CHAPTER 10

FEEDING BEHAVIOUR OF LESSER WEEVER (*ECHIICHTHYS VIPERA*) AND DAB (*LIMANDA LIMANDA*) IN THE C-POWER WIND FARM

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ABSTRACT

This chapter focuses on the feeding behaviour of lesser weever (*Echiichthys vipera*) and dab (*Limanda limanda*) in and around the C-Power wind farm. To find out if the presence of wind farms is influencing the feeding behaviour of both demersal fish, stomach content analyses were performed for both demersal fish species originating from the wind farm and from a nearby control area. Results on stomach fullness, frequency of occurrence and numerical percentage of prey taxa, prey diversity and prey species composition are discussed.

The fullness index and prey diversity of lesser weever was not affected by the presence of the wind farm. However, the diet composition did change: lesser weever consumed significantly more of the species

Jassa herdmani - which is typically associated with hard substrates and highly available in the wind farm- in both the control and to a greater extent in the impact area.

The fullness index of dab also displayed no significant differences. The prey diversity and the diet composition of dab however, were influenced by the presence of the wind farm. The ampipods *Nototropis swammerdamei* and *J. herdmani* and the tube-building polychaete *Lanice conchilega* were responsible for those differences. The latter species is a well-known ecosystem-engineer with the potential to enhance habitat complexity and heterogeneity. Its presence might have led to a significant higher prey diversity in the wind farm and hence in the diet of dab.

The differences in feeding behaviour between wind farm and control area can in part be related to the presence of the wind farm, its associated fauna and the expanding reef effect.

10.1. INTRODUCTION

With the construction of wind farms, artificial hard substrates are introduced into a natural sandy environment, and act as artificial reefs (Petersen & Malm, 2006; Langhamer, 2012). These hard substrates are in general rapidly colonized by an epifaunal community (Petersen & Malm, 2006; Kerckhof *et al,* 2010; De Mesel *et al.,* 2013; De Mesel *et al,* 2015) which may provide food for fish that aggregate around these structures (May *et* al, 2005; Reubens *et al,* 2011; Reubens *et al,* 2013; Wilhelmsson *et al*, 2006). Also demersal fish from the surrounding soft substrates profit from the presence of the wind turbines.

In 2012, a feeding behaviour study on dab *Limanda limanda* was performed which showed that dab had fuller stomachs in the C-Power wind farm compared to the control area (Derweduwen *et al.*, 2012). Furthermore, the hard substratum species *Phtisica marina* solely occurred in the stomachs of fish originating from the wind farm area. Other diet studies on pouting (Reubens *et al,* 2011; Reubens *et al*, 2013) indicated that the amphipod *Jassa herdmani* and the crab *Pisidia longicornis* - both (sub)dominantly present on the foundation of the wind farms (Kerckhof *et al,* 2010; De Mesel *et al*, 2013; De Mesel *et al.,* 2015) - were important prey species. However, these prey species were not found in the diet study of Derweduwen *et al.* (2012). This is probably due to the fact that the hard substratum epifaunal community was not yet fully developed and stable two years after construction (Jensen, 2002; Gray, 2006; Petersen and Malm, 2006) and hence the effects on the soft sediment were also still limited (Bergström *et al,* 2012; Bergström *et al.*, 2013, Wilhelmsson *et al.,* 2006; Vandendriessche *et al*, 2013; Vandendriessche *et al.*, 2015). In this study, which is conducted five years after construction, we may expect different effects of the windmills and its hard substrate community on the demersal fish of the soft sediment.

Both the artificial reef effect associated with the physical presence of the turbine foundations and scour protection (Reubens *et al*, 2011; Reubens *et al*, 2013; Derweduwen *et al*, 2012) and the exclusion of fisheries activities from wind farms and their safety buffers may change the food availability and subsequent diet of demersal fish within the wind farm (Berkenhagen *et al.*, 2010; Kaiser & Ramsay, 1997; van Hal *et al.*, 2012).

The research questions we want to answer in this study are the following.

- Do demersal fish have fuller stomachs inside versus outside the wind farm and 5 years after versus before the construction of the wind farm? In other words, do they feed more inside the wind farm, after it was constructed?
- Do fish have a different diet composition inside versus outside the wind farm and before versus 5 years after the construction of the wind farm?
- Do demersal fishes feed on hard substratum species associated with the wind farm constructions?

