FACULTY OF SCIENCES

The effect of offshore windfarms on hyperbenthic communities in the Belgian Part of the North Sea

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1. Introduction

Independent and sustainable energy production is becoming increasingly important over time and offshore wind farms (OWFs) play a vital role in meeting those energy requirements. The first construction of OWFs in the Belgian part of the North Sea (BPNS) started in 2009. In 2020, the first large offshore wind zone, consisting of 9 concession zones and 399 wind turbines, was finalised. Today, this allows for a production capacity of 2.2 GW of green electricity. Further plans have been made to increase this production to 4.5 GW through the construction of three more offshore wind farm zones. According to the Belgian Offshore Platform (BOP) offshore wind production in the BPNS must increase to 5.7 GW to, in combination with solar power, supply half of Belgium's electricity requirement from renewable sources by 2030 (*Belgian Offshore Platform, 2023*).

The construction and presence of these OWFs is not without impact on marine life, but not all impacts are negative. A first positive aspect is that OWFs function as artificial reefs, providing opportunities for various marine life forms (Degraer et al., 2020). After construction of these wind turbines, a long-term colonization process starts on the submerged part of the turbine and the deposited rocks which function as scour protection. Degraer et al. (2020) found that these epifouling communities reach a climax state after 6+ years. A More recent study by Zupan et al. (2023) however, claims that these communities may either not reach an equilibrium or that this climax might show temporal and cyclical variation. A second positive impact of OWFs is the fact that these artificial constructions might also function as steppingstones for species with planktonic life stages, allowing them to reach previously inaccessible regions (Adams et al., 2014). The creation of this new habitat, combined with this steppingstone effect can however leave the area vulnerable for the invasion of non-indigenous species, which might negatively affect native habitats (Glasby et al., 2006).

The Belgian OWFs are being researched extensively. Since 2009, a monitoring report is being published each year (*Ovidio, 2023*). These reports contain a multitude of studies that took place in the Belgian OWFs that year. Combined with a variety of other studies around the world, knowledge is now becoming increasingly available about the impact of OWFs on their surroundings. Besides impacting the biotic environment, OWFs also change the abiotic environment as introduction of these turbines places hard substrates in a previously soft-substrate environment. They further affect environmental conditions through alteration of local currents, granulometry, and hydrology (Leonhard & Pedersen, 2005, Janssen et al., 2005, Coates et al., 2014). The presence of epifouling fauna colonizing the foundations has an impact as well. They gather food from the water column and deposit organic matter as faecal pellets onto the ocean floor, which in turn enriches the sediment around the turbines (Maar et al., 2009). The combination of all these processes causes the sea floor sediment near the turbines to become more fine-grained and organically enriched (Janssen et al., 2005, Coates et al., 2014). The lower tidal flow and higher food availability near the foundation alters the characteristics of the macrobenthic communities close to the turbines. These communities show changes in species abundance, diversity, and composition (Coates et al., 2014, Dannheim et al., 2020, Degraer et al. 2020). The process is then further strengthened by the colonization of newly formed hard substrate by hard substrate-associated macrobenthos, which were previously not present in the area (Dannheim et al., 2020). This ultimately leaves macrobenthic communities close to the foundations to be significantly different compared to those further away from the turbines (Braeckman et al., 2020). Recently it has also been shown that the extend of the area in which turbines alter their environment could become quite large over time (Lefaible et al., 2023).

The OWFs do not only affect the smallest of sea creatures, but also impact larger species groups like fish, birds and mammals. For fish, the impact differs between species. Fish usually leave the site during construction but return once construction is finished (Vaissière et al., 2014). Lindeboom et al. (2011) found no significant increase in species richness but noticed changes in several individual species within the OWF sites. Species like Sole (*Solea solea*) and Whiting (*Merlangius merlangus*) found refuge in the OWF and increase in numbers compared to baseline areas while other species like Lesser Weever (*Echiichthys vipera*), showed a decline in population sizes. Similar patterns are seen in seabirds as impact on birds varies between species as well. A sizeable number of species avoid OWFs, and the impact might change seasonally (Peschko et al., 2020). Wind turbines may provide a movement/migration barrier or leave birds prone to collision. Disturbance from operating turbines and associated ship and helicopter traffic may also cause birds to leave the area (Reid et al., 2023). The Northern Gannet (*Morus bassanus*) for example is a species very prone to the impact of the OWFs (Goodale et al., 2020). On the contrary, for a good number of species there seems to be no change in abundance, while others might even use the sites as feeding grounds and thus show an increase in numbers in the area. (Rothery et al., 2009). Mammals like porpoises and seals clearly evade the sites when under construction, due to the very loud piledriving, but quite often do return to the sites after construction. Ongoing traffic from maintenance vessels does however decrease their abundance (Gall et al., 2021).

To this day, a gap in the knowledge still, is the effect on hyperbenthos. A limited amount of research leaves the impact of OWFs on this group mostly undescribed. Hyperbenthos are a group of species that are also known as suprabenthos, demersal zooplankton and benthopelagic plankton depending on the environment in which they occur (Mees & Jones, 1997). Within hyperbenthos, a distinction can also be made between merohyperbenthos and holohyperbenthos. Merohyperbenthos spends only part of their early lifetime in the hyperbenthos, while holohyperbenthos refers to species that spend (periods of) their adult life in the hyperbenthos (Hamerlynck & Mees 1991). Species can also be divided even further based on their size: meiohyperbenthos have a size between 32µm and 1000µm, while macrohyperbenthos are not smaller than 0.5mm or 1mm (Mees & Jones, 1997). For this study, only hyperbenthos larger than 1mm were considered. Taxonomically, hyperbenthos consists for the most part of amphipods, mysids, decapod shrimp and juvenile fish (De Neve et al., 2020). Hyperbenthos plays in important role in benthic - pelagic foodweb coupling. Shrimp and Mysid species for example will feed on phyto- and zooplanktonic biomass and serve in turn as food for higher trophic levels (Mees & Jones, 1997). Mysids are omnivores and, by feeding on zooplankton, have the ability to structure planktonic communities. Copepods (although rarely found in hyperbenthic samples in the BPNS) are also an important part of the diet of many post-larval and juvenile fish. Mysids will then replace the copepods as main food source during further development of these fish (Mees & Jones, 1997). Hyperbenthos form an important part of the diet of both commercial and non-commercial fish species (Mees & Jones, 1997). Since OWFs have been shown to be a refuge from fishing and nursing site for fish species (Lindeboom et al., 2011), it would thus benefit these predators even more if the OWFs proved to be a more suitable habitat for hyperbenthic species. An increased hyperbenthos density also benefits cephalopods like sepiolid squids, who have been proven to feed on hyperbenthos (preferably mysids) in Nordic waters (Bergstrom, 1985). Lastly, certain commercially popular crustaceans like shrimp & lobsters also feed on hyperbenthos (Mees & Jones, 1997), which again makes possible increased hyperbenthos densities economically interesting if the OWFs show to act as a nursing ground or source for surrounding areas.

2. Objective

This study aims to expand the knowledge of hyperbenthos within a socio-economical important context, the global movement towards green, renewable energy. It investigates the impact of operational OWFs on the hyperbenthos communities in the transitional and offshore areas of the BPNS to help fill in the gaps about how OWFs alter the environment in which they are built. The main research question is thus: how does the presence of an OWF impact the hyperbenthos communities living above the soft sediment between the turbines? As fouling fauna grows on the turbines, an increase in organic material occurs in the surrounding area because of epifouling (Degraer et al., 2020). The turbines also alter local sediment composition, increase the available organic matter and locally decrease currents (Maar et al., 2009, Coates et al., 2014, Leonhard & Pedersen., 2005). Based on these facts, it was hypothesized that the introduced changes could be favourable for hyperbenthos and thus lead to an increase in biodiversity, density, and biomass.

3. Material & methods

3.1. Study area

The sampling was performed in three operational OWFs located at the eastern side of the Belgian part of the North Sea (BPNS). Due to time limitations, only two concession zones were further analysed in the lab, namely Norther and Belwind. The C-Power samples are yet to be further analysed.

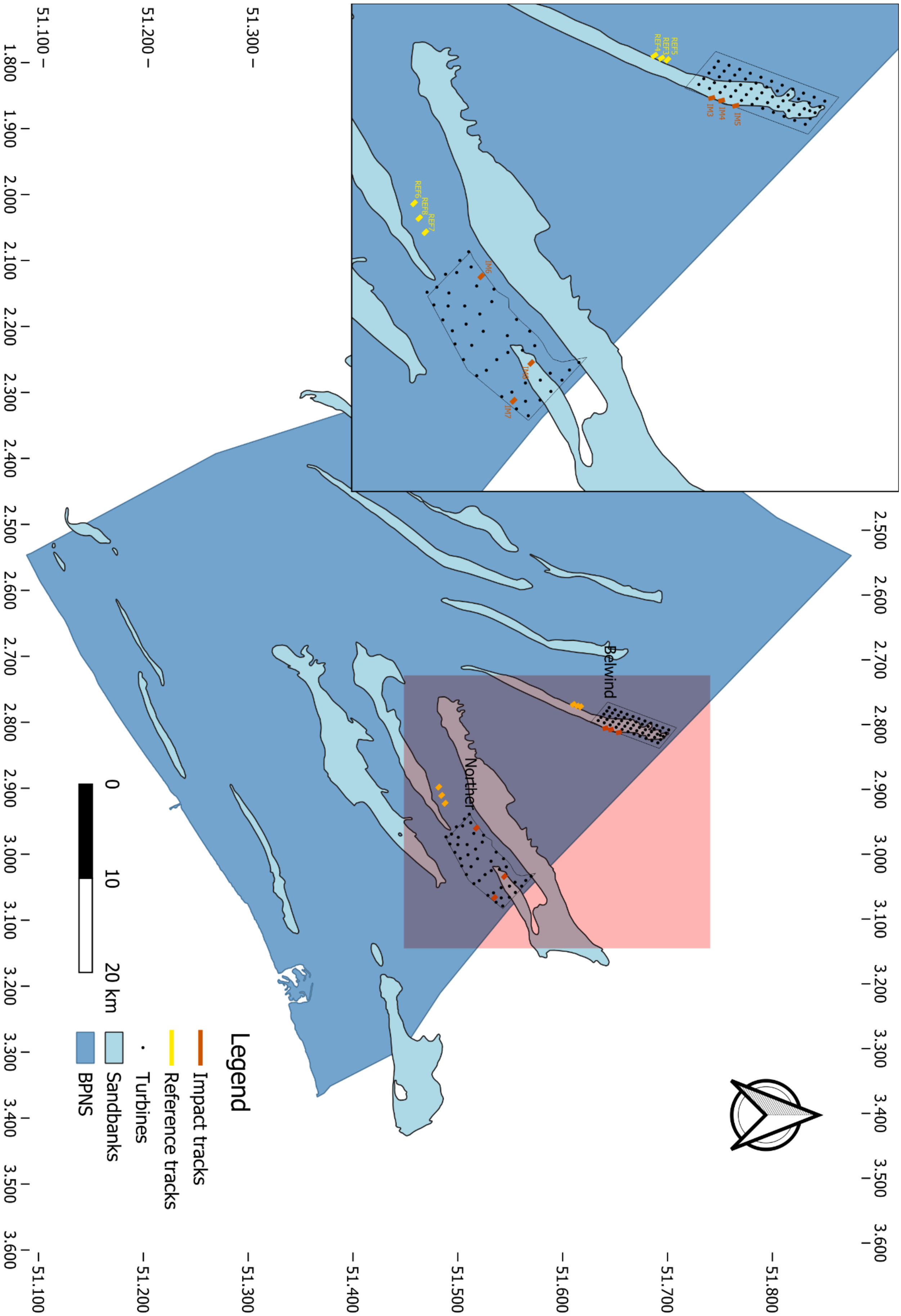

Figure 1: Map of a the BPNS, showing the locations of the hyperbenthos sample tracks that were analysed, and their respective concession zones. Positions of the tracks taken outside the OWFs are shown with a green line (REF3-5, REF6-8) and the tracks taken inside are indicated in red (IM3-5, IM6-8).

