline studies on hyperbenthic distributions (Mees & Jones 1997; Parry *et al*. 2021). Within the available literature, there is also a strong focus on two habitat types, namely estuaries and the surf zone on beaches, while there is little information on hyperbenthic communities in deeper, offshore areas (Mees & Jones 1997). A study by Dewicke *et al*. (2003) represents one of a few extensive surveys on hyperbenthic spatial patterns in the Belgian part of the North Sea (BPNS). This study detected six biological communities that could be linked with the different subtidal sandbank systems. Trends in community density and biomass principally followed an onshore-offshore gradient, while an east-west gradient was observed for diversity, which was most pronounced in the onshore areas (Dewicke *et al*. 2003). In general, hyperbenthic abundance and diversity were significantly lower at the offshore Hinder Banks compared to the Flemish and Zeeland Banks located closer to the coast, and it was concluded that distance to the coastline, current direction and habitat heterogeneity were the most important structuring factors for larger-scale hyperbenthic distribution patterns (Dewicke *et al.* 2003).

Despite the limited knowledge of this ecosystem component, its importance in the functioning of marine ecosystems has been widely recognised (Mees & Jones 1997; Dewicke *et al.* 2003; McGovern *et al.* 2018; De Neve *et al.* 2020). Due to their vertical position within the marine system, at the interface between sediment and water column, it is expected that these organisms play an important role in the exchange of energy, nutrients and biomass between the seabed and the water column (Mees & Jones 1997; De Neve *et al.* 2020). Besides their importance for bentho-pelagic coupling, hyperbenthos also fulfil a supporting role as a food source for higher trophic levels (Mees & Jones 1997; De Neve *et al.* 2020). For example, stomach content analysis revealed that several hyperbenthic taxa, and certainly mysids, constitute a major component of the diet of many demersal fish and epibenthic crustaceans throughout different stages of their life cycles (post-larval, juvenile and adult), which also implies that the "hyperbenthic zone" functions as a nursery area for these often commercially important species (Mees & Jones 1997; De Neve *et al.* 2020). Because of the strong analogy between hyperbenthic distribution patterns and those of other benthic assemblages such as macrobenthos, it is believed that these organisms might also be influenced by the altered habitat characteristics created by the presence of wind turbines (Dannheim *et al.* 2020; Lefaible *et al.* 2019b, 2021). On the one hand, altered hydrodynamics around the turbines create "refugium" areas with decreased current velocities, which might increase the settlement of passively dispersing, planktonic species within those areas and provide sheltered against hydrological forcing for actively swimming hyperbenthic organism (Mees & Jones 1997). On the other hand, the combined effects of organic enrichment and increased deposition of organic matter by the turbine-associated epifauna (Dannheim *et al.* 2020) may attract more mobile species that can actively migrate to these areas with higher food availability, as has been demonstrated for several demersal and benthic fish species (Vandendriessche *et al.* 2013; Reubens *et al.* 2014). It is therefore hypothesised that these turbine-related effects on the sediment could create more favourable conditions and the concomitant establishment of richer hyperbenthic communities within the OWFs. Moreover, it is not allowed to trawl in these areas, and thereby the OWFs function as de facto marine protected areas (MPAs). Bottom fishing activities negatively affect benthic and pelagic ecosystem components and can be considered as one of the most important disturbances within the marine realm (Johnson 2002). It is therefore expected that the removal of this pressure within the concession zones could allow the recovery of seabed-water interface habitats and communities, with concomitant positive impacts on higher trophic levels due to their important function as a nursery area and food supply for many fish and crustaceans (Vandendriessche *et al.* 2013; Reubens *et al.* 2014). As a result, monitoring hyperbenthos could also be a propitious method to assess long-term impacts of the "fishery exclusion effect" of OWFs.

The main objective of this study is to investigate potential OWF-related impacts on hyperbenthic communities for two concession zones that differ in terms of local habitat characteristics, turbine foundation types and timing of construction. To achieve this, samples were collected from areas located inside and outside the OWFs to perform a spatial analysis in which we want to test whether OWF areas harbor enriched hyperbenthic communities. Furthermore, it will also be verified whether the sampling method (*i.e.* sample collection and processing) resulted in a qualitative description of the hyperbenthic communities within these areas. Through these findings, we want to contribute to the general knowledge of this poorly studied ecosystem component and highlight the importance of their inclusion within OWF monitoring surveys.

2. Material and methods

2.1. Study areas

Sampling was performed in two operational OWFs situated in the eastern concession zone of the Belgian Part of the North Sea (BPNS), namely C-Power and Norther. Whereas these concession zones are located relatively close to each other, they differ in terms of local habitat characteristics, turbine foundation types and timing of construction. C-Power was constructed on the Thornton bank (TB) at an intermediate distance from the coastline (30 km) relative to the most offshore situated OWF (Rumes & Brabant 2017). This OWF is composed of 6 gravity-based and 48 jacket foundations and became fully operational in 2013 (Rumes & Brabant 2017). Norther is located 23 km from the Belgian coastline, southeast from the TB and represents the most nearshore OWF within the concession area (Lefaible *et al.* 2021). The construction phase of the Norther OWF ended in 2019, with the installation of 44 monopiles, which also makes this OWF the most recent operational park (Lefaible *et al.* 2021).

2.2. Sampling strategy

A feasibility study performed in 2018 revealed that sampling locations based on existing epibenthic tracks (1 km towing distance) at C-Power did not result in adequate hyperbenthos samples due to a high accumulation of sediment within the sample collectors (Lefaible *et al*. 2018). Therefore, it was proposed to perform several (3 tracks), shorter (150 m towing distance) replicate tracks in which local topography (*i.e.* position of sand ridges) is taken into account, based on digital terrain models for the C-Power study site provided by the Federal Public Service Economy (FOD Economie). The new sampling strategy was applied during a one-day sampling cruise (3/11/2021) on board the *Simon Stevin* in the C-Power and Norther concession areas where three replicate tracks were taken within each concession area and reference areas outside the OWFs (Fig. 1). The hyperbenthic sledge on the *Simon Stevin* consists of two nets: the lower net samples water depths from 0.2 to 0.5 m, and the upper net from 0.5 to 1 m above the bottom; both nets have a 1 mm mesh size. A flowmeter was installed in the lower net to calculate the volume of water filtered during each sampling event. An average volume of 71 ± 21 m^3 was filtered through the nets during the sampling in and around the C-Power OWF. Average volumes were lower in the inside the OWF ($n = 3$, 65 ± 19 m^3) compared to the reference area ($n = 3$, 77 ± 23 m^3). For the Norther study site, an average volume of 40 ± 5 m^3 of water was filtered through the nets at the Norther study site and the average amount of water flow was comparable between areas inside and outside the OWF (38 ± 5 m^3 and 42 ± 5 m^3). Tows were carried out during daytime conditions and hauled counter current at the lowest towing speed possible (1.5 knots) according to the strategy applied by Dewicke *et al*. (2003) to allow a descriptive comparison with results found in this study.