10.2. MATERIAL AND METHODS

SAMPLING

In autumn 2009, 2010, 2012 and 2013 samples for stomach analyses were collected at several impact locations within the C-Power wind farm, located on the Thornton bank in the Belgian part of the North Sea and at an adjoining reference location using an 8m shrimp trawl (see chapter 8 ‘Effects of Belgian wind farms on the epibenthos and fish of the soft sediment’).

Per station, a number of specimens of lesser weever (*Echiichthys vipera*), dab (*Limanda Limanda*) and whiting (*Merlangius merlangus*) were collected and injected with formaldehyde (35 %) for preservation. The length of lesser weever varied between 70 and 155 mm and the length of dab varied between 123 and 270 mm. All individuals of both species were subdivided into three length categories: small (S), medium (M) and large (L) (see Table 1). The specimens were stored in formaldehyde (8 %) until analysis.

Table 1. Length categories (in mm) for the two studied fish species, dab (L. limanda) and lesser weever (E. vipera).

	Dab	**Lesser weever**
Small	<151	<101
Medium	151-170	101-130
Large	>170	>130

LABORATORY TREATMENT

The intact stomachs were removed by cutting above the oesophagus and below the large intestine. An incision was made along the longitudinal axis and the contents were emptied on a sieve (0.125 mm), rinsed and put into a Petri dish with a few drops of deionised water. All prey items encountered in the stomachs, were counted and identified using a binocular microscope. Prevailing protocols for accreditation were followed

(BELAC – ISO 16665), using the current determination keys and the correct names based on WoRMS (Vandepitte *et al.* 2010) If possible, prey items were identified to species level. Some prey items were classified into a higher taxonomic level (e.g. order) due to fragmentation or partial digestion.

Both fish and stomach contents were placed into separate vials for further investigation and subsequent drying. After identification, the stomach contents were placed in pre-weighed porcelain or aluminium foil cups, dried at 60°C for 48 hours, weighed, incinerated in ceramic cups at 500°C for 2 hours and cooled to room temperature in a desiccator for 2 hours before weighing again in order to obtain ash weights and to calculate ash free dry weights (AFDW) of the stomach contents. An overview of all the analysed fishes is given in annex 2.

DATA ANALYSIS

Analyses were done for lesser weever and dab but not for whiting since there were not enough control samples for this species. For the analysis of the stomach content data, several indices were used. The fullness index (FI) was used, where S_i is the ash-free dry weight (AFDW) of the stomach content in milligram (mg) and W_i is the ash-free dry weight (AFDW) of the fish (mg).

$$FI = \frac{S_i}{W_i} x100$$

For a number of fishes, only the Wet Weight (WW) was determined. WW of the fish was then converted to AFDW with the common formula AFDW ≈ 20% of WW (Edgar and Shaw, 1995; Van Ginderdeuren, 2013).

Also the percentage of empty stomachs was calculated for each fish species and station.

The frequency of occurrence and numerical percentage of prey items were calculated to characterise the stomach contents (Hyslop, 1980). The frequency of occurrence (*FO%*) calculates the percentage of the total number of stomachs in which a specific prey species occurs where FO_i is the number of stomachs in which the species '*I*' occurs, and FO_t is the total number of full stomachs.

$$FO\% = \frac{FO_i}{FO_t} x100$$

The diet composition was expressed as a numerical percentage (*N%*):

$$N\% = \frac{number\ of\ individuals\ of\ prey\ type\ i}{total\ number\ of\ ingested\ prey\ items} x100$$

The prey species richness in fish stomachs was estimated by S, the number of species in a stomach. The Shannon-Wiener Index H'(log*e*) was used to calculate prey species diversity.

Statistical analyses were performed using the Plymouth routines in multivariate ecological research (PRIMER)e-package + PERMANOVA add-on, version 6.1.6 (Andersen *et al.*, 2007). Prior to multivariate analysis the prey abundance data were standardised (De Crespin de Billy *et al.* 2000) and a similarity matrix was constructed using the Bray-Curtis index of similarity. For the community analysis, the multivariate techniques SIMPER (similarity percentages procedure) and PCA (Principal Component Analysis) were used to investigate the feeding strategy of lesser weever and dab and to highlight the important prey items in their diet.

The statistical analyses are based on the "Before After Control Impact" (BACI)-design (Smith *et al.,* 1993). The analysed factors are "time", "area" and "length category". The factor "time" has two levels: Before *versus* After the construction of the wind farm, also noted as B and A. The "After"-period implies the presence of the 3-dimensional wind farm (from spring 2011 onwards).