Norther consists of 44 monopile turbines, and came in operation in 2019, making it the second most recent operational OWF in the BPNS, after Seamade. The depth of the Norther area ranges between 20 and 35 m *(Projects - Belgian Offshore Platform,* 2023). Unlike any other concession zone, it is also not built on top of a sandbank but is built at the edge of the Thornton bank. This makes Norther a unique study site compared to both other sites in regards of the physical environment. This OWF is the closest to the shoreline (23km from the Zeebrugge coastline) and has a more heterogeneous sediment composition. The sediment in Norther consists of fine-grained sediment, mixed with much coarser material. This is in contrast with the well sorted, medium coarse sands that can be found in more offshore locations like the Belwind OWF (Lefaible et al., 2021).

C-power is the second closest concession zone and built on the Thorntonbank. It became operational in 2009, making it the oldest operational concession zone in the BPNS. It has an average distance of 30 km to the coast, a depth between 14 and 28m, and consists of six gravity-based turbines and 48 jacket turbines. *(Projects - Belgian Offshore Platform,* 2023).

The last concession zone, Belwind, is built on the Hinder banks. It became operational in 2010 and consists of 55 monopiles. It is located on average 49 km away from the shoreline and has a depth between 15 and 37 m. *(Projects - Belgian Offshore Platform,* 2023).

3.2. Sampling strategy

The hyperbenthos was sampled in the second half of October of 2022, over a period of multiple days during campaign 25 and 26 of the *RV Belgica*. Samples were acquired during daytime and for each site triplicate tracks of 150m were taken parallel to the sand ridges of each sandbank (figure 1). This was shown to be the most accurate way to acquire representative samples and prevent a lot of substrate from ending up in the samples according to a preliminary feasibility study by Lefaible et al. (2018). For each concession zone (later also referred to as 'impact'), samples were also taken at representative baseline ('reference') zones, outside of the OWFs. During sampling, the ship cruised at a speed of 1.5 kt against the currents.

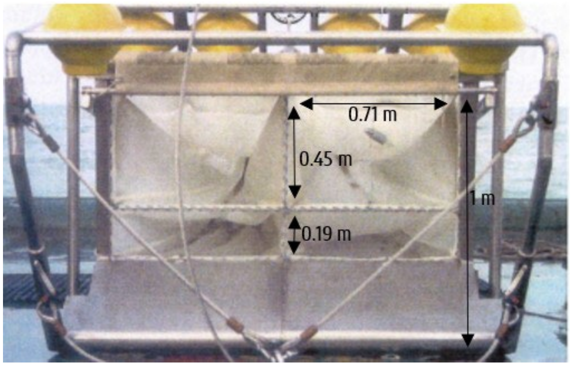

Figure 2: The net used for sampling, indicating net opening dimensions and total height of active sampling area.

The samples were acquired using a hyperbenthic sledge (figure 2), consisting of four nets (two lower and two upper nets), each with a 1mm mesh size. During sampling only one lower and one upper net was used. The sled also has a curtain in front of the net openings, linked to a mechanism that allows it to open when touching the ocean floor. This prevents any pelagic specimen from getting caught in the net during sampling. In the opening of the upper net, a flowmeter was mounted to measure the volume of water passing through. At the other end, a collector bottle was mounted on each net at a 45° angle, capturing everything that was filtered out through the nets.

3.3. Sample analysis

After collection, the samples were sieved and transferred to plastic bottles to be fixed on a 4% formaldehyde-seawater solution so they could be transferred to the lab. In the lab, the samples were rinsed once more to get rid of any unwanted sediment that was caught in the sample. After this, all individuals were identified to the lowest possible taxonomic level, counted, and fixed on formaldehyde in separate vials per species. When relevant, distinctions were made between development stages of species (larva, megalopa, and juvenile). If identification to species level was not possible, they were registered on a higher taxonomic level and considered 'unique' if no other species would be identified at the same taxonomic level. Species caught in the net that did not belong to the hyperbenthos were removed from the dataset before continuing further analysis. Furthermore, all hydrozoa were excluded from the analysis due to the fact that the individuals were all heavily fragmented in all of the samples that contained them. This made it impossible to acquire a correct estimation of their densities. As a final step, the biomass for each species per sample was determined as blotted wet weight (balance precision 0.0001g). The density of each species was normalized to the number of individuals per $100m^3$ using the following formula: $ind./100\,m^3 = \text{number individuals} / (\text{surface net} * \text{number of turns flowmeter} * 0.33)$

** 100* and the same normalization was performed for the biomass of species. A division of the flowmeter turns was required since three rotations of the flowmeter result in a single-unit increase.

3.4. Statistical analysis

The statistical analysis was performed in an overall analogous way as the previous hyperbenthic study in the OWFs by Lefaible et al. (2022). The data of both years was however not readily comparable due to the usage of a different type of hyperbenthic sled.

As a primary data analysis, the Shannon-Wiener diversity index (H'), species richness (S') and Pielou's evenness (J') were calculated from the raw count data. In addition, the relative contribution of higher-level taxonomic groups towards the local densities were calculated to determine hyperbenthic community composition patterns both inside and outside the OWFs. A species accumulation curve (SAC) was constructed for each set of tracks (impact and reference for both Belwind and Norther) to assess the level of accuracy of the monitoring survey. This was then followed by the calculation of the non-parametric richness estimators Jacknife1, Chao1, and Bootstrap to estimate the number of species missing from the samples. To evaluate the possible impact of the OWFs on the hyperbenthic community, a spatial comparison was performed to test for differences in species richness, The Shannon-Wiener index, Pielou's evenness, species density and biomass between the impact and reference sites. To estimate the possible differences between the structural univariate indices under study, a three-way ANOVA (Factors: "park", "net position" and "impact type" with levels: "Belwind" and "Norther", "lower" and "upper", "impact" and "reference" respectively) was performed for each index. To test for assumptions of normality and homogeneity of variances, Shapiro-Wilkinson and Levene tests were used respectively. A Log transformation was required for the density and biomass data to meet the assumption of normality. After the ANOVA analysis, a post-hoc analysis, using the Tukey test was performed to detect any significant differences between each sampling site.

A three-way PERMANOVA analysis was performed with both the untransformed species densities and relative species densities to assess any OWF-related effects on the community composition of Norther and Belwind. A pairwise Adonis comparison was then done to find any pairwise differences between sample groups. The assumption of homogeneity of multivariate dispersions was tested with a PERMDISP test and to visualize any patterns in community composition, non-metric multidimensional scaling (nMDS) plots were constructed by the means of a Bray-Curtis matrix.

Finally, a Species Indicator Analysis was performed to determine which species were responsible for the differences in community composition between the impact and reference areas, between the Belwind and Norther area and between the upper and lower nets.

4. Results

4.1. Hyperbenthos distribution patterns

Across the entire sampling effort, a grand total of 52 hyperbenthic species were found, originating from 9 different higher taxa (Class/Order). The total density for all sampling sites ranged from 80 to 15084 individuals per 100m^3 (figure 3) of which the lower net samples of the Norther reference site were a clear outlier. Part of this can possibly be explained by the significant amount of sediment that got caught in the net, which may be the cause of the large amount of *Monocorophium sp.* amphipods that were present in the samples. These samples did however also contain a large count of *Crangonidae sp.* shrimp and *Bodotria sp.* cumacean individuals.

In reards of biomass, the samples varied between 0.049 and 36.884 g/100m^3 (figure 4). As a result of the high density, the lower net samples of the Norther reference area again formed an outlier. The sample with the highest density outside of this area only held 4.574 g/100m^3. It is also worth noting that, even though the lower net samples from the Norther reference site had a very high density, the total density and biomass of the upper net samples are the second lowest in terms of both indices, after the upper net samples of the Belwind reference site.

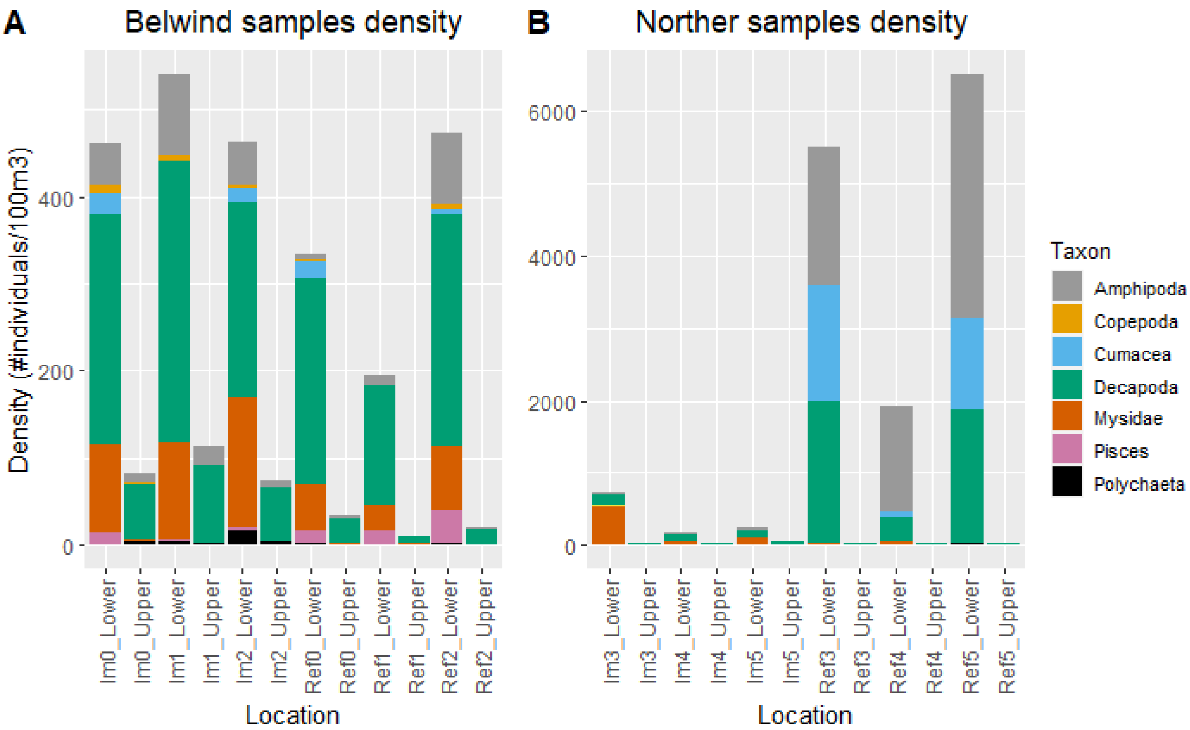

Figure 3: Total hyperbenthic densities (ind. / 100m3) per sample for the areas within ('Im') and outside ('Ref') the Norther and Belwind concession zones. Further subdivided in lower net (Lower) and upper net (Upper) samples. Includes contributions of lower-level taxa towards the total densities.