2.3. Sample processing and analysis

Samples obtained from the lower and upper nets were immediately rinsed on board with seawater over a 1 mm sieve, collected into separate 1L pots and preserved with seawater-buffered 4% formaldehyde. In the laboratory, organisms were sorted, counted and identified to the lowest possible taxonomic level. Further specification on the life stage of the organism (megalopa, larva and juvenile) was also applied for certain classes such Polychaeta, Malacostraca (infraorder: Caridea, Anomura, Brachyura) and Actinopterygii. Following DeWicke *et al* (2003), typical macrobenthic and non-hyperbenthic species (juvenile and adult fish and decapods, fish eggs and ectoparasitic organisms) were removed from the dataset. Total densities for each sample were obtained by standardizing the data to individuals per 100 m^3 as follows: ind./100 m^3 = number individuals /(surface net * number of turns flowmeter*0.3) * 100. The multiplication by 0.3 within this formula was added to account for the fact that the flowmeter used in this study increased by one unit for every three rounds. Due to time limitations, biomass could not be determined and the upper-net samples collected in the Norther OWF still need to be processed. Diversity indices were calculated based on raw count data and included species richness (S), Shannon-Wiener diversity index (H') and Pielou's evenness (J'). Species reported on a higher taxonomic level were considered as "unique" if no other representative of the same taxon level was present or if they were distinctly different (morphospecies).

Relative contributions of the major observed high-level taxonomic groups (Class and Order) and species to overall total

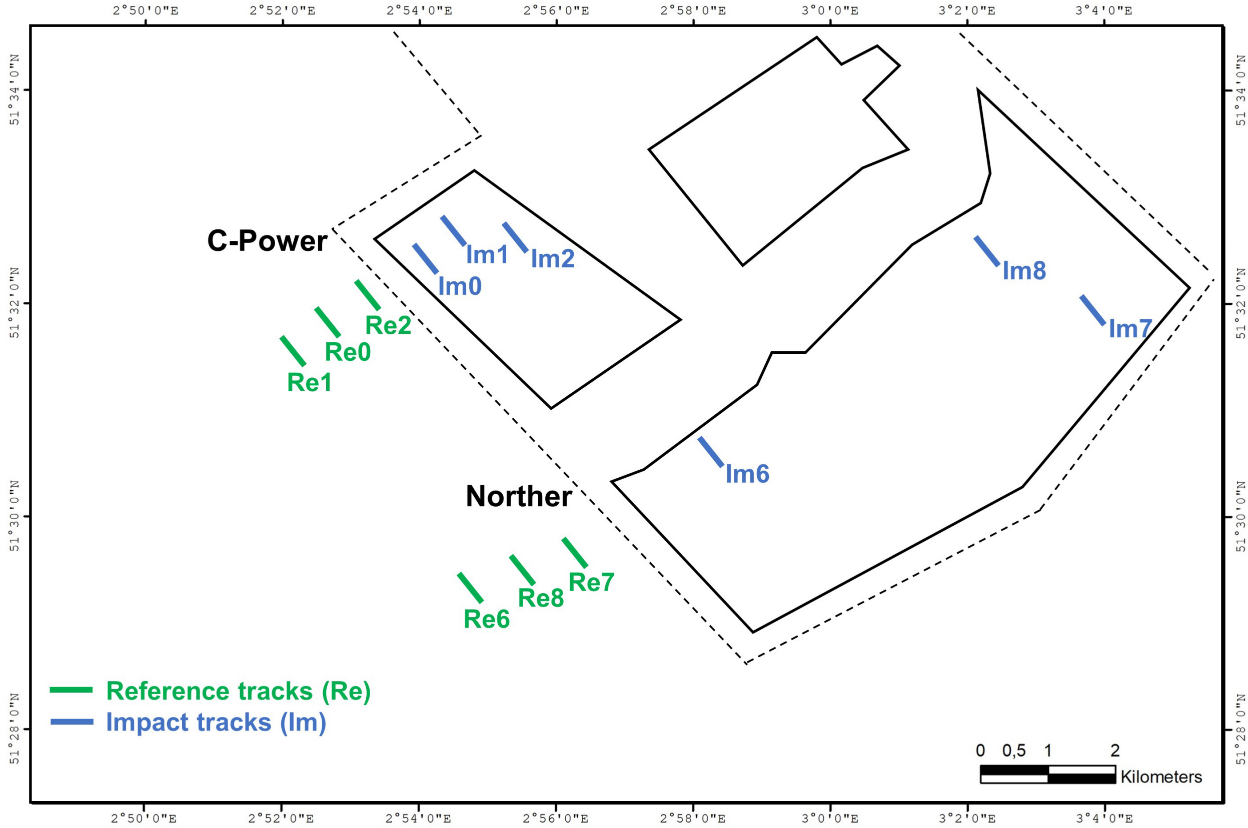

Figure 1. Location of the two windfarm concession areas under study (upper: C-Power, lower: Norther) sampled during the 2021 monitoring campaign. Positions of the tracks are depicted with green lines for the reference tracks (Re: Re0-3 and Re6-7) taken outside the OWFs and blue lines for the impact tracks (Im; Im0-2 and Im6-7) taken inside each OWF.

densities for each sample were calculated and used to describe the hyperbenthic community composition patterns for the inside and outside areas at each OWF. In addition, Species Accumulation Curves (SACs) were constructed for the lower net samples from each area (inside and outside the OWFs) for C-Power and Norther to assess the level of accuracy of the monitoring survey. Only the lower net samples were used to allow a comparison between both OWFs as the upper net samples of the Norther OWF could not be included within this study. Moreover, several non-parametric richness estimators (Chao1, Jacknife1, Bootstrap) were also calculated to estimate the number of unrecorded species.