The factor "area" also has two levels: Control *versus* Impact, also noted as C and I. An effect of 'time" solely gives an indication of natural temporal variation, both in control and impact areas. An effect of "area" demonstrates natural spatial variation, both before and after the construction of the wind farm. An interaction between "time" and "area" indicates that there is a wind farm effect on the prey density, diversity or species composition. Pair-wise tests then could reveal where the differences are situated.

The factor "length category" has three levels: Small, Medium and Large. Since this factor has no significant effect on prey species diversity, prey species composition nor fullness index, all length categories were pooled for further analyses.

10.3. RESULTS

LESSER WEEVER (*ECHIICHTHYS VIPERA*)

Fullness Index (FI) and % empty stomachs

In general, the percentage of empty stomachs was relatively low, especially after the construction. There were more empty stomachs in the impact area than in the control area, both before (23% *vs.* 15%) and after the construction (16% *vs.* 6%) (Table2).

The fullness index FI ranged between 1.1 (±0.3) (AC) and 1.7 (±0.8) (BC) (Figure 1 left). Although the effect of 'length category' on the fullness index was not significant, the representation per length category (Small, Medium and Large) shows a slightly more detailed picture (Figure 1 right). In general, lesser weever had fuller stomachs in the impact area (I). (Figure 1).

Also the factors 'time' (B/A) and 'area' (C/I) seemed to have no effect on the fullness index of lesser weever.

Table 2. Percentage of empty stomachs of lesser weever, Before (B) and After (A) the construction of the wind farm, in Control (C) and Impact (I) areas.

		% empty stomachs
B	C	15
	I	23,21
A	C	6,38
	I	15,66

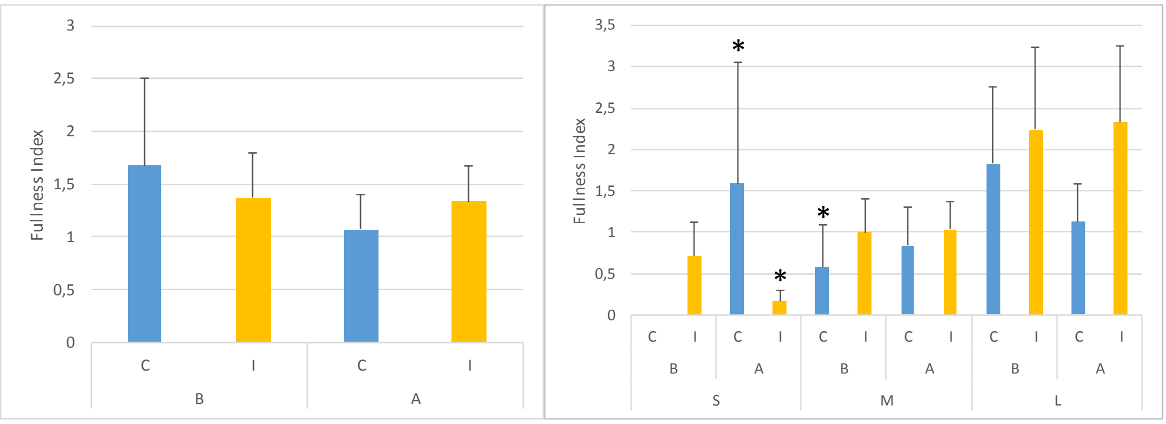

Figure 1. Fullness Index (±SE) for lesser weever (E. vipera), Before (B) and After (A) the construction of the wind farm, in Control (C) and Impact (I) areas (left) and also for small (S), medium (M) and large (L) individuals (right). * encompasses less than five samples for that combination of factors.

Diversity of the diet

The number of species nor the Shannon-Wiener Index H'(loge) was affected by the factors 'time' and 'area' nor by the interaction between those factors. So, no significant differences could be demonstrated between control and impact area, between before and after the construction of the wind farm, nor between any combination of those factors. This implies that the wind farm did not affect the diversity of the diet of lesser weever (Figure 3).

Notable however, is the species *Pisidia longicornis*, a hard-substratum Decapoda which only occurred in the wind farm area, after it was constructed (Figure 2).

Figure 2. *Pisidia longicornis*.