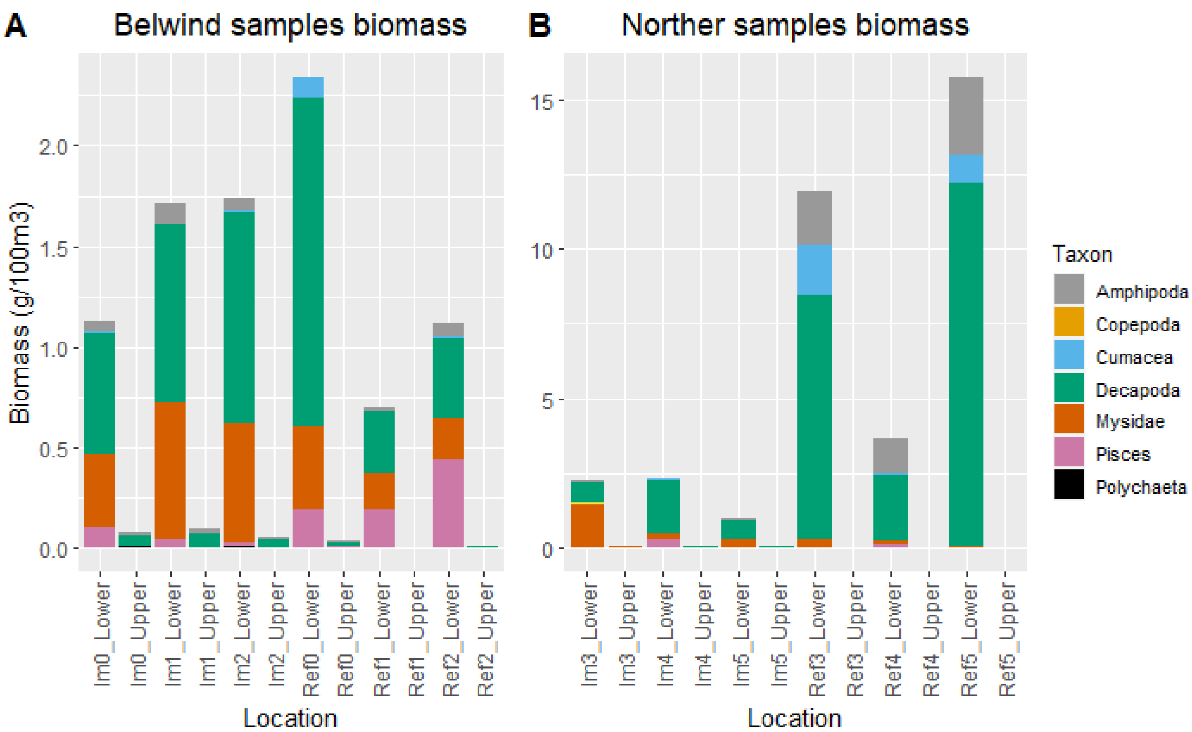

Figure 4: Total hyperbenthic Biomass (g / 100m3) per sample for the areas within (Im) and outside (Ref) the Norther and Belwind concession zones. Further subdivided in lower net (Lower) and upper net (Upper) samples. Includes contributions of lower-level taxa towards the total biomass. Note that seemingly empty samples are not empty but have a very low biomass.

Overall, the largest contributing groups towards the community composition were the Amphipoda (40%), Decapoda (35%) and Cumacea (17%) in terms of density. The most represented species were *Monocorophium sp., Crangonidae sp.* and *Bodotria sp.* for their respective taxa, as well as across the entire sampling effort. For biomass, the biggest contributors were Decapods (66%), Amphipods (13%) and Mysidae (11%). The largest contributing species for these taxa were *Crangonidae sp., Monocorophium sp.* and *Hippolyte varians* . across the entire sampling effort *Bodotria sp.* did still have a higher biomass than *Monocorophium sp.*

The cumulative number of species are shown in figure 5 in function of the sampling effort with n = 3 for each sample area. The resulting species accumulation curves (SACs) indicate that the largest share of newly found species are collected between the first and second sample, while the curves seem to level off slightly towards n = 3. For all samples, the diversity estimators tend to predict a higher number of species compared to what was found in the samples. Across samples the SE tends to vary but is often quite high. The Norther reference area has the highest amount of estimated missing species and has an overall high standard error value for each estimator. When using an estimator where less weight is given to rare species, like the Bootstrap estimator, the estimated number of species does come closer to the recorded number of species.

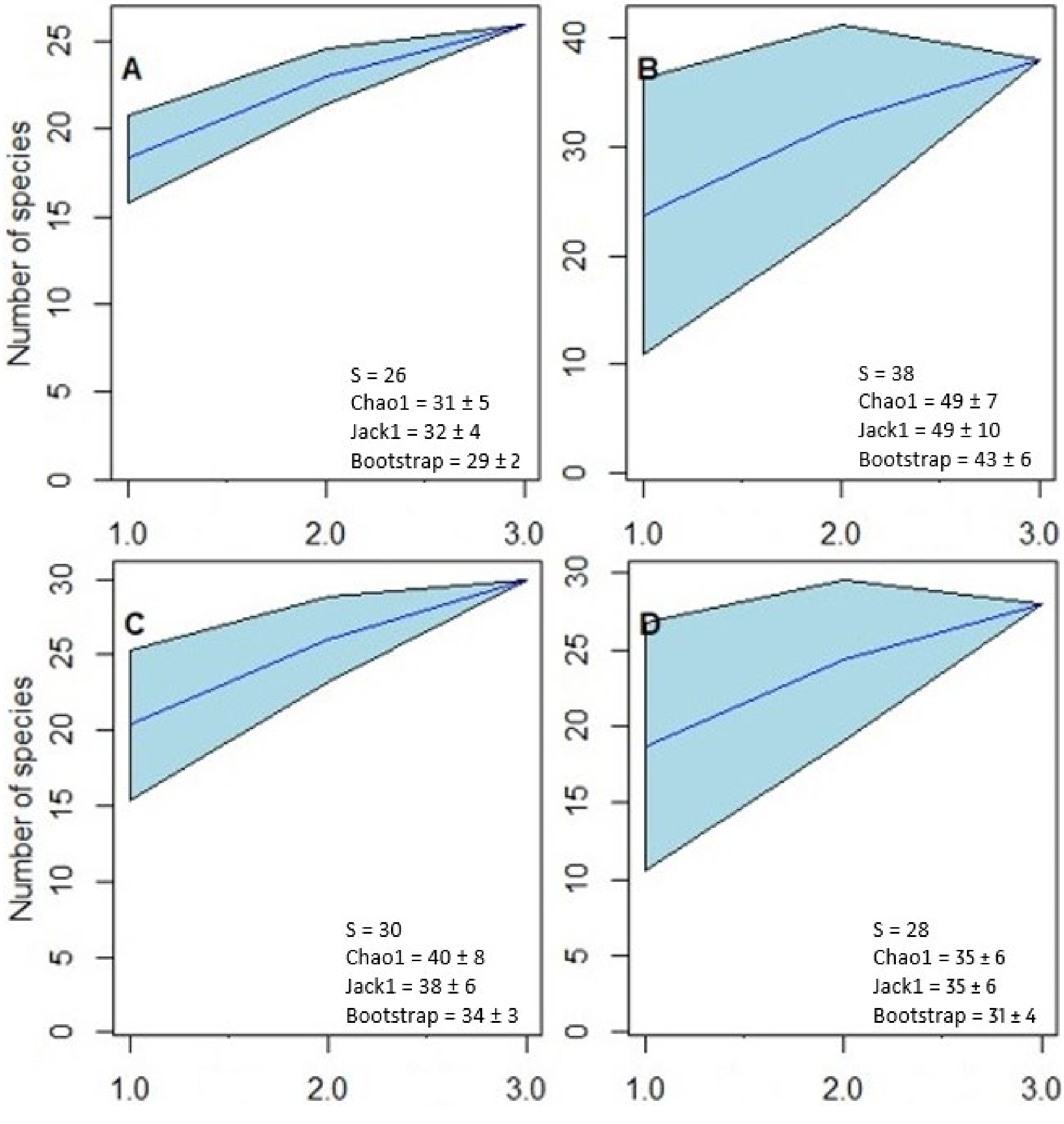

Figure 5: Species Accumulation Curves (SACs) were plotted for the samples collected within the Norther (A) and Belwind (C), as well as the samples taken outside these study sites, (graph B and D). The species richness (S) and richness estimators Chao1, Jacknife1, and Bootstrap (mean ± SE) were provided for each area.

Table 1: Results of the three-way anova analysis.

	Density (ind / 100 m^3)	Biomass (g / 100m^3)	Species richness (S')	Shannon-Wiener index (H')	Pielou's eveness index (J')
Park		*			
Impact type					
Net Position	***	***	***	**	**
Park : Impact type	***	*			*
Park : Net Position	**			*	***
Impact type : Net Position	***	***			*
Park : Impact type : Net Position					

Significance codes: '***' p < 0.001, '**' p < 0.01, '*' p < 0.05

4.2. Hyperbenthos distribution & diversity

Hyperbenthos density differed significantly between the two parks and net position, net position and impact type, and park and impact type (3-way ANOVA all two-way interactions significant, Table 1). The Belwind impact zone had a slightly higher hyperbenthos density compared to the reference zone, while the inverse is true for Norther (Tukey post-hoc test $p < 0.05$, figure 6A, table2, table 3). Overall densities were also significantly higher in Norther compared to Belwind, but only in the lower nets (Tukey post-hoc test $p < 0.05$, figure 6A). Lastly, both lower and upper net samples from the reference sites had a higher density compared to the impact zone (Tukey post-hoc test $p < 0.05$, figure 6A).