OWF-related effects were investigated by means of a spatial comparison to test differences between the sampled areas (inside OWF vs outside OWF) for the structural univariate indices under study. For the C-Power study site, this was done by a two-way ANOVA (Factors: “Position” and “Area” with levels: “lower”, “upper” and “inside”, “outside”), while a one-way ANOVA (Factor: “Area” with levels “inside”, “outside”) was used for the Norther study site. Assumptions of normality and homogeneity of variances were tested by Shapiro-Wilk and Levene tests, respectively, and log transformations were performed if these assumptions were not met. Moreover, a posteriori power analyses were performed to assess the statistical power of the applied sampling design to detect differences between the areas inside and outside the OWFs for two important univariate community descriptors (total densities and Shannon-Wiener diversity). This was done by means of the pwr.anova.

test (k = , n = , f = , sig.level = , power =) function, based on the fitted models. In order to allow a correct comparison between the two OWFs under study, these models were fitted for the lower net samples only. The power analysis function was based on four parameters, namely the sample size (k and n), the effect size (f), the significance level (default of 0.05) and the power or probability to detect an effect. Within this analysis, the sample size (n) corresponds to the three lower samples, taken within each subgroup (k = 2, inside vs outside). The effect sizes (f) were calculated through the etaSquared() function, for the sum of square values obtained from the fitted models. This allowed to calculate the required sample size per subgroup to detect effects with a power of 0.80 (*i.e.* 80% likelihood to detect an effect). In addition, the statistical power was calculated for the currently applied sampling design (k =2 with n = 3) and to predict the statistical power under two theoretical scenarios of increased sampling effort (k = 2 with n = 6, k = 2 with n = 12). Comparable to the univariate analysis, a two-way and one-way Permanova test was performed to assess OWF-related effects on the community composition for C-Power and Norther, respectively. Homogeneity of multivariate dispersions was tested using the PERMDISP routine (distances among centroids). Species Indicator Analysis (SIA, package “indicspecies”) to test which species were most responsible for the differences in community composition. Patterns in terms of community composition were visualized by means of non-metric multidimensional scaling (nMDS) plots, constructed through a Bray-Curtis matrix. All the multivariate analyses were based on relative abundance data, to exclude effects of differences in total abundances between samples.

3. Results

3.1. Hyperbenthic distribution patterns

Within the C-Power study site, a total of 54 species was found, originating from 11 higher taxa (Class/Order) and total densities per sample ranged from 930 ind. 100 m^{-3} to 2197 ind. 100 m^{-3} (Fig. 2). Relative contribution calculations showed that community compositions were clearly dominated by three major groups. Cnidaria, identified as medusa of the class Hydrozoa, accounted for 50 % of total densities. Malacostracan crustaceans of the order Amphipoda also contributed meaningfully to overall abundances (29 %)

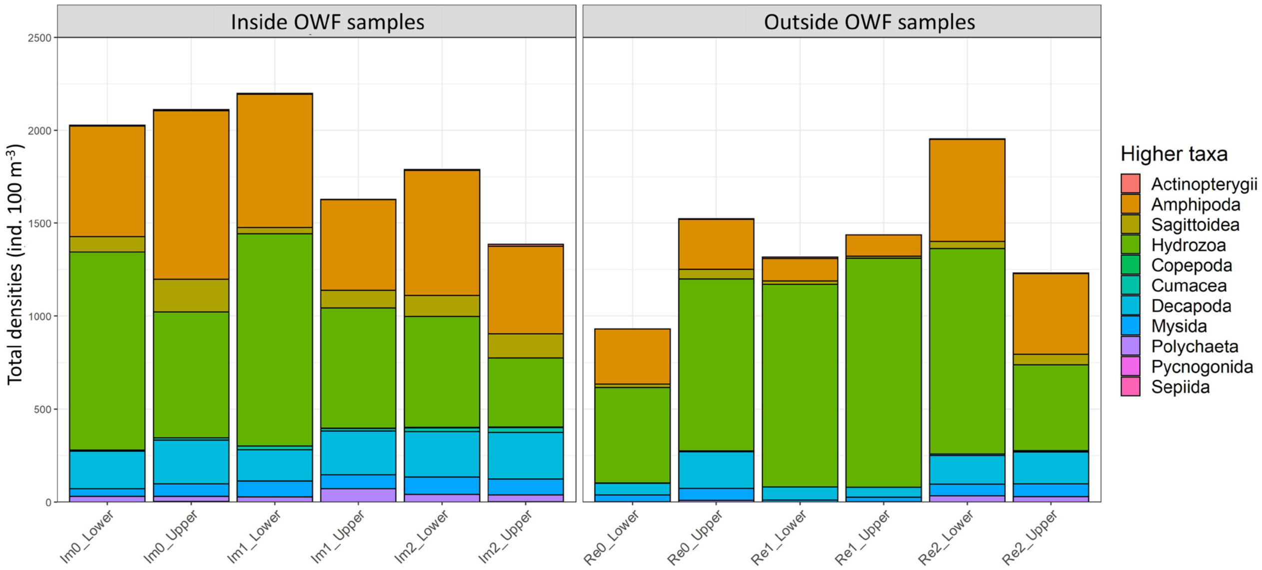

Figure 2. Total hyperbenthic densities (ind. 100 m^{-3}) per sample for the areas located inside (impact) and outside (reference) the C-Power study area. Relative contributions of the major lower-level taxa to overall densities are also included.

and this group was mainly represented by organisms of the family Atylidae (*Nototropis* sp.). A third dominant group was larval and megalopa life stages of different Decapoda, such as *Pisidia longicornis* and *Macropodia rostrata*, making up about 11 % of total densities. Sagittoidea (*Sagitta elegans*) and Mysida (mainly *Mesopodopsis slabberi*, *Acanthomysis longicornis* and *Gastrosaccus spinifer*), contributed to a lesser extent (4%) to overall densities. All of these species were detected in each sample and are therefore considered as characteristic hyperbenthic species within the C-Power samples.