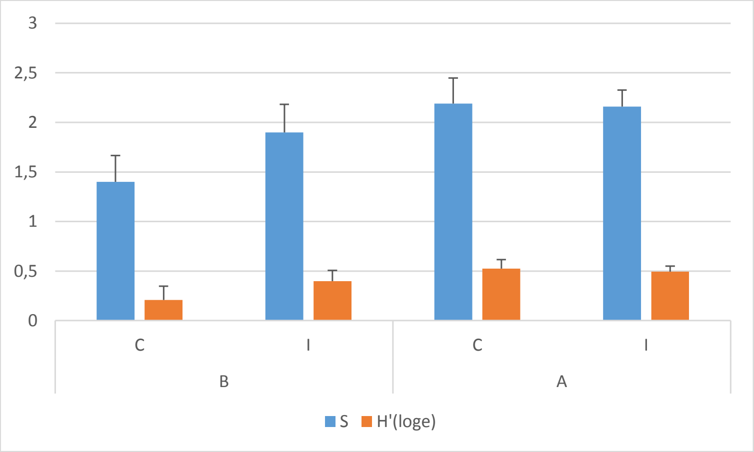

Figure 3. Average number of prey species/stomach S (±SE) and Shannon-Wiener Index H' (±SE) for lesser weever (E. vipera) before (B) and after (A) the construction of the wind farm, in control (C) and impact (I) areas.

Numerical percentage (N%) and Frequency of occurrence (FO%)

Before (B) the construction of the wind farm Decapoda (mainly Brachyura sp.) and Mysida (mainly *G. spinifer* (Figure 5 left) and unidentified Mysidae sp) were numerically the most important prey taxa in the diet of lesser weever (*E. vipera*) (Figure 4). Those taxa were also the most frequently encountered ones (see frequency of occurrence (FO%) (Table 3). After the construction of the wind farm, Decapoda and Mysida were still important prey taxa. However, the Amphipoda became more important, especially in the wind farm (I) were they dominated the diet of lesser weever with a numerical percentage (N%) of 57 % and a frequency of occurrence (FO%) of 79 % (Figure 4 and Table 3). This could mainly be attributed to the dominance (average number of 3±0.47) of the hard substrate Amphipoda *Jassa herdmani* (Figure 5 right) after the construction of the wind farm, both inside (AI) and outside the wind farm area (AC) (Figure 6). Indeed, a significant difference (p=0.01) in numbers of *J. herdmani* could be detected for the factor 'time' but no difference in numbers of *J. herdmani* were found between control and impact area.

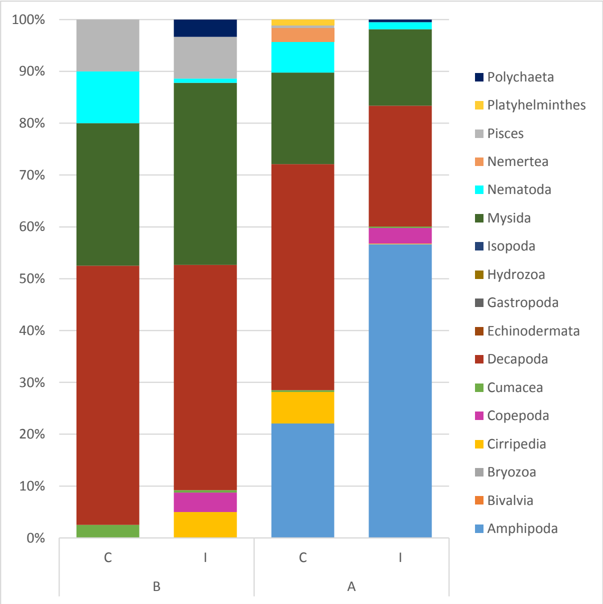

Figure 4. Representation of the diet composition of lesser weever (E. vipera) based on numerical percentages (N%) of prey items, before (B) and after (A) the construction of the wind farm, in control (C) and impact (I) areas.

Table 3. Frequency of occurrence (FO%) of the different prey taxa of lesser weever (E. vipera), Before (B) and After (A) the construction of the wind farm, in Control (C) and Impact (I) areas.

	B		A	
	C	I	C	I
Amphipoda	-	-	29,7	79
Bivalvia	-	-	-	-
Bryozoa	-	-	-	-
Cirripedia	-	6,7	8,1	6,5
Copepoda	-	6,7	-	1,6
Cumacea	10	3,3	-	3,2
Decapoda	60	56,7	67,6	43,6
Echinodermata	-	-	-	-
Gastropoda	-	-	-	-
Hydrozoa	-	-	-	-
Isopoda	-	-	-	-
Mysida	40	50	37,8	40,3
Nematoda	10	3,3	13,5	1,6
Nemertea	-	-	-	-
Pisces	10	23,3	2,7	-
Platyhelminthes	-	-	2,7	-
Polychaeta	-	3,3	-	1,6

Figure 5. *Gastrosaccus spinifer* (left) and the hard-substratum species *Jassa herdmani* (right) © Hans Hillewaert.