Hyperbenthos biomass differed significantly among park and impact type, as well as net position and impact type. (3-way ANOVA significant interactions, Table 1). Belwind's impact samples had a higher biomass than the reference samples, while there was no significant difference in Norther (Tukey post-hoc test $p < 0.05$, figure 6B, table2, table 3). Norther reference samples were significantly higher in biomass than the Belwind reference samples (Tukey post-hoc test $p < 0.05$, figure 6B). Both in the reference and impact areas, the lower net samples had a significantly higher

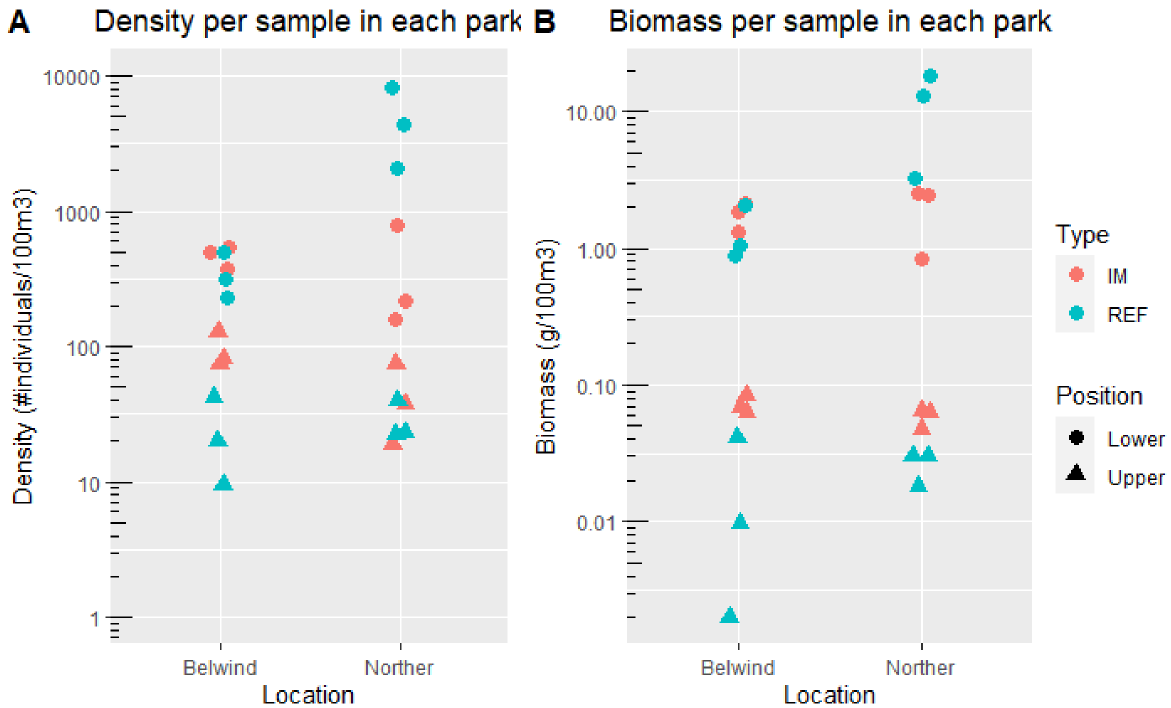

Figure 6 : Hyperbenthos species density (A) and biomass (B) for all samples inside (IM) and outside (REF) in each concession zone. Subdivided into lower net (Lower) and upper net (Upper) samples.

biomass than the upper nets. The upper nets of the impacted areas had a higher biomass than the ones in the reference areas (Tukey post-hoc test $p < 0.05$, figure 6B).

Species richness only differed between net positions, while the Shannon-Wiener index differed among park and net position. Pielou's evenness differed among park and net position, net position and impact type, and park and impact type (3-way ANOVA significant results, Table 1). Species richness was overall higher in lower net samples. The Shannon Wiener index showed the same trend, but only for the Belwind samples (Tukey post-hoc test $p < 0.05$, figure 7A, 7B). Pielou's evenness was significantly higher in the Belwind reference area compared to the Norther reference area. It was also higher when comparing the lower nets of the Belwind area with those of the Norther area. Pielou's evenness was higher in the Norther upper nets compared to the Norther lower nets. It was also overall significantly higher in the upper nets of the reference areas, compared to the lower nets (Tukey post-hoc test $p < 0.05$, figure 7C).

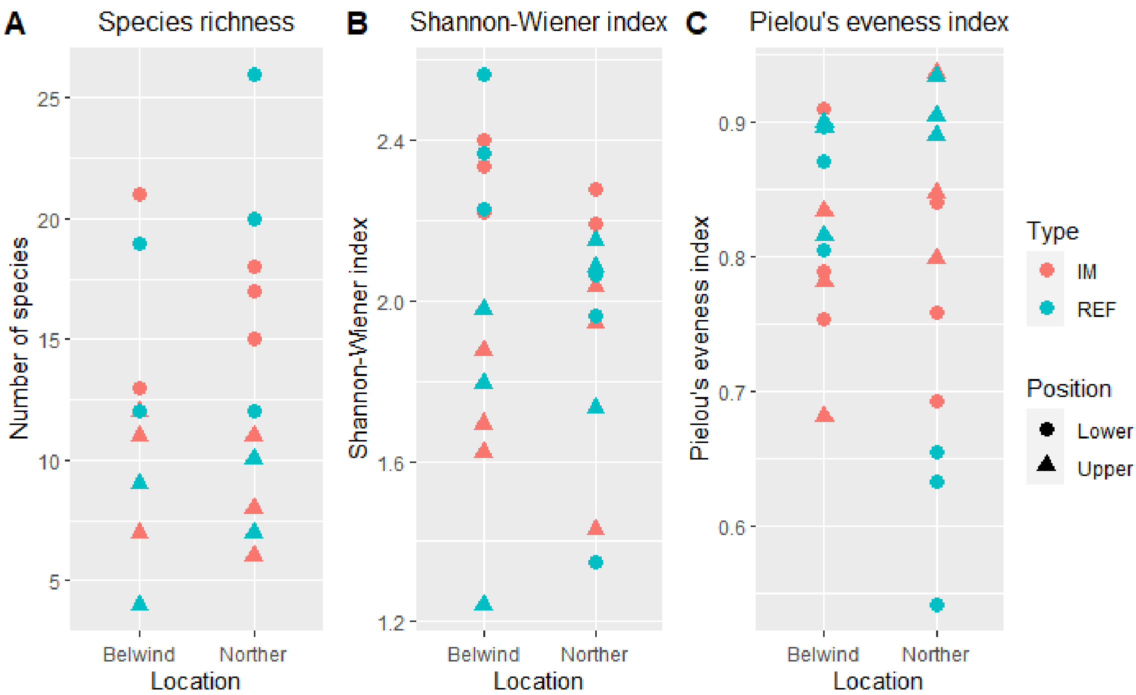

Figure 7: Hyperbenthos species richness (A), Shannon-Wiener index (B) and Pielou's evenness (C) for all samples inside (IM) and outside (REF) in each concession zone. Subdivided into lower net (Lower) and upper net (Upper) samples.

Table 2: Overview of calculated community descriptors (mean ± SE and p-values) taken inside and outside the Belwind OWF.

Belwind – univariate results	Inside OWF	Outside OWF
Total density (N, ind./100 m3)	5778 ± 39	355 ± 83
Total biomass (B, g/100m3)	1.60 ± 0.20	1.40 ± 0.50
Number of species (S)	28 ± 3	24 ± 4
Shannon-Wiener (H')	2.30 ± 0.17	1.82 ± 0.21
Pielou's evenness (J')	0.62 ± 0.05	0.63 ± 0.06

Table 3: Overview of calculated community descriptors (mean ± SE and p-values) taken inside and outside the Norther OWF.

Norther – univariate results	Inside OWF	Outside OWF
Total density (N, ind./100 m3)	422 ± 164	4670 ± 1398
Total biomass (B, g/100m3)	1.91 ± 0.42	10.47 ± 3.57
Number of species (S)	25 ± 2	28 ± 5
Shannon-Wiener (H')	2.36 ± 0.02	2.43 ± 0.10
Pielou's evenness (J')	0.62 ± 0.05	0.59 ± 0.07

4.3. Multivariate analysis

A three-way PERMANOVA analysis of the untransformed data (visualized in an nMDS plot in figure 8) shows that, when including community composition in terms of density, there is a significant three-way interaction "park, impact type and net position" (3-way PERMANOVA, $p < 0.05$). Due to a not large enough sample size (3 replicates for each sample group) not enough permutations can be made to perform an Adonis pairwise comparison for the three-way interaction. Subsequently, possible trends could only be interpreted from the nMDS plot (figure 8). In this plot, the impact samples form a more compact cluster compared to the reference samples. This shows that samples of the impact areas were more similar to each other, while community composition of the reference samples varied more. The lower net samples of Norther reference samples are overall quite dissimilar to the others and there are multiple species highly associated with this area as well.

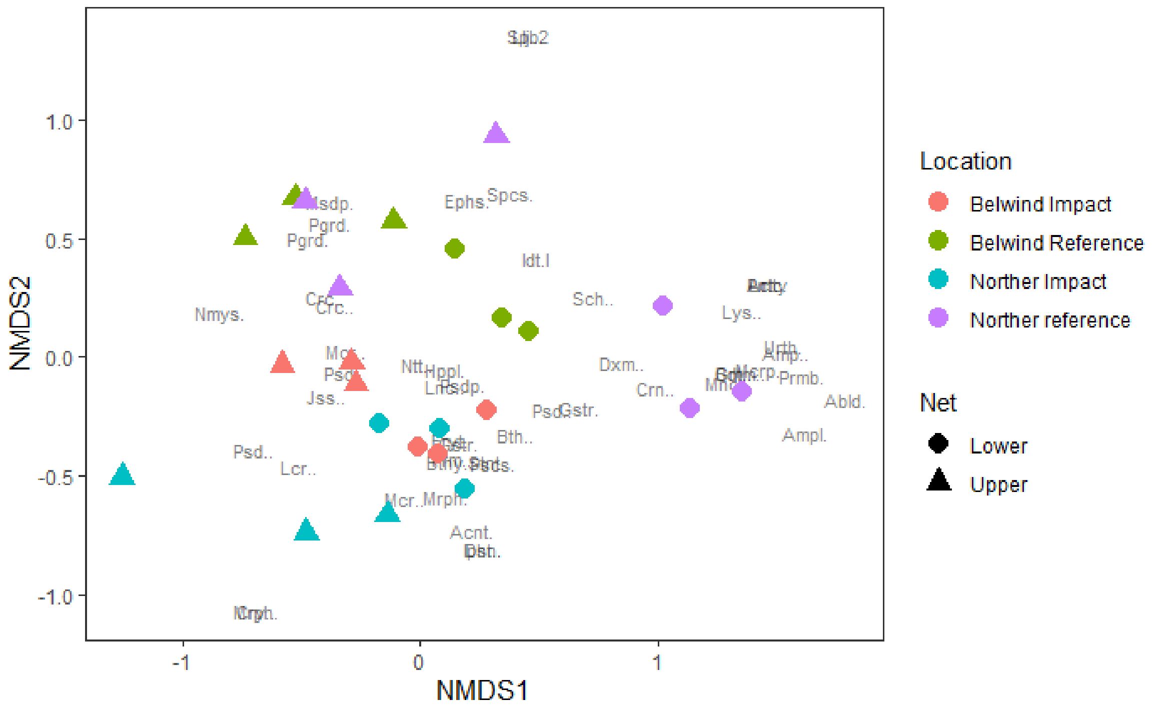

Figure 8: Non-metric multidimensional scaling (nMDS) plot of hyperbenthos densities for the lower and upper samples net taken at each location and for each species. Based on untransformed abundance data.