The lower net samples of the Norther OWF contained a total number of 40 species, divided over 9 higher level class and order taxa (Fig. 3). Total densities showed considerable variation and ranged from 1205 ind. 100 m^{-3} to 10157 ind. 100 m^{-3}. One sample (Im8) taken inside the OWF, clearly deviated from the other ones in terms of total densities, which could be attributed to the extreme abundance of hydromedusae (9990 ind. 100 m^{-3}). If this group was not considered, this sample actually proved to be the most impoverished one in terms of total abundance (166 ind. 100 m^{-3}) and diversity (S: 17, H: 0.12). Community composition at the Norther site was dominated by Hydrozoa (Hydromedusa) and Amphipoda (*Nototropis* sp.), which collectively contributed about 80% to total abundances. However, Mysida also comprised about 7% and were mainly represented by the species *Gastrosaccus spinifer*, *Schistomysis* sp. and *Mesopodopsis slabberi*. Megalopa and larval life stages of the Decapoda group (*Pisidia longicornis* and morphospecies 1) comprised another 5% to overall abundances, while many other groups contributed to a lesser extent: Sagittoidea (*Sagitta elegans*, 2%), Polychaeta (*Lanice conchilega* larva, 1.5%) and Actinopterygii (fish larvae, 1%). All of the above-mentioned species were shared among all samples except for the sample Im8 sample. The latter sample did not contain the species *Sagitta elegans*, *Schistomysis* sp. and fish larva, and overall community composition was comparable to the one described for the C-Power site.

Figure 4 visualizes the cumulative number of species recorded as a function of the sampling effort (lower net samples, n = 3) for the different areas under study. The resulting SACs indicate that the largest share of newly recorded species are found

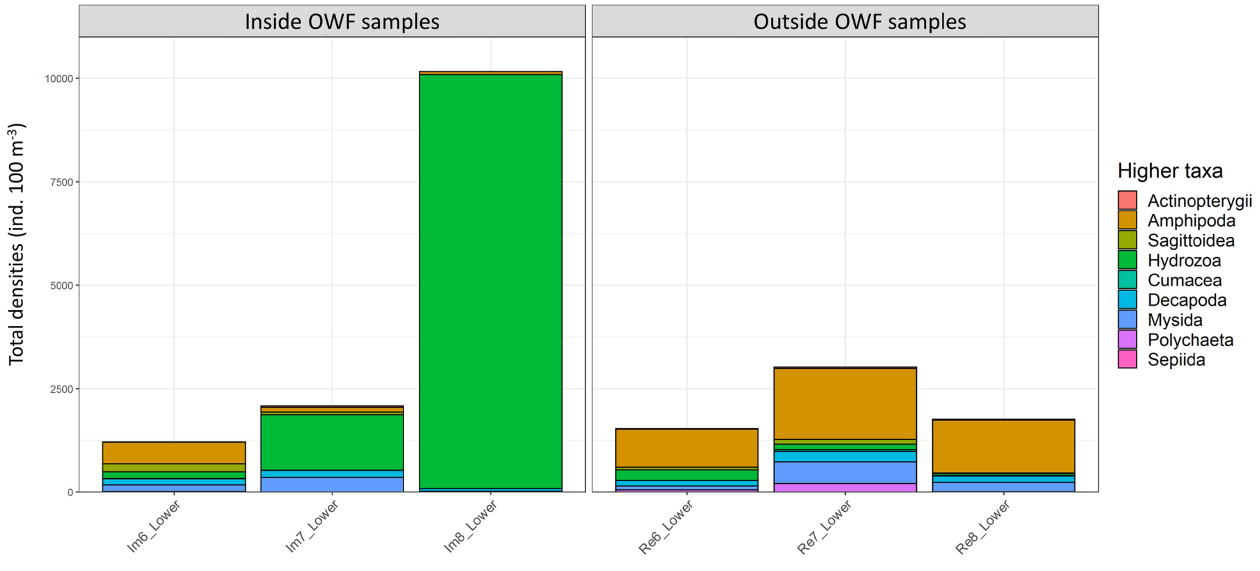

Figure 3. Total hyperbenthic densities (ind. 100 m^{-3}) per sample for the areas located inside (impact) and outside (reference) the Norther study area. Relative contributions of the major lower-level taxa to overall densities are also included.

between the first and second sample, while the curves seem to already level off slightly towards n = 3, especially for the samples taken inside the C-Power OWF (Fig. 4A) and the reference area outside the Norther OWF (Fig. 4D). Based on the richness estimator values, it appears the number of "undetected" species was found to be rather low, but some variation was seen between the sampled areas. Highest absolute differences between the estimated richness values and the recorded species richness (S) were found for the samples inside the Norther OWF and those collected outside the C-Power OWF, ranging between 4 and 8 species. For the other two sampling areas (inside the C-Power OWF and outside the Norther OWF) these values ranged between 1 and 5 species.

3.2. OWF-related impacts: inside vs outside areas

Results from the two-way ANOVA analysis for the C-Power study site revealed that the average values for all the univariate variables were comparable between the lower