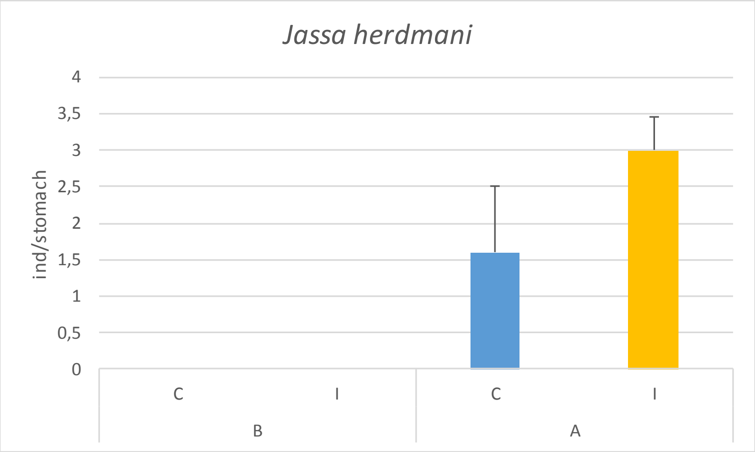

Figure 6. Average number (ind/stomach±SE) of J. herdmani before (B) and after (A) the construction of the wind farm, in control (C) and impact (I) areas.

Community analysis

Statistical analyses revealed several important differences in diet composition of lesser weever, both between control and impact areas (p=0.0004) and before and after construction (p=0.0001). Also, the overall BACI-effect turned out to be significant (p=0.003) which implies that the wind farm had a substantial effect on the diet composition of lesser weever (Table 4).

In the wind farm, the species community differed significantly before and after the construction (BI-AI; p=0.0001). Notable differences were also detected between control and impact areas after the construction (AC-AI; p=0.0001). Both phenomena could mainly be explained by two species: *J. herdmani* and *G. spinifer.* The former was clearly more abundant in the wind farm after the construction (AI) (see first paragraph). The latter however, showed a higher numerical abundance in the wind farm before (BI) than after (AI) the construction and had also a higher abundance after the construction in the control area (AC) than in the impact area (AI).

The PCA-plot illustrates above-mentioned and gives an indication of the most important prey species/taxa (Figure 7), which are also represented in Table 4.

Table 4. p-values for the different factors and their interaction effect on the diet composition of lesser weever (E. vipera) and the characteristic species/taxa for each group (AI= after impact; AC= after control; BI= before impact; BC = before control) based on SIMPER-analyses.

FACTOR	Pair-wise tests	p	Group	Characteristic species/taxa
Time (B/A)		0.0001	AI	*J. herdmani, G. spinifer*
Area (C/I)		0.0004	AC	*J. herdmani, G. spinifer, Processa modica,* Caridea sp.
BA x CI		0.003	BI	Mysidae sp., *G. spinifer,*
	B/A within I	0.0001	BC	*G. spinifer*
	B/A within C	0.09		
	C/I within A	0.0001		
	C/I within B	0.07		