A second PERMANOVA analysis was performed with relative abundance data (visualized in an nMDS plot in figure 9). In this case, the PERMDISP test gave a significant result ($p=0.041$), this indicates that the differences found between the sample groups, may not be a true location effect but may partially be the result of differences in dispersions between the sample groups. The PERMANOVA resulted in a significant two-way interaction between park and impact type, as well as between impact type and net position. Subsequent Adonis pairwise comparisons revealed a significant difference in community composition between the impact and reference areas for both Belwind and Norther. It also showed a significant difference for each pairwise comparison of the impact type-net position interaction. (impact area lower nets vs impact area upper nets, impact area lower nets vs reference area lower nets, impact area upper nets vs reference area upper nets and reference area lower nets vs reference area upper nets)

4.4. Indicator species analysis

Indicator species analysis showed that only *Mesodopsis slabberi* (Mysidae) was significantly associated with the impact sites (p-value < 0.05). There were no species significantly associated with the reference sites. No species were characteristic for the upper nets either, while a variety of species was associated with the lower nets.

A comparison between Norther and Belwind revealed that *Liocarcinus holsatus* larvae, *Pisidia longicornis* larvae, *Macropodia rostrata* megalopa, *Paramysis* sp., and *Eurytemora velox* were characteristic for Belwind (p-value < 0.05). Megalopa of the *Paguridae*, *Schistomysis sp.* and *Microprotopus maculatus* were characteristic for the Norther area (p-value < 0.05).

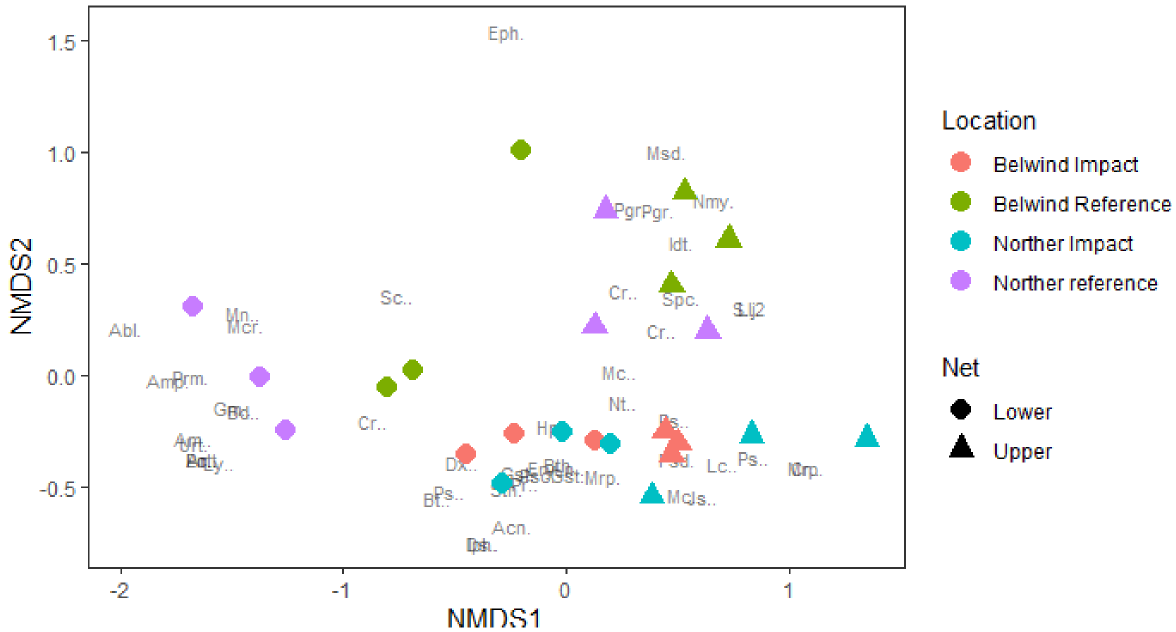

Figure 9: Non-metric multidimensional scaling (nMDS) plot of relative hyperbenthos densities for the lower and upper net samples taken at each location and for each species. Based on relative abundance data.

5. Discussion

5.1. The Impact of offshore windfarms on hyperbenthic fauna

The introduction of offshore windfarms on soft sediment seabeds establishes new artificial hard substrates throughout the entire water column. Through this, they alter the environment in a variety of ways. The turbines act as an artificial reef on which species can settle and increase overall habitat heterogeneity (Degraer et al., 2020). The area can also function as steppingstones for species to cross an otherwise impassable region (Adams et al., 2014). Epifouling fauna growing on the turbine base drop faecal pellets which increases food availability in the area (Maar et al., 2009, Lefaible et al., 2023). Overall, this makes the sediment more fine-grained and increases the amount of organic matter in the sediment close to the turbines (Janssen et al., 2005). Several studies already focused on the influence of these effects on macrobenthic densities and showed that these populations tend to grow richer in the area surrounding the turbines (Maar et al., 2009, Coates et al., 2014, Hutchinson et al. 2020). Additionally, there is a possibility of this process extending the reach of the artificial reef effect to more distant regions surrounding the turbines (Lefaible et al., 2023).

In the monitoring survey on hyperbenthos in the Belgian OWFs in 2021, it was suggested that these impacts also caused the significant higher hyperbenthos density in the C-Power and Norther OWF, as well as the higher H' and J' in C-Power. (Lefaible et al., 2022). In 2022, S', H' and J' were not impacted by the presence of OWFs, neither in Belwind or Norther. The higher hyperbenthos densities and biomass found in 2022 within Belwind agree with the overall increased densities in OWFs found in 2021. The results found for density inside the Norther OWF in 2022 did not align with what was found in 2021, the results for Belwind in 2022, nor the general hypothesis that OWFs provide a more favourable habitat for hyperbenthos (Lefaible et al., 2022, Lefaible et al., 2021). Instead, Norther showed significantly higher hyperbenthos densities outside of the impact area in 2022, while there was no significant difference for biomass.

In 2022, community composition differed between impact and reference areas for both Norther and Belwind when relative abundance data was used, and a similar trend could also be observed in the nMDS plot for the untransformed abundance data. In 2021, the one-way PERMANOVA analysis revealed a significant difference in community composition between both areas in C-Power, but not in Norther. This means that, again, Norther appears contradictory.

Belwind was sampled and analysed for the first time in 2022. This means no direct comparisons could be made with previous sampling efforts. The results for Belwind in 2022, just like overall results found in 2021, do align with the hypothesis that OWFs built on soft sediments impact the hyperbenthos in a way that could be regarded as beneficial.

Furthermore, the OWFs could alter the local environment enough for hyperbenthos community composition within the OWF to become distinct from non-impacted areas.

The findings discovered in Norther in 2022 however, present a contrasting perspective to these findings. The results from 2022 partially contradict the ones obtained in 2021 and also refute the hypothesis that OWFs are beneficial for hyperbenthos density and biodiversity. A part of this could be explained by the young age of the Norther OWF (operational since 2019). Multiple studies already showed than an area impacted by the construction of an OWF needs more time to recover (Degraer et al. 2020, Zupan et al., 2023). Another potential explanation for this different result in Norther is the habitat heterogeneity in the area (ref, Dewicke?). A lack of sediment samples taken close enough to the sampling track locations made it however unable to include the effect of sediment in the study.

Important to also note is that in 2021 a different model of hyperbenthic sledge was used, which made it impossible to directly compare the data gathered 2021 and 2022. It also means that part of the variation between the results of both years, could be explained by the sampling material. Furthermore, only the lower nets were analysed for the Norther OWF in 2021. For these reasons any comparison made between this and last year's results of the Norther OWF need to be taken with a grain of salt and cannot be seen as fully concise. Density results of Norther in 2022 (including lower + upper nets) very clearly contradict last year's results (only including lower nets) as species density was this time much higher outside the OWF, compared to inside. If the analysis of 2021 Included Norther's upper net samples, it might have possibly skewed the results to be more in line with this year's, but future analysis will be required.

5.2 Vertical segregation of hyperbenthic communities

In research focused on the hyperbenthos, researchers frequently use two-levelled hyperbenthic sledges, consisting of lower and upper nets, to consider the possibility of diverse hyperbenthic distributions across the sampled water column (Mees & Jones, 1997). Overall, research tends to be inconclusive about vertical segregation. Some studies show a clear difference between lower and upper nets (Mees & Jones, 1997), while others show the distribution to be homogeneous (Lefaible et al., 2022). 2021's study showed no significant difference between upper and lower nets. This time, a very clear difference was found between the lower and upper nets. The lower nets consistently had higher densities and biomass, a higher S', a higher H' (in Belwind only), as well as a lower J' in the reference areas and Norther area. An advantage of this year's sampling effort was the fact that the sled had a sloped metal plate under the lowest net (see figure 2), which allowed to sample the water column to (almost) the sea floor. Being able to sample more of the water column made it possible to better include certain species groups, like mysids, that are typically associated with the lowest centimetres of the water column (Parry et al. 2021). This made it possible to create a more complete image of hyperbenthos diversity. The net used on Simon Stevin in 2021 did also not have a rolling curtain, as such also sampling water column species, which could lead to contamination of the samples. Improved sampling material and a more extensive dataset could partially explain why the results of 2022 contradict the results about vertical segregation of 2021 and are more in line with Mees & Jones (1997). Overall, this means that the results of 2022 add to the ongoing discussion about the vertical segregation of hyperbenthos and makes it hard to draw any solid conclusion.

5.3. Spatial variation in Hyperbenthos distribution

While Norther is the closest OWF concession zone to the Belgian coastline, Belwind is much further out at sea. Dewicke et al. (2003) found that, next to an east-west gradient, an obvious onshore-offshore gradient exists in the BPNS. They also found six distinguishable hyperbenthos communities that exist within the BPNS. The onshore-offshore gradient exhibits prominent variations in density and biomass, which are connected to factors such as turbidity, salinity, and sediment composition. Sediment becomes coarser and contains less organic matter further away from the coast and more offshore locations also have a higher salinity due to influences from the southern Atlantic (Dewicke et al., 2003, Djenidi et al., 1996). On the other hand, coastal waters close to the shore exhibit distinct characteristics due to the influence of freshwater runoff from the nearby land. As a result, the water is more turbid, and the distribution of mud becomes uneven and scattered (Dewicke et al.,2003). The tides create periods of suspension and re-suspension of particles which not only serves as a source of nourishment for organisms that feed on these particles in the water column, but also for a diverse range of organisms that feed on the sediment surface (Dewicke et al.,2003). According to

Dewicke et al. (2003), this explained a higher density and biomass of hyperbenthos in the onshore communities, a pattern which was also found in this study. Lower nets were found to have a higher density in the Norther area compared to Belwind, and biomass was overall also higher in the Norther area.

Planktonic holohyperbenthic species like hydromedusae, ctenophores and chaetognaths tend to increase in abundance towards more offshore areas. Offshore areas also cope with a stronger ebb-dominated current making hydrodynamical forces and habitat heterogeneity the most important structuring factors for hyperbenthic distributions in the BPNS. For Hyperbenthos communities this means that the found species are typically more planktonic, as they are less dependent on the deposit of organic material (Dewicke et al., 2003). Heavy fragmentation of all Cnidaria in the collected samples unfortunately made it unable to include them in the analysis, which makes it unable to draw any conclusion about these planktonic hyperbenthos.