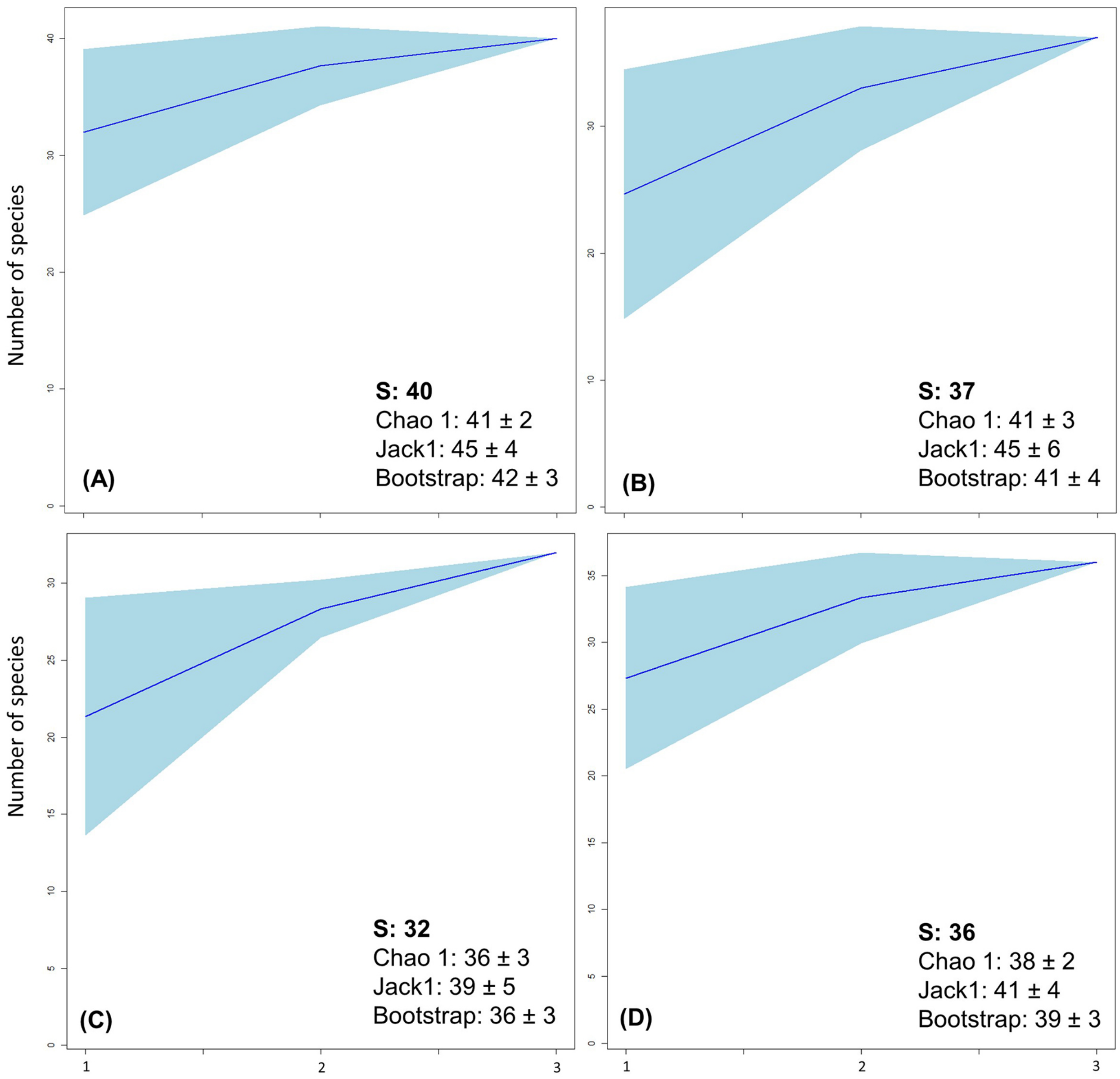

Figure 4. Species Accumulation Curves (SACs) for the samples taken inside (graphs A and C) and outside (graphs B and D) the C-Power (upper) and Norther (lower) study site. For every area, the species richness (S) is given, together with several richness estimators: Chao1, Jacknife1 and Bootstrap (mean ± SE).

and upper net samples (TN/S/H'/J', factor "Position": $p > 0.05$) and that no significant interactions were found (TH/S/h'/J', factors "Area" and "Position": $p > 0.05$). In contrast, all of the univariate community descriptors showed higher average values inside the OWF compared to the reference area located outside the concession zone (Table 1) and significant spatial differences were detected between the inside and outside areas for the total abundance (TN, factor "Area": $p = 0.049$), the Shannon-Wiener diversity (H', factor "Area": $p = 0.024$) and the Pielou's evenness (J', factor "Area": $p = 0.033$). The statistical power analysis was performed for the sample size estimation, based on the models to compare the total abundance (TN) and Shannon-Wiener diversity (H') between the inside and outside areas at C-Power. The effect sizes were found to be 0.94 and 1.04 for TN and H' respectively. Given these sample sizes, a significance level of 0.05 and a power of 0.80, the projected sample size per subgroup or area (inside vs outside) would be $n = 6$ for TN and $n = 5$ for H'. Based on the applied model, with $n = 3$ (3 lower samples for each subgroup) and the calculated effect sizes, the computed power proved to be 0.42 for TN and 0.49 for H'. If the number of samples per subgroup would be hypothetically multiplied by two ($n = 6$), the estimated power would increase to 0.83 and 0.90 for TN and H respectively, while an additional increase to $n = 12$ would further increase the estimated power to approximately 1 (0.99) for both variables.

A similar trend was found for the community composition, which was significantly different between the areas under investigation at C-Power (two-way Permanova, factor "Area": $p = 0.030$), which is also visualized through an nMDS plot in Figure 5. Indicator Species Analysis, revealed that a total of 8 species were significantly associated with the "impact group" and the majority of them corresponded to the highest ranked species from the relative abundance analysis. The most distinct species associated with the impact samples (p-value < 0.01, '**') included: *Bodotria* sp. (Cumacea) and *Crangonidae* sp. (Decapoda, Caridea) and other species (p-value < 0.05, '*') included *Sagitta elegans* (Chaetognatha), *Macropodia rostrata* megalopa (Decapoda), *Nototropis* sp. (Amphipoda), *Lanice conchilega* larva (Polychaeta), *Pseudocuma* sp. (Cumacea) and *Pariambus typicus* (Amphipoda). For the reference samples, the SIA only revealed a strong association with one taxon, namely *Hydromedusa* (Cnidaria, p-value < 0.05, *).

While average total densities were higher for the samples taken inside the Norther OWF compared to the reference samples, an opposite trend was found for the diversity indices (Table 2). Except for the average total abundance (TN, factor "Area": $p = 0.046$), the one-way ANOVA analysis did not reveal any significant differences for the other structural community descriptors between the impact and reference samples (S/H'/J', factor "Area: $p > 0.05$, Table 2).

Table 1. Overview of calculated community descriptors (mean ± SE and p-values) for the spatial comparison between samples taken inside and outside the C-Power OWF.

C-Power – univariate results	Inside OWF	Outside OWF	Main effect (Area)
Total densities (N, ind. 100 m^{-3})	1856 ± 140	1399 ± 152	p = 0.049 (*)
Number of species (S)	35 ± 3	28 ± 2	p = 0.058 (NS)
Shannon-Wiener (H')	1.80 ± 0.10	1.26 ± 0.20	p = 0.024 (*)
Pielou's evenness (J')	0.51 ± 0.03	0.37 ± 0.05	p = 0.033 (*)

*Signif. codes: '***'p < 0.001, '**'p < 0.01, '*'p < 0.05, 'NS'p > 0.05*

Table 2. Overview of calculated community descriptors (mean ± SE and p-values) for the spatial comparison between samples taken inside and outside the Norther OWF.