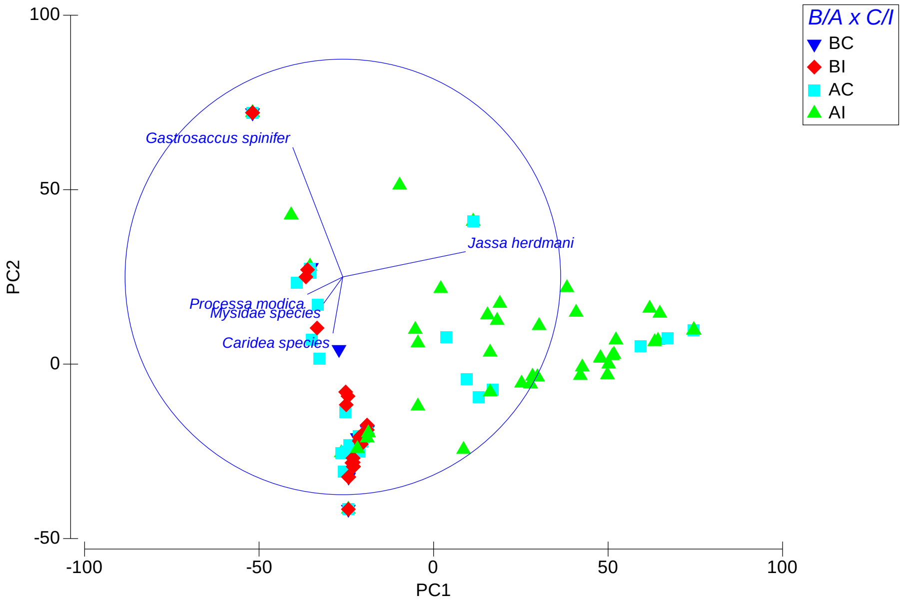

Figure 7. PCA (Principal Component Analysis) plot based on numerical prey abundances of lesser weever (E. vipera) with indication of the most important prey taxa. Axes 1 and 2 explain 24.4% and 13.2% of the total variation respectively.

DAB (*LIMANDA LIMANDA*)

Fullness Index (FI) and % empty stomachs

The percentage of empty stomachs varied between 11 and 18 % and was approximately equal before and after the construction and in control and impact areas (Table 5).

The fullness index varied between 0.03 (±0,01) AC) and 0.29 (±0.11) (BI) (Figure 8 left). The 'length category' again had no significant effect on the fullness index of dab but was visualised in Figure 8 (right) to get a more detailed image. The fullness index was generally higher in the wind farm area (I) than in the control area (C), both before and after the construction (Figure 8). However, this overall difference in fullness index between wind farm and control area was not significant.

The fullness index was lower ‘after’ construction than ‘before' (Figure 8 left). In the impact area however, this was only the case for the ‘large’ individuals (Figure 8 right). Still, the factor ‘time’ was not significant for the interpretation of the fullness index values.

Table 5. Percentage of empty stomachs of dab (L. limanda), Before (B) and After (A) the construction of the wind farm, in Control (C) and Impact (I) areas.

		% empty stomachs
B	C	17,65
	I	11,11
A	C	14,29
	I	15,15

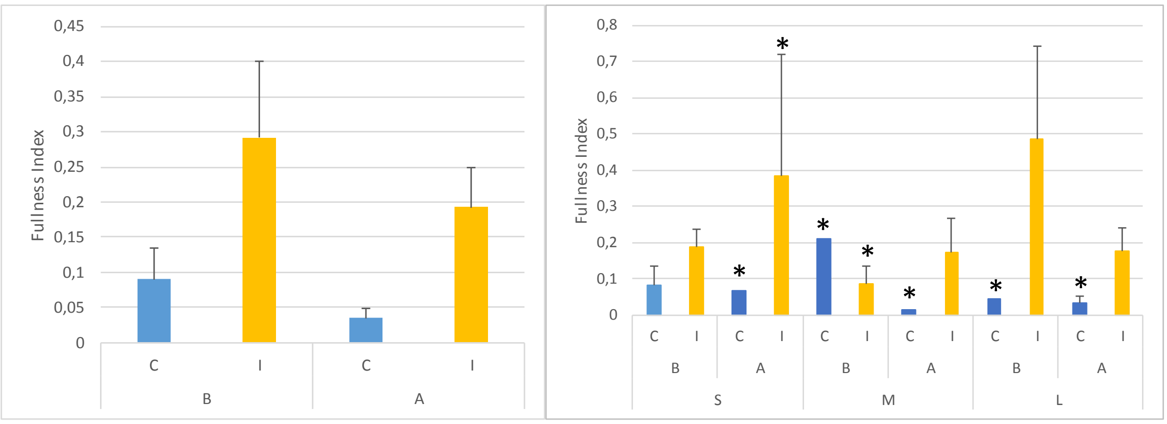

Figure 8. Fullness Index (±SE) for dab (L. limanda) Before (B) and After (A) the construction of the wind farm, in Control (C) and Impact (I) areas (left) and also for small (S), medium (M) and large (L) individuals (right). * encompasses less than five samples for that combination of factors.

Diversity of the diet

The average number of prey species was significantly (p=0.01) higher in the fish stomachs originating from the impact area (S=4±0.5) than in those originating from the control area (2±0.1). The same is true for the Shannon-Wiener diversity index H’ (0,9±0,1 *versus* 0,3±0,1, p= 0.001). This difference was most explicit after the construction of the wind farm (A) (Figure 9).

Species or taxa which only occurred in fish stomachs originating from the wind farm area (I) and were not present in fish stomachs originating from the control area (C) were *N. swammerdamei, L. conchilega, J. herdmani* Calanoida *sp.,* Copepoda *sp.,* Brachyura juvenile, Hydrozoa *sp., Liocarcinus pusillus,* Palaemonidae *sp.,* Eteoninae *sp.,* Gammaridea *sp., Abludomelita obtusata* and Echinodermata.