The Norther concession zone is located in what Dewicke et al. (2003) identified as the Zeeland transitional community; a community dominated by *Liocarcinus holsatus* larvae. While this species was found by Dewicke et al. (2003) to be most commonly associated with the Zeeland transitional community (Norther area), it was significantly associated with the more offshore Belwind area in this study instead. Brachyura larvae, Crangonidae and *Liocarcinus* sp. were most often found in the transitional areas by Dewicke et al. (2003). Despite a high prevalence of Crangonidae in the Norther reference area, indicator species analysis did not reveal it to be a characteristic species for Norther. Mysid species (and *Schistomysis* in particular) were described by Dewicke et al. (2003) to reach higher densities in onshore environments. This was confirmed in this study, as well as in 2021 (Lefaible et al., 2022) as this species was characteristic for the more onshore Norther area. Belwind is instead located more offshore in the Hinder community. This community was, according to Dewicke et al. (2003), more evenly represented by a higher number of species like *Pilumnus hirtellus* and *Pisidia longicornis* . Next to four other species, *Pisidia longicornis* was this year also characteristic for the Belwind (Hinder) community.

5.4. Sampling quality assessment

The sampling was performed with a hyperbenthic sledge; a piece of equipment that has been used over a multitude of studies already (Dewicke et al. 2003, Lefaible et al. 2022, Mees & Jones, 1997). While being dragged along the sea floor, the sledge samples the first meter of the water column. The model used in this study divides this layer of water into two nets and was equipped with a rolling curtain, designed to only open when being dragged along the seabed. Hyperbentic sledges are designed to prevent the accumulation of sediment into the nets, but this is not always as successful. Despite their popular use, the catch efficiency of sledges remains under debate and as such, found densities and biomass remain an estimate (Mees & Jones, 1997). In this study, the samples taken in the Norther reference area in particular were filled with a lot of sediment, contaminating the samples with individuals who were living on, or in the sediment. Clear non-hyperbenthic species like *Asterias rubens* and *Psammechius miliaris* were therefore removed from the dataset.

Furthermore, the SACs and species diversity indicators predicted a considerable number of missed species for certain samples, which shows that an increased sampling effort might be required. This is however in contradiction with the results found in the feasibility study by Lefaible et al. (2019). Lastly, lab analysis of the collected samples is a highly time-consuming activity which resulted in the C-Power samples taken this year to not be analysed. A broader study will require more people to actively analyse the samples in the lab.

Lastly, the inclusion of biomass this year allowed to create a broader picture of the impact of the OWFs on hyperbenthos. Interesting is that it did not follow the exact same trends as the density analysis. While a higher density equalled a higher biomass in the Belwind impact area, the higher densities in the Norther reference area did not result in a significantly different biomass between the Norther impact and reference zone.

6. Conclusion

While an overall, solid conclusion could not be drawn from the analysed samples, there were some trends that could be distinguished. First of all, diversity indicators S', H' and J' were not significantly different inside or outside the analysed OWFs. Secondly, densities did differ inside and outside the OWFs. In the Norther area they were much higher outside the OWF, while the opposite was true in the Belwind area. Thirdly, biomass was also higher inside the Belwind impact area but did not significantly differ for Norther. Lastly, multivariate analysis with relative abundance data did reveal significant differences in community composition between impact and reference areas. Long term recovery and succession in an OWF do require a continued, and preferably more intense, study effort to achieve a full image on the impact of OWFs on hyperbenthos.

7. Summary

7.1 English summary

Independent and sustainable energy production is becoming increasingly important and offshore wind farms (OWFs) play a vital role in meeting those energy requirements. Since 2009, windfarms have been built and will continue to be constructed in the Belgian Part of the North Sea (BPNS) (*Belgian Offshore Platform, 2023*). It is however important to consider the impact these structures have on marine ecology and biodiversity. These turbines have introduced abiotic changes on hydrology, granulometry and food availability (Maar et al., 2009, Coates et al., 2014) and act as an artificial reef in the soft, sandy sediment (Degraer et al., 2020). This has been shown to have an impact on macrobenthic biodiversity (Lefaible et al., 2023). In extension, certain fish, bird and mammal species may profit from the presence of the OWFs, while others rather avoid them (Lindeboom et al. 2011, Peschko et al., 2020, Gall et al., 2021). The impact on hyperbenthos does however remain unclear. Hyperbenthos are small animals that live in the first meter above the sea floor and consist for the most part of amphipods, mysids, decapod shrimp and juvenile fish. They play an important role in bentho-pelagic coupling as they consume phyto-, and zooplankton and serve in turn as food for higher trophic levels. This means they also serve as food for economically important fish and crustacean species (Mees & Jones, 1997).

This study aims to expand the knowledge of hyperbenthos within a socio-economical important context, the global movement towards green, renewable energy. It investigates the impact of operational OWFs on the hyperbenthos communities in the transitional and offshore areas of the BPNS to help fill in the gaps about how OWFs alter the environment in which they are built. The main research question is thus: how does the presence of an OWF impact the hyperbenthos communities living above the soft sediment between the turbines? As fouling fauna grows on the turbines, an increase in organic material occurs in the surrounding area because of epifouling (Degraer et al., 2020). The presence of these turbines also leads to more fine-grained sediment, higher availability of organic matter and locally decreases currents (Janssen et al., 2005, Leonhard et al., 2005, Coates et al., 2014). Based on these facts, it was hypothesized that the introduced changes could be favourable for hyperbenthos and thus lead to an increase in biodiversity, density, and biomass.

In October of 2022, samples were collected in the Belwind, C-Power and Norther concession zones. To take the samples, a two-levelled hyperbenthic sled was used. For each concession zone, samples were taken in- and outside the OWF, with triplicate tracks each. The samples taken for Norther and Belwind were then sorted in the lab and statistical analysis was performed. First, species accumulation curves (SACs) were constructed, and diversity estimators were calculated. Second, A three-way ANOVA analysis was performed to reveal any differences species richness (S'), Shannon-Wiener index (H'), Pielou's evenness (J'), density, and biomass between all sample types. (Factors: "park", "net position" and "impact type" with levels: "Belwind" and "Norther", "lower" and "upper", "impact" and "reference" respectively). Two PERMANOVA analyses were also conducted to determine any differences in community composition, based both on untransformed and relative abundance data. These were then visualized in an nMDS plot. Lastly, an indicator species analysis was performed.

The ANOVA analyses and subsequent Tukey post-hoc tests revealed the following results: (1) the Belwind impact zone had a higher hyperbenthos density compared to the reference zone, while the inverse is true for Norther. Overall densities were also significantly higher in Norther compared to Belwind, but only in the lower nets. Lastly, both lower and upper net samples from the reference sites had a higher density compared to the impact zone. (2) For biomass, the Norther reference samples were significantly higher than the Belwind reference samples. Both in the reference and impact areas, the lower net samples had a significantly higher biomass than the upper nets. The upper nets of the impacted areas also had a higher biomass than the ones in the reference areas. (3) S' was overall higher in lower net samples. (4) H' showed the same trend as S', but only for the Belwind samples. (5) J' was significantly higher in the Belwind reference area compared to the Norther reference area. It was also higher when comparing the lower nets of the Belwind area with those of the Norther area. J' was higher in the Norther upper nets compared to the Norther lower nets. It was also overall significantly higher in the upper nets of the reference areas, compared to the lower nets.

The three-way PERMANOVA analysis performed with relative abundance data resulted in a significant two-way interaction between park and impact type, as well as between impact type and net position. Subsequent Adonis pairwise comparisons revealed a significant difference in community composition between the impact and reference areas for both Belwind and Norther. It also showed a significant difference for each pairwise comparison of the impact type-net position interaction. (impact area lower nets vs impact area upper nets, impact area lower nets vs reference area lower nets, impact area upper nets vs reference area upper nets and reference area lower nets vs reference area upper nets). The three-way PERMANOVA analysis of the untransformed data resulted in a significant three-way interaction. Due to a not large enough sample size (3 replicates for each sample group) not enough permutations could be made to perform a pairwise comparison for the three-way interaction. Subsequently, possible trends could only be interpreted from the nMDS plot, which appeared to be mostly similar to the results of the analysis of relative abundance data.

This study was the second year of research on the impact of OWFs on hyperbenthos. In 2021, the Norther and C-power OWF were studied (Lefaible et al., 2022), while this time, in 2022, Norther and Belwind were studied. Between both years, a different type of hyperbenthic sledge was used as well. This means only the global results of the two years could be compared. Over both years, only C-Power revealed a higher H' and J' within the OWF, while all other sampling sites revealed no difference between the impact and reference areas. Both C-Power and Norther in the 2021 study, as well as Belwind in 2022, showed an increase in density within the OWFs. The results for Norther in 2022 did however indicate densities that were a lot higher outside the Norther OWF. This means that, up until now, the hypothesis was proven true for densities in three out of four cases. The effect on biodiversity appears to still be minimal, as only an increased biodiversity was found in C-power in 2021. A difference in community composition was also found in three out of four cases. Only Norther in 2021 did not reveal such a difference.

There is no decisive reason why the Norther OWF appears to be so contradictive, but it could be explained by the young age of the Norther OWF (operational since 2019). Multiple studies already showed than an area impacted by the construction of an OWF needs a lot of time to recover (Degraer et al., 2020, Zupan et al. 2023). Another potential explanation for this different result in Norther is the habitat heterogeneity in the area. A lack of sediment samples taken close enough to the sampling track locations made it however unable to include the effect of sediment in the study.

In research focused on the hyperbenthos, two-levelled hyperbenthic sledges are frequently used. They consist of lower and upper nets, so possible vertical segregation across the water column can be analysed. Overall, research tends to be inconclusive about this vertical segregation. Some studies show a clear difference between lower and upper nets (Mees & Jones, 1997), while others show the distribution to be more homogeneous (Lefaible et al., 2022). 2021's study showed no significant difference between upper and lower nets, while in 2022 the lower nets consistently had higher densities and biomass, a higher S', a higher H' (in Belwind only), as well as a lower J' in the reference areas and Norther area. An advantage of this sampling effort was the fact that the sled had a sloped metal plate under the lowest net, which allowed to sample the water column to (almost) the sea floor. This not only allowed to sample more of the water column, but also made it possible to better include certain groups, like mysids, who are typically associated with these lowest centimetres of the water column (Parry et al. 2021). This allowed to create a more complete image of hyperbenthos diversity. Improved sampling material and a more extensive dataset could thus explain why the results about vertical segregation of 2022 contradict those of 2021.

Certain differences between the Belwind and Norther OWF could also be observed, because of the spatial variation in hyperbenthos distribution. While Norther is the closest OWF concession zone to the Belgian coastline, Belwind is much further out at sea. Dewicke et al. (2003) found that an obvious onshore-offshore gradient exists in the BPNS. They also found six distinguishable hyperbenthos communities that exist within the BPNS. According to Dewicke et al. (2003), hyperbenthos have a higher density and biomass in the more onshore communities and is this connected to factors such as turbidity, salinity, and sediment composition. A similar pattern was also found in this study. Lower nets were found to have a higher density in the Norther area compared to Belwind, and biomass was overall higher in the Norther area.