Norther – univariate results	Inside OWF	Outside OWF	Main effect (Type)
Total densities (N, ind. 100 m^{-3})	4483 ± 3488	2105 ± 567	$p = 0.046$ (*)
Number of species (S)	22 ± 3	28 ± 2	$p = 0.174$ (NS)
Shannon-Wiener (H')	1.20 ± 0.69	1.55 ± 0.16	$p = 0.565$ (NS)
Pielou's evenness (J')	0.37 ± 0.21	0.47 ± 0.04	$p = 0.622$ (NS)
*Signif. codes: '***' p < 0.001, '**' p < 0.01, '*' p < 0.05, 'NS' p > 0.05*			

Comparable to the power analysis conducted for C-Power, sample size estimations were performed for the total abundance (TN) and Shannon-Wiener diversity (H') between the inside and outside areas at the Norther OWF. These analyses revealed that the effect sizes were considerably lower (TN: 0.41 and H': 0.32) compared to C-Power and that approximately 24 samples and 39 samples would be necessary for each subgroup (inside vs outside) given a significance level of 0.05 and a power of 0.80. Based on the applied model, with n = 3 (3 lower samples for each subgroup) and the calculated effect sizes, the computed power proved to be 0.12 for TN and 0.09 for H'. If the number of samples per

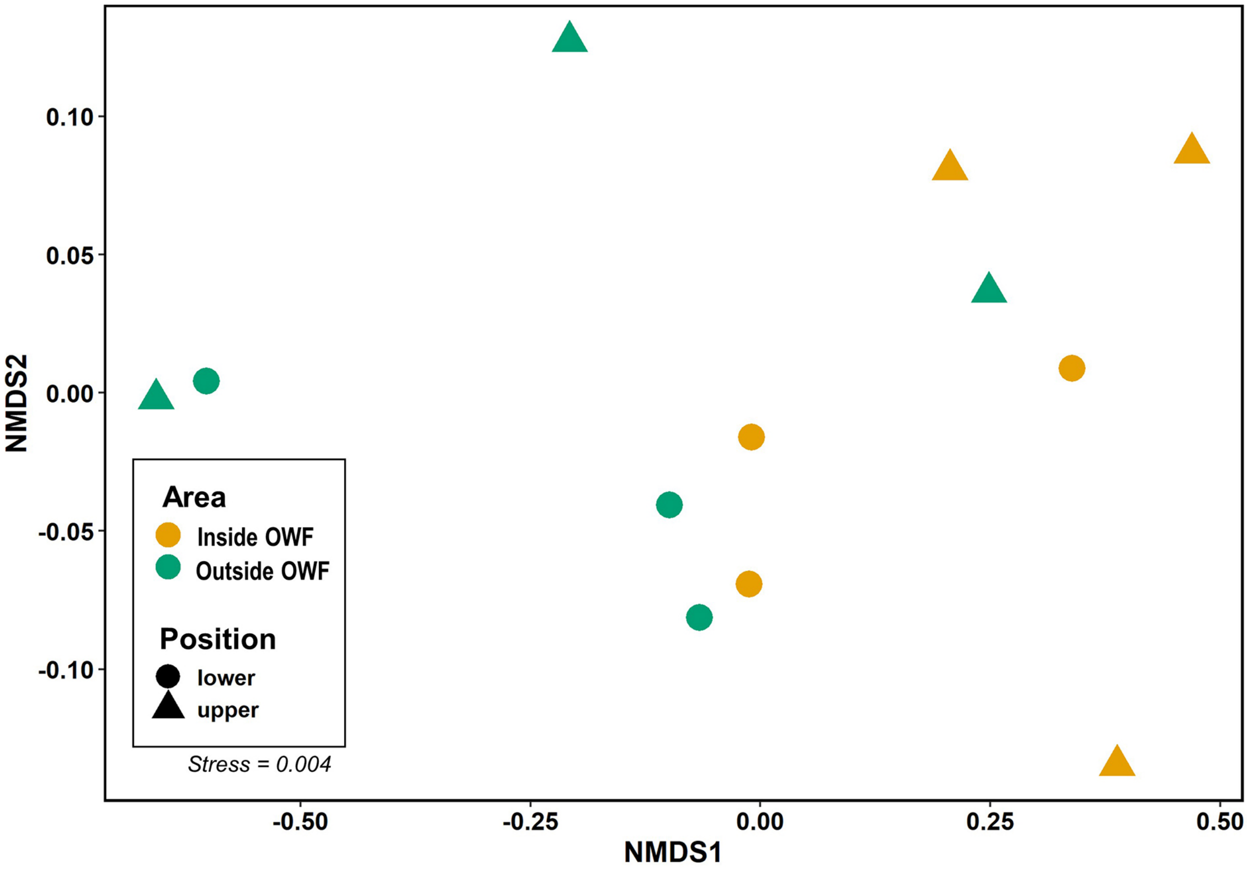

Figure 5. Non-metric multidimensional scaling (NMDS) plot for the lower and upper samples collected for the area inside (orange) and outside (green) the C-Power OWF, based on relative abundance data.

subgroup would be hypothetically multiplied by two (n = 6), the estimated power would increase to 0.25 and 0.17 for TN and H respectively, while an additional increase to a sample size of n = 12 would further increase the estimated power to approximately 0.48 and 0.32 for TN and H' respectively. Community composition analysis showed no significant differences between both areas at Norther (one-way Permanova, $p > 0.05$), and the Species Indicator Analysis did not show a selected number of species for any of the groups under study.

4. Discussion

4.1. Spatial distribution patterns of hyperbenthic communities

Extensive sampling by Dewicke *et al.* (2003) in the BPNS revealed the presence of a clear onshore-offshore gradient in terms of hyperbenthic distributions and identified a total of six hyperbenthic communities situated within onshore, transitional and offshore clusters. It was concluded that hydrodynamical forces and habitat heterogeneity can be considered as the most important structuring factors for hyperbenthic distributions in this North Sea area (Dewicke *et al.* 2003). Offshore areas are controlled by strong ebb-dominated currents (SW-direction) and are characterized by coarser sediments and lower settlement rates of suspended material (Dewicke *et al.* 2003). Hyperbenthic communities in these areas such as the Hinder offshore and Zeeland transitional communities are therefore mainly composed of planktonic taxa, which are less dependent on deposited organic matter, such as hydromedusae, chaetognaths (Sagittoidea) and early life stages of many benthic organisms (merohyperbenthos) which also have a planktonic lifestyle (Dewicke *et al.* 2003). In terms of geographic location, the C-Power OWF is situated at the boundary between the transitional and offshore clusters and its presence within this convergence zone could also lead to passive accumulation of organisms and concomitant higher abundances of merohyperbenthic taxa (Mees & Jones 1997; Dewicke *et al.* 2003). These patterns were observed in C-Power where densities were dominated by the planktonic hydromedusae and, to a lesser extent, chaetognaths, together with individuals of *Nototropis* sp., belonging to the merohyperbenthos. Other important representatives of the latter group were larval stages of the decapod crustaceans *Pisidia longicornis* and *Macropodia rostrata*, in agreement with results of Dewicke *et al.* (2003). It can therefore be concluded that hyperbenthic communities at C-Power are located at a node receiving influences from the three surrounding communities (Hinder offshore, Flemish and Zeeland transitional) described by Dewicke *et al.* (2003).