The factor ‘time’ nor the interaction between ‘area’ and ‘time’ had a significant effect on the prey diversity indices. Still, when comparing ‘Before’ and ‘After’ construction, it seems that the number of prey species has declined in the control area and increased in the wind farm area.

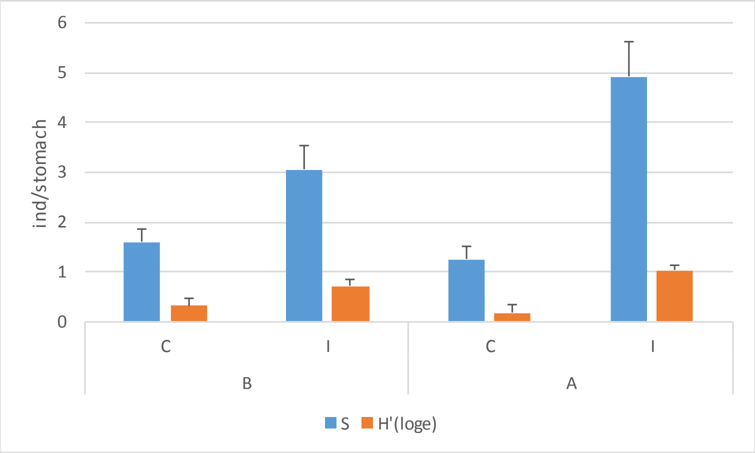

Figure 9. Average number of prey species/stomach S (±SE) and Shannon-Wiener Index H’ (loge) (±SE) for dab (L. limanda) before (B) and after (A) the construction of the wind farm, in control (C) and impact (I) areas.

Numerical percentage (N%) and Frequency of occurrence (FO%)

The diet composition of dab varied a lot, both for the factor ‘area’ (Control/Impact) as for the factor ‘time’ (Before/After). Nematoda were only present before the construction of the wind farm, particularly in the control area (BC) (N%=41.67 and FO%=44.44). Decapoda were numerically the most dominant taxon (N%=48.18) in the impact area before the construction (BI). Also the frequency of occurrence was the highest (FO%=65) of all taxa (Figure 10 and Table 6). Decapoda completely disappeared from the diet of dab in the control zone after construction. Cirripedia occurred solely after construction, especially in the control area (AC) where they were present in half of the stomachs (N%=37.5 and FO%=50) and were of equal importance as the Polychaeta, both numerically as concerning the frequency of occurrence.

The relatively high numerical percentage of Amphipoda in the impact area and the absence of that taxon in the control area is striking (Figure 10 and Table 6), particularly their dominance after the construction (N%=76.92 and FO%=51.68) is remarkable. This difference (between C and I) could particularly be attributed to the relatively high number of the Amphipoda *Nototropis swammerdamei* (5±1.68) in the wind farm (Figure 11). Moreover, there was a significant wind farm effect (BACI-effect) on the numerical abundance of *N. swammerdamei* (p=0.02).

Jassa herdmani (2±1.16) and *Lanice conchilega* (1±0.44) were also important species and occurred only in the wind farm and not in the control area (see previous paragraph and Figure 11 and Figure 12).

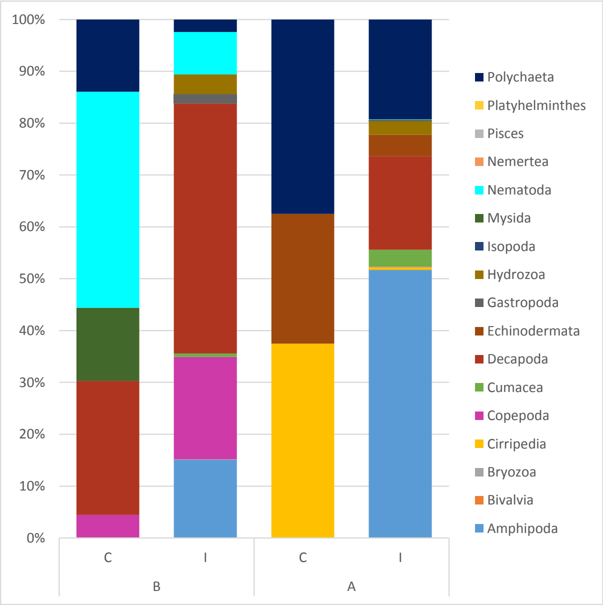

Figure 10. Representation of the diet composition of dab (L. limanda) based on numerical percentages (N%) of prey items, before (B) and after (A) the construction of the wind farm, in control (C) and impact (I) areas.