The Norther concession zone is located in what Dewicke et al. (2003) identified as the Zeeland transitional community; a community dominated by *Liocarcinus holsatus* larvae. Brachyura larvae, Crangonidae and *Liocarcinus* sp. were generally most found in the transitional areas by Dewicke et al. (2003). Belwind is instead located more offshore in the Hinder community. This community was more evenly represented by a higher number of species like *Pilumnus hirtellus* and *Pisidia longicornis* (Dewicke et al., 2003). While *Liocarcinus holsatus* larvae were found by Dewicke et al. (2003) to be most commonly associated with the Zeeland transitional community (Norther area), it was significantly associated with the more offshore Belwind area in this study instead. *Pisidia longicornis* larvae were, as described by Dewicke et al. (2003), also characteristic in the Belwind area. Mysid species (and *Schistomysis* in particular) were found to reach higher densities in onshore environments by Dewicke et al. (2003). The results of this study, as well as the one conducted in 2021 (Lefaible et al., 2022) confirm with this as this *chistomysis* was characteristic for the more onshore Norther area.

The sampling strategy for this study was however not perfect. The samples taken in the Norther reference area in particular, were filled with a lot of sediment, contaminating the samples with individuals who were living on or in the sediment. Despite the design of the sampling strategy and hyperbenthic sleds themselves, to try and prevent this as much as possible, the aggregation of sediment in the samples remains a known issue that can occur. Species that were clearly not hyperbenthic, were however removed from the dataset before performing the statistical analysis. Furthermore, the SACs and species diversity estimators tend to predict a considerable number of missed species for certain samples, which shows that an increased sampling effort might be required. This is however in contradiction with the results found in the feasibility study by Lefaible et al. (2019). Lastly, lab analysis of the collected samples is a highly time-consuming activity, resulting in the C-Power samples taken this year to not be analysed. Lastly, Inclusion of biomass in the analysis did prove to be relevant, as biomass did not always follow the same trends as density.

While an overall, solid conclusion could not be drawn from the analysed samples, there were some trends that could be distinguished. First of all, diversity indicators S', H' and J' were not significantly different inside or outside the analysed OWFs. Secondly, densities did differ inside and outside the OWFs. In the Norther area they were much higher outside the OWF, while the opposite was true in the Belwind area. Thirdly, biomass was also higher inside the Belwind impact area but did not significantly differ for Norther. Lastly, multivariate analysis with relative abundance data did reveal significant differences in community composition between impact and reference areas. Long term recovery and succession in an OWF do require a continued, and preferably more intense, study effort to achieve a full image on the impact of OWFs on hyperbenthos.

7.2 Dutch summary

Onafhankelijke en duurzame energieproductie wordt steeds belangrijker en offshore windparken (OWP) spelen een vitale rol in het bereiken van deze vereisten. Sinds 2009 worden er dan ook offshore windmolenparken gebouwd in het Belgische deel van de Noordzee (BPNS) (*Belgian Offshore Platform*, 2023). Het is echter van belang dat tijdens deze ontwikkelingen er rekening wordt gehouden wordt met de impact die ze kunnen hebben op het mariene leven. Eerdere studies toonden al aan dat er een toename is in biodiversiteit op en rond de basis van de turbines omdat deze dienst doen als een artificieel rif op het zachte, zanderige sediment (Degraer et al., 2020). De turbines veroorzaken ook veranderingen in de lokale abiotiek door wijzigingen in hydrologie, granulometrie en voedselbeschikbaarheid (Leonhard et al., 2005, Coates et al., 2014). Eerder werd al aangetoond dat dit een positieve impact heeft op de densiteit en biodiversiteit van macrobenthos (Lefaible et al., 2023). Er is echter ook sprake van een impact op vissen, vogels en zeezoogdieren (Lindeboom et al. 2011, Peschko et al., 2020, Gall et al., 2021). Hoe de windmolenparken hyperbenthos juist beïnvloeden, is echter nog onduidelijk. Hyperbenthos zijn kleine diertjes die in de eerste meter boven de zeebodem leven en ze bestaan voornamelijk uit amphipoda, aasgarnalen, garnalen en juveniele vissen. Ze spelen een belangrijke rol in bentho-pelagische koppeling omdat ze phyto- en zoöplankton consumeren, en vervolgends zelf dienstdoen als voedsel voor hogere trofische niveaus (Mees & Jones, 1997).

Deze studie beoogt om de kennis over de hyperbenthos te vergroten binnen een socio-economische context, namelijk de globale beweging richting groene, hernieuwbare energie. De impact van operationele OWPs op hyperbenthos gemeenschappen in de transitie- en offshore regio's van de BPNS werd onderzocht om zo te helpen de gaten in te vullen over hoe de OWPs de omgeving veranderen in welke ze gebouwd worden. De ondderzoeksvraag is dus: hoe beïnvloedt de aanwezigheid van OWPs de hyperbenthosgemeenschappen die leven boven de zeebodem tussen de turbines? Fouling gemeenschappen groeien op de turbines en verhogen door epifouling de hoeveelheid organisch materiaal in de omgeving (Degraer et al., 2020). De aanwezigheid van de turbines leidt ook tot fijnkorreliger sediment, meer beschikbaar organisch materiaal en vertragen lokaal de stroming (Janssen et al., 2005, Coates et al., 2014, Leonhard et al., 2005). Gebaseerd op deze feiten werd de hypothese gesteld dat de geïntroduceerde veranderingen gunstig zijn voor hyperbenthos en dus kan leiden tot een toename in biodiversiteit, densiteit en biomassa.

In oktober 2022 werden er stalen verzameld voor de Belwind, C-Power en Norther concessiezones. Om de stalen te verzamelen werd een tweeledige hyperbenthosslede gebruikt. Voor elke concessiezone werden stalen verzameld binnenin de OWP en in een referentiezone erbuiten, elk met drievoudige replicaten. De stalen genomen voor Belwind en Norther werden in het lab verder gesorteerd en geïdentificeerd. Eerst werden soorten accumulatie curves opgesteld en werden diversiteitsschatters berekend. Daarna werd een drie-wegs ANOVA-analyse uitgevoerd om verschillen in soortenrijkdom (S'), Shannon-Wiener index (H'), Pielou's evenness (J'), densiteit en biomassa te achterhalen. (Factors: "park", "netpositie" en "impact type" met respectievelijke niveaus: "Belwind" en "Norther", "onder" en "boven", "impact" en "referentie"). Twee PERMANOVA-analyses (één met ongetransformeerde en één met relatieve abundanties) werden ook uitgevoerd om mogelijke verschillen in gemeenschapssamenstelling te bepalen. Deze werden ook voorgesteld aan de hand van een nMDS plot. Als laatste werd ook een indicatorsoorten analyse uitgevoerd.

De ANOVA-analyses en daaropvolgende post-hoc Tukey tests toonden het volgende aan: (1) De densiteit van Belwinds impact zone was hoger dan die referentiezone, terwijl het omgekeerde waar is voor Norther. Hyperbenthosdensiteiten waren voor Norther ook hoger dan Belwind in de onderste netten. Als laatste hadden zowel de onderste en bovenste netten van de referentiezone een hogere densiteit dan die van de impact zones. (2) De Biomassa van de Norther referentiestalen waren significant hoger dan die uit Belwind, zowel voor de impact als de referentiezone. De onderste netten hadden ook altijd een hogere biomassa dan de onderste en de bovenste netten uit de impact zones hadden een hogere biomassa dan die uit de referentiezones. (3) S' was steevast hoger voor de onderste netten. (4) Hetzelfde gold voor H', maar dan enkel in Belwind. (5) J' was steeds hoger in de Belwind referentiezone vergeleken met die van Norther. Het was ook hoger in Northers bovenste netten dan de onderste en was ook significant hoger in de bovenste netten van de referentiezone, vergeleken met de onderste.

De drie-wegs PERMANOVA-analyse gebaseerd op relatieve abundanties resulteerde in een significante twee-wegs interactie tussen park en impact type, alsook tussen impact type en netpositie. De Adonis paarsgewijze vergelijking toonde aan dat er een significant verschil is in gemeenschapssamenstelling tussen de impact en referentiezones, zowel voor Belwind als Norther. Er was ook een significant verschil voor de elke paarsgewijze vergelijking voor de impact type – netpositie interactie. (impact zone onderste netten vs impact zone bovenste netten, impact zone onderste netten vs referentiezone onderste netten, impact zone bovenste netten vs referentiezone bovenste netten en referentiezone onderste netten vs referentiezone bovenste netten). De drie-wegs PERMANOVA-analyse van de niet-getransformeerde data resulteerde in een significante driewegsinteractie. Door een te kleine staalgrootte (3 replicaten per groep) konden er niet genoeg permutaties plaats vinden om een paarsgewijze vergelijking uit te voeren. Als gevolg konden enkel de trends geïnterpreteerd worden uit de nMDS plot. De bleken vrij gelijkaardig aan de resultaten van de analyse met relatieve abundanties.

Deze studie was het tweede jaar waarin de impact van OWPs op de hyperbenthos onderzocht werd. In 2021 werden Norther en C-power bestudeerd (Lefaible et al., 2022) terwijl dit jaar, in 2022, Norther en Belwind werden geanalyseerd. In beide studies werd ook een ander model hyperbenthos slede gebruikt, wat ervoor zorgt dat enkel de globale resultaten met elkaar vergeleken konden worden. C-Power was de enige OWP die een hogere H' en J' vertoonde in de impact zone, terwijl voor alle andere parken er geen verschil was. Zowel C-Power als Norther in 2021, alsook Belwind in 2022, toonden een hogere densiteit aan binnen de OWPs. In 2022 toonden de resultaten voor Norther echter aan dat de densiteit binnen de OWP een pak lager was. Globaal betekent dit dat in drie van de vier gevallen, hyperbenthos densiteit hoger was binnen de OWPs. Biodiversiteit was echter maar één keer hoger, namelijk in C-Power in 2021. Een verschil in gemeenschapssamenstelling werd ook drie van de vier keer vastgesteld, met uitzondering van Norther in 2021.

Er is geen finale reden waarom de resultaten van Norther zo tegenstrijdig lijken maar het zou ten den dele kunnen veroorzaakt worden door de recente bouw van Norther (operationeel sinds 2019). Meerdere studies toonden al aan dat gebieden waarin een OWP gebouwd worden meer tijd nodig hebben om te herstellen (Degraer et al., 2020, Zupan et al. 2023). Een andere mogelijke reden kan de heterogeniteit van het gebied zijn, maar een gebrek aan sedimentstalen genomen dicht bij de staalname tracks maakten het onmogelijk om sedimenttypes op te nemen in de studie.