The Norther OWF can also be situated within the Zeeland transitional area, but is located more nearshore compared to C-Power and is not characterized by the presence of a subtidal sandbank (Lefaible *et al.* 2021). Nearshore areas are mainly influenced by flood-dominated currents and are believed to experience less intense hydrological forcing (Dewicke *et al.* 2003). This also implies that these areas experience higher suspended matter concentrations compared to offshore areas, with locally increased levels of organic matter deposition (Dewicke *et al.* 2003). The majority of the samples collected at the Norther study site were clearly less dominated by the typical planktonic groups such as hydromedusae and Chaetognatha, and showed higher abundances of merohyperbenthic groups, such as amphipods or larval decapods, compared to the samples collected at C-Power. In addition, organisms of the order Mysida contributed more to total abundances in Norther compared to C-Power. Mysids are motile, omnivorous organisms that often reach high densities in areas with increased food availability and are important representatives of nearshore hyperbenthic communities as their distribution is also strongly linked to the existing onshore-offshore gradient (Mees & Jones 1997; Dewicke *et al.* 2003; Parry *et al.* 2021). The importance of this group and especially the occurrence of the "characteristic" species

Schistomysis sp., which is typically associated with more onshore located hyperbenthic communities, implies that the community composition at the Norther study site differs from more offshore situated OWFs (Dewicke *et al*. 2003). Previous studies within the Norther OWF already revealed that this area is very heterogeneous both in terms of abiotic and biotic conditions and constitutes a mosaic of habitat types (Lefaible *et al.* 2021). This finding was again confirmed by the strong variation in terms of hyperbenthic distributions that was found in this study within the impact area. Two impact tracks (Im6 and Im7) were taken within an area that has been categorized as Habitat Type 1 (HT1), which is characterized by fine, organically enriched sediments that contain significant amounts of coarser material (Lefaible *et al.* 2021). In contrast, the third impact sample (Im8) was collected in the Habitat Type 3 (HT3), which corresponds with medium-coarse sands with relatively low organic matter and is typically associated with subtidal sandbank systems (Lefaible *et al.* 2021).

4.2. OWF-related effects in the hyperbenthic zone

The introduction of underwater structures within mobile, soft-sediments alters hydrological conditions in the wake of the turbine, resulting in the creation of sheltered areas with the deposition of finer sediments and increased retention of deposited organic matter (Danheim *et al.* 2020. The turbines also provide new habitat for the colonization of hard substrate species which enhances overall habitat complexity and biodiversity through the so called artificial reef effect (Danheim *et al.* 2020; Degraer *et al.* 2020). Established epifouling communities (> 6 years) are mainly composed by suspension feeders such as *Mytilus edulis* and have the ability to organically enrich the surrounding sediment through the deposition of faecal pellets (Degraer *et al.* 2020). Moreover, biofouling "drop-offs" from the turbines can occur, which might expand the impact footprint of the artificial reef effect to areas located further away from the turbines (Lefaible *et al.* in prep.). Previous studies within the C-Power OWF have already revealed that the long-term presence of the jacket foundations and combined impacts of the artificial reef effect and altered hydrological conditions (sediment fining and organic enrichment), have led to a shift towards richer macrobenthic communities at distances of approximately 38 meters from the turbines (Lefaible *et al.* 2019a; Braeckman *et al.* 2020; Lefaible *et al.* in prep.).

It is therefore considered that certain results within this study such as the lower average water flow together with a higher hyperbenthic abundance and diversity inside the C-Power OWF compared to the reference area, could be a consequence of these turbine-related impacts. Moreover, significantly different hyperbenthic communities were found between the areas inside and outside the OWF and these findings also seem to support the hypothesis that the creation of more favourable conditions related to turbine-induced habitat changes could result in enriched hyperbenthic communities (Lefaible *et al.* 2021). For example, a relative abundance analysis showed that mysids such as *Mesopodopsis slabberi* and *Gastrosaccus spinifer* occurred more frequently inside the OWF. These motile and omnivorous/ detrivorous organisms reach higher densities in areas of increased food availability, which might be a reason for their higher presence within the OWF (Mees & Jones 1997; Dewicke *et al*. 2003). A species indicator analysis also revealed that some holohyperbenthic groups such as cumaceans (*Diastylis* sp., *Bodotria* sp.) and amphipods (*Parambius typicus*) were strongly associated with the samples collected inside the C-Power concession zone. These surface deposit feeding species are, however, not typical for the transitional areas but are rather characteristic for onshore communities (Dewicke *et al.* 2003). Moreover, organisms of *Parambius typicus* are also often attached to substrata such as large-bodied infauna, algae and hydroids.

Their increased abundance could therefore also be a result of the cease of fisheries activities within C-Power. Bottom fishing physically disturbs the seafloor in various ways and benthic communities are strongly affected through the removal of epibenthic organisms and structures (Johnson 2002). This, together with increased abundances of larvae of the tube-building polychaete *Lanice conchilega* and fish larvae, could indicate that the seabed-inhabiting fauna has been freed of the disturbances associated with commercial fishing. These findings are in line with the proposed hypothesis that cumulative effects of the long-term (> 10 years) presence of the jackets and cease of fishery activities ("fisheries exclusion effect") might result in enriched hyperbenthic communities within the OWF area. However, it should be emphasized that this hypothesis remains suggestive and should therefore be interpreted with caution. Subtidal sandbank systems are highly dynamic habitats and are known to exhibit strong small-scale heterogeneity both in terms of abiotic and biotic conditions (Mestdagh *et al*. 2020; Cheng *et al*. 2021). Therefore, future research is highly recommended to determine whether these differences are induced by the OWF presence, or simply a result of natural spatial variation between the areas sampled inside and outside C-Power.

In contrast to C-Power, no clear spatial differences were detected between the areas located outside and inside the Norther OWF, while strong variation was found among the samples taken inside the concession zone. This lack of conclusive results may of course be related to the fact that this OWF has been built quite recently and has only been operational for two years. It is therefore likely that the communities within this OWF probably still have to recover from the short-term effects associated with the construction phase, which would also explain why the samples collected inside the OWF were generally poorer compared to the reference samples in terms of density and diversity. An additional explanation is the fact that the presence of strong natural spatial variability (habitat types) in combination with a relatively low number of replicates (3 tracks) is insufficient to test OWF-related effects in a robust way, which was confirmed by the power analysis conducted within this study. Future monitoring studies are therefore important to investigate potential long-term effects, in which the applied sampling design should be able to incorporate the habitat heterogeneity that is found within this OWF.

4.3. Sampling quality assessment

Horizontal hauling devices such as the hyperbenthic sledge used in this study, are often preferred as the equipment of choice when sampling for hyperbenthic communities (Hamerlynck & Mees 1991; Mees & Jones 1997; Dewicke *et al*. 2003). They are designed to sample at least the lower one metre of the water column such that no contamination with the sediment occurs (Mees & Jones 1997). The level of success, however, strongly depends on local factors such as depth, degree of exposure, bottom topography and weather conditions, which also implies that heavier and more robust equipment (ship size and power, lifting gear) is necessary to sample deeper and offshore habitats in which the OWFs under study are located (Mees & Jones 1997). Compared to the feasibility study performed in 2018, the extent of the sampling effort within this study did however indicate that the adjusted sampling strategy can be considered effective: both OWFs were sampled during a one-day sampling campaign, for which each towing event proved to be successful, resulting in the desired number of quantitative samples.

Besides the actual sample collection, it is also important to assess the sampling quality or "catch efficiency" of the obtained samples (Del Vecchio *et al*. 2019). Species found within the hyperbenthal zone are often highly mobile and migrate through this zone at specific times (day/year) or at certain life-history stages (Mees & Jones 1997; Dewicke *et al*. 2003). Consequently, it has proven rather difficult to provide complete population assessments of hyperbenthic communities as they comprise a wide array of different species and exhibit dynamic distributions in space and time (Mees & Jones 1997). Due to a lack of scientific literature on hyperbenthic spatial patterns within these areas and the absence of true baseline studies, it is also challenging to verify whether the obtained dataset can be considered as "representative". The samples in this study turned out to be quite rich in terms of abundance and diversity, and sample processing (sorting, counting, identifications) proved to be a very time-consuming process, which even resulted in an incomplete dataset for the Norther study site. The very high number of hydromedusae specimens that were found in this study may be attributed to the timing of the sampling (*i.e.* daytime conditions) as these organisms are often concentrated within the hyperbenthic zone during the day (Mees & Jones 1997). In terms of community composition, the hyperbenthic descriptions within this study corresponded to the communities described by Dewicke *et al.* (2003), which currently is the only one that can be used as a "baseline" study. The SACs and species richness estimators did, however, show that an asymptote had not yet been reached and a fraction of undetected species could still be expected, especially within the Norther OWF. Assuming that these communities have similarities with more onshore situated clusters and the strong habitat heterogeneity within this OWF, it is likely that increased sampling effort will be required to fully characterize the hyperbenthic communities within this concession zone.

A final aspect to consider is the level of the vertical distinction that was applied for the hyperbenthic sampling in this study. Two-level (lower and upper nets) hyperbenthic sledges are often used to account for potential varying hyperbenthic distributions throughout the sampled water column (Mees & Jones 1997). The effective height that marks the distinction between the lower and upper samples can be variable and also depends on the water depth, but the majority of hyperbenthic studies use a sledge with a lower net up to about 50 cm, comparable to our study (Mees & Jones 1997). Within the currently available literature, findings regarding vertical segregation of hyperbenthic communities are rather inconclusive as some studies show that much higher densities are found within the lower net samples, while other studies report homogenous hyperbenthic distributions between the different nets (Mees & Jones 1997). Results in our study correspond to the latter, as no significant positional differences were found in terms of densities, diversity and composition between the lower and upper nets for the C-Power study site. However, it must also be considered that the lower net of the hyperbenthic sledge used in this study was situated at 20 cm, which means that the lowermost centimetres of the water column (0–20 cm) were not sampled. Certain taxa such as mysids are known to be epibenthic during the day and reside near the bottom, while they move up to the water column during the night (Mees & Jones 1997; Parry *et al.* 2021). As a result, the densities and diversity of mysids, which are characteristic hyperbenthos, could be under-represented with the applied sampling methodology.

4.4. Challenges and opportunities

The results obtained from this study proved to be valuable as they allowed to enhance our knowledge on the distribution of hyperbenthic communities within these areas, and to assess the strength of the applied sampling design to investigate the hyperbenthic enrichment hypothesis. Obtaining complete, qualitative data appeared to be a time-consuming process due to the high densities and diversity that characterized these samples. Sample processing activities such as sorting, counting and especially species-level identification were fairly labour intensive and required specific taxonomic expertise. Evidently, this constraint creates a trade-off between the sampling effort and the number of replicates that can be processed per study site. We therefore conclude that a sampling design in which 12 tracks are sampled (24 lower and upper samples) represents the maximum number of replicates that can be processed qualitatively within an annual monitoring program.

The sampling quality assessment performed within this study revealed that an increase of the sampling effort would strongly enhance the ability to fully characterize the hyperbenthic communities and the statistical power to detect any spatial differences between the areas inside and outside the OWFs. This appears to be especially true for the Norther study site, which can be attributed to the fact that several habitat types are found within this concession zone. It is therefore proposed to increase the sampling effort to 6 tracks inside and 6 tracks outside the OWFs and to revise the positions and number of tracks for the Norther study site based on previous macrobenthos studies to account for the habitat heterogeneity (Lefaible *et al.* 2021). It can then be further discussed how the actual sample processing can be spread over a longer period of time depending on the focus of the research objectives and reporting. Given the fact that no vertical segregation was detected between the lower and upper net samples within this study, it might also be considered to limit the actual reporting to the lower net samples only to decrease the amount of time spend on sample processing.

While the spatial differences and trends found for the C-Power study area support the proposed hypothesis of hyperbenthic enrichment, it remains unclear whether this can also be linked to aberrant abiotic conditions between the two areas due to actual turbine-related impacts. We therefore strongly recommend including relevant abiotic variables at the sediment-water column boundary within the design to strengthen our ability to assess this hypothesis more thoroughly. This could be achieved by applying a similar methodology used by Dewicke *et al.* (2003), in which every tow was preceded by the deployment of a multi-corer. From the obtained cores, the near-bottom water will be collected and used for pigment analysis. In addition, the upper sediment layers (0–3 cm) could be used to determine seabed conditions in terms of granulometry and organic matter. With these proposed adjustments future studies will be able to obtain the most cost-effective methodology to maximize the efficient use of monitoring resources and research objectives outcomes.

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