In onderzoek gefocust op hyperbenthos, worden tweeledige hyperbenthos sledes vaak gebruikt. Ze hebben een onderste en bovenste net zodat verticale segregatie in de waterkolom kan worden geanalyseerd. De effectieve aanwezigheid van deze verticale segregatie blijft echter betwist. Sommige studies tonen een duidelijk contrast aan tussen de bovenste en onderste netten (Mees & Jones, 1997), terwijl in andere de verdeling homogeen blijkt (Lefaible et al., 2022). In de studie van 2021 was er geen significant verschil tussen de bovenste en onderste netten. In 2022 vertoonden de onderste netten echter consequent een hogere densiteit, biomassa, S' en H' (enkel in Belwind), alsook een lagere J' in de referentiezones en in Norther tegenover Belwind. Het voordeel van deze staalname was het feit dat de slede een schuine plaat had onder het onderste net, waardoor tot vrijwel tegen de zeebodem gesampeld kon worden. Door dichter bij de zeebodem te samplen, kon er een vollediger beeld gevormd worden van de hyperbenthos diversiteit (Parry et al. 2021). Ook had de slede een rolgordijn dat ervoor zorgde dat organismen enkel in de netten terecht konden komen wanneer de slede effectief op de zeebodem was. Deze verbeterde staalname zou ervoor kunnen zorgen dat de resultaten van 2022 tegenstrijdig zijn met die uit 2021.

Er konden ook verschillen waargenomen worden tussen Belwind en Norther als gevolg van ruimtelijke variatie in Hyperbenthos distributie. Terwijl Norther de OWP het dichtste bij de kustlijn is, bevind Belwind een stuk verder in zee. Dewicke et al. (2003) beschreef een duidelijke onshore-offshore gradient in de BPNS. Daarnaast beschreven ze ook zes verschillende hyperbenthos gemeenschappen in de BPNS. Volgens Dewicke et al. (2003) is de densiteit en biomassa hoger voor meer onshore gemeenschappen en is verbonden aan verschillen in turbiditeit, saliniteit en sedimentsamenstelling. Dezelfde trend werd ook gevonden in deze studie. De onderste netten hadden een hogere densiteit voor Norther vergeleken met Belwind, en biomassa was ook hoger voor Norther.

De Norther OWP bevindt zich in wat Dewicke et al. (2003) beschreef als de Zeeland transitie gemeenschap. Een gemeenschap gedomineerd door *Liocarcinus holsatus* larvae. Brachyura larvae, Crangonidae en *Liocarcinus* sp. werden verder het vaakst gevonden in transitie gemeenschappen. Belwind is gelegen in de offshore Hinder gemeenschap. Deze gemeenschap wordt gekenmerkt door een meer evenredige verdeling van een groter aantal soorten zoals *Pilumnus hirtellus* and *Pisidia longicornis* (Dewicke et al., 2003). Terwijl *Liocarcinus holsatus* larvae door Dewicke et al. (2003) vooral toegewezen werden aan de Zeeland transitie gemeenschap (Norther regio), toonde deze studie aan dat ze significant geassocieerd werden met de offshore Belwind regio. *Pisidia longicornis* larvae werden, zoals beschreven door Dewicke et al. (2003) vooral gevonden in de Belwind regio. Aasgarnalen (en vooral *Schistomysis*) werden als indicatorsoorten bevonden voor het meer onshore Norther zowel in deze studie, als in 2021 (Lefaible et al., 2022). Ook dit is in lijn met de beschrijving van Dewicke et al. (2003).

De staalname strategie was voor deze studie echter niet perfect. Vooral de stalen genomen in de Norther referentiezone bevatten veel sediment, wat de stalen contamineerde met soorten die in of op de zeebodem leefden. Desondanks het ontwerp van de staalname strategie, alsook de hyperbenthos sledes zelf om dit te proberen voorkomen, blijft dit een gekend probleem tijdens staalnames. Soorten die duidelijk niet tot de hyperbenthos behoorden, werden wel uit de dataset verwijderd. Ook de SACs en diversiteitsschatters voorspelden vaak een vrij groot aantal gemiste soorten. Dit was in tegenstelling tot de resultaten van de haalbaarheidsstudie door Lefaible et al. (2019). Als laatste bleek analyse van de stalen in het lab ook een tijdrovend werk. Dit zorgde ervoor dat de stalen die genomen werden voor C-Power dit jaar niet konden worden geanalyseerd. Als laatste bewees de opname van biomassa in de analyse ook nuttig te zijn, omdat biomassa niet altijd dezelfde trends volgde als densiteit.

Terwijl een algemene, duidelijke conclusie nog niet getrokken kon worden uit de geanalyseerde stalen, waren er wel enkele trends die zichtbaar waren. Als eerste waren de diversiteitsindicators S', H' en J' nooit significant binnen en buiten de OWPs. Ten tweede was densiteit wel verschillend binnen en buiten de OWPs. In Belwind was de densiteit hoger in de impact zone, terwijl het omgekeerde waar was voor Norther. Ook biomassa was hoger binnenin Belwind, maar was niet verschillend voor Norther. Als laatste toonde multivariate analyse aan dat de gemeenschapssamenstelling binnenin de OWFs significant verschilde van die in de referentiezones. Langdurig herstel en successie binnen de OWFs maken het echter duidelijk dat een continue, en ook meer intensieve, inspanning nodig is om een volledig beeld te kunnen krijgen over de impact van OWFs op hyperbenthos.

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Appendix 1: List of species

Amphipoda

Abludomelita obtusata
Ampelisca brevicornis
Amphilochidae sp.
Aora typica
Bathioporeia sp.
Bathyoporeia sarsi
Dexaminidae sp.
Gammaridae sp.
Jassa sp.
Leucothoidae sp.
Liljeborgia kinahani
Lysianassidae sp.
Microprotopus maculatus
Monocorophium sp.
Nototropis sp.
Pariambus typicus
Pontocrates altamarinus
Pseudoprotella phasma
Stenothoe marina
Urothoe elegans

Copepoda

Eurytemora velox

Cumacea

Bodotria sp.
Diastylis sp.
Iphinoe trispinosa
Pseudocuma sp.

Decapoda

Carcinus maenas larve
Carcinus maenas megalopa
Corystes cassivelaunus larve
Crangonidae sp.
Hippolyte varians
Liocarcinus holsatus larve
Macropodia rostrata larve
Macropodia rostrata megalopa
Morphospecies larve
Morphospecies megalopa
Paguridae larve
Paguridae megalopa
Pisidia longicornis larve
Pisidia longicornis megalopa
Species shrimp
Species shrimp 2

Euphausiacea

Euphausiaceae sp

Isopoda

Idiotea linearis

Mysidae

Acanthomysis longicornis
Gastrosaccus sanctus
Gastrosaccus spinifer
Mesodopsis slabberi
Neomysis integer
Paramysis sp.
Schistomysis sp.

Pisces

Pisces juvenile

Polychaeta

Lanice conchilega juvenile

Appendix 2: Statistical results

Results of three-way ANOVA analysis with species richness as response variable

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Park	1	1.04	1.04	0.0758	0.7867	
Type	1	0.04	0.04	0.0030	0.9568	
Position	1	477.04	477.04	34.6939	2.281e-05	***
Park:Type	1	18.38	18.38	1.3364	0.2646	
Park:Position	1	1.04	1.04	0.0758	0.7867	
Type:Position	1	5.04	5.04	0.3667	0.5533	
Park:Type:Position	1	0.04	0.04	0.0030	0.9568	
Residuals	16	220.00	13.75			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Results of three-way ANOVA analysis with Shannon-Wiener index as response variable

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Park	1	0.05389	0.05389	0.8083	0.381952	
Type	1	0.00935	0.00935	0.1402	0.712978	
Position	1	0.78733	0.78733	11.8090	0.003389	**
Park:Type	1	0.01174	0.01174	0.1760	0.680392	
Park:Position	1	0.50494	0.50494	7.5735	0.014176	*
Type:Position	1	0.06255	0.06255	0.9382	0.347158	
Park:Type:Position	1	0.16617	0.16617	2.4923	0.133969	
Residuals	16	1.06675	0.06667			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Results of three-way ANOVA analysis with Pielou’s evenness as response variable

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Park	1	0.010405	0.010405	2.6267	0.1246192	
Type	1	0.000597	0.000597	0.1507	0.7029646	
Position	1	0.047774	0.047774	12.0599	0.0031392	**
Park:Type	1	0.023402	0.023402	5.9074	0.0272120	*
Park:Position	1	0.070730	0.070730	17.8549	0.0006433	***
Type:Position	1	0.026766	0.026766	6.7566	0.0193663	*
Park:Type:Position	1	0.007067	0.007067	1.7839	0.2003568	
Residuals	16	0.063382	0.003961			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Results of three-way ANOVA analysis with density as response variable

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Park	1	0.853	0.853	3.3054	0.0878255	.
Type	1	0.055	0.055	0.2143	0.6496510	
Position	1	52.716	52.716	204.1805	1.579e-10	***
Park:Type	1	7.055	7.055	27.3255	8.298e-05	***
Park:Position	1	2.934	2.934	11.3652	0.0038892	**
Type:Position	1	5.557	5.557	21.5238	0.0002728	***
Park:Type:Position	1	1.006	1.006	3.8955	0.0659418	.
Residuals	16	4.131	0.258			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Results of three-way ANOVA analysis with biomass as response variable

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Park	1	3.146	3.146	7.5189	0.0144681	*
Type	1	0.723	0.723	1.7282	0.2071698	
Position	1	109.777	109.777	262.3523	2.403e-11	***
Park:Type	1	3.372	3.372	8.0598	0.0118483	*
Park:Position	1	0.678	0.678	1.6209	0.2211592	
Type:Position	1	6.765	6.765	16.1685	0.0009875	***
Park:Type:Position	1	0.161	0.161	0.3836	0.5443825	
Residuals	16	6.695	0.418			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Results of three-way PERMANOVA analysis with species density as response variable

```
                   Df SumOfSqs      R2       F Pr(>F)    
Park                1   0.2979 0.06519  3.8639  0.003 ** 
Type                1   0.7898 0.17288 10.2460  0.001 ***
Position            1   1.2873 0.28176 16.6989  0.001 ***
Park:Type           1   0.2378 0.05204  3.0842  0.010 ** 
Park:Position       1   0.1642 0.03593  2.1295  0.047 *  
Type:Position       1   0.3614 0.07911  4.6888  0.002 ** 
Park:Type:Position  1   0.1970 0.04312  2.5555  0.018 *  
Residual           16   1.2334 0.26997                   
Total              23   4.5687 1.00000                   
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Permutation test for adonis under reduced model
Permutation: free
Number of permutations: 999
```

Results of three-way PERMANOVA analysis with relative species density as response variable

```
                   Df SumOfSqs      R2       F Pr(>F)    
Park                1   0.3318 0.05652  2.7363  0.011 *  
Type                1   1.0692 0.18211  8.8162  0.001 ***
Position            1   1.2963 0.22079 10.6888  0.001 ***
Park:Type           1   0.3233 0.05507  2.6658  0.013 *  
Park:Position       1   0.1565 0.02666  1.2908  0.247    
Type:Position       1   0.5187 0.08834  4.2769  0.001 ***
Park:Type:Position  1   0.2348 0.04000  1.9364  0.065 .  
Residual           16   1.9404 0.33050                   
Total              23   5.8710 1.00000                   
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Permutation test for adonis under reduced model
Permutation: free
Number of permutations: 999
```