Table 6. Frequency of occurrence (FO%) of the different prey taxa of dab (L. limanda) Before (B) and After (A) the construction of the wind farm, in Control (C) and Impact (I) areas.

	B		A	
	C	I	C	I
Amphipoda	-	35	-	76,9
Bivalvia	-	-	-	3,9
Bryozoa	-	5	-	-
Cirripedia	-	-	50	7,7
Copepoda	11,1	40	-	3,9
Cumacea	-	5	-	11,5
Decapoda	33,3	65	-	50
Echinodermata	-	-	25	7,7
Gastropoda	-	15	-	-
Hydrozoa	-	20	-	15,4
Isopoda	-	-	-	7,7
Mysida	22,2	-	-	7,7
Nematoda	44,4	20	-	3,9
Nemertea	-	-	-	-
Pisces	-	-	-	-
Platyhelminthes	-	-	-	-
Polychaeta	33,3	10	50	61,5

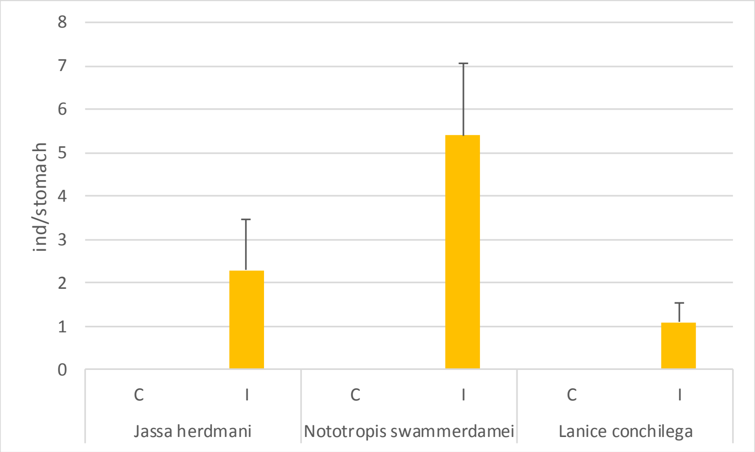

Figure 11. Average number (ind/stomach±SE) of J. herdmani,, N. swammerdamei and L. conchilega in the stomachs of dab (L. limanda) before (B) and after (A) the construction of the wind farm, in control (C) and impact (I) areas.

Figure 12. *Nototropis swammerdamei* (left) and *Lanice conchilega* (right) © Hans Hillewaert (left) and P. Legranche (right).

Community analysis

Statistical analyses of the prey species composition of dab indicated a significant interaction between 'time' (B/A) and 'area' (C/I) (p=0.0001), which implies that the wind farm does have an effect on the prey species composition.

Before the construction of the wind farm, the prey species composition differed significantly in control and impact areas (BC-BI) due to higher abundances of Nematoda and Brachyura in the control and impact area, respectively (p=0.005). After the construction however, the dominance of Cirripedia in the control area and of *N. swammerdamei* in the impact area were responsible for the significant difference between 'areas' (AC-AI) (p=0.0005). Looking into more detail to the impact area, differences in prey species composition before and after the construction (BI-AI) were particularly caused by the dominance of *N. swammerdamei* after the construction (A) and of Brachyura before the construction (p=0.0001) (Table 7). In the control area, Nematoda dominated before the construction, whereas Cirripedia were the most occurring taxon after the construction (AC-BC) (p=0.01)

SIMPER-analyses also revealed the most characteristic species/taxa for each combination of factors which are described in Table 7. The most structuring taxa are also represented in the PCA-plot (Figure 13).

Table 7. p-values for the different factors and their interaction effect on the diet composition of dab (*L. limanda*) and the characteristic prey species/taxa for each group (AI= after impact; AC= after control; BI= before impact; BC = before control) based on SIMPER-analyses.

FACTOR	**Pair-wise tests**	**p**	**Group**	**Characteristic species/taxa**
Time (B/A)		0.0001	AI	*N. swammerdamei*, *L. conchilega*, *J. herdmani*
Area (C/I)		0.0001	AC	Cirripedia sp.
BA x CI		0.0001	BI	Brachyura sp., Paguridae sp., Copepoda sp.
	B/A within I	0.01	BC	Nematoda sp.
	B/A within C	0.0001		
	C/I within A	0.0005		
	C/I within B	0.005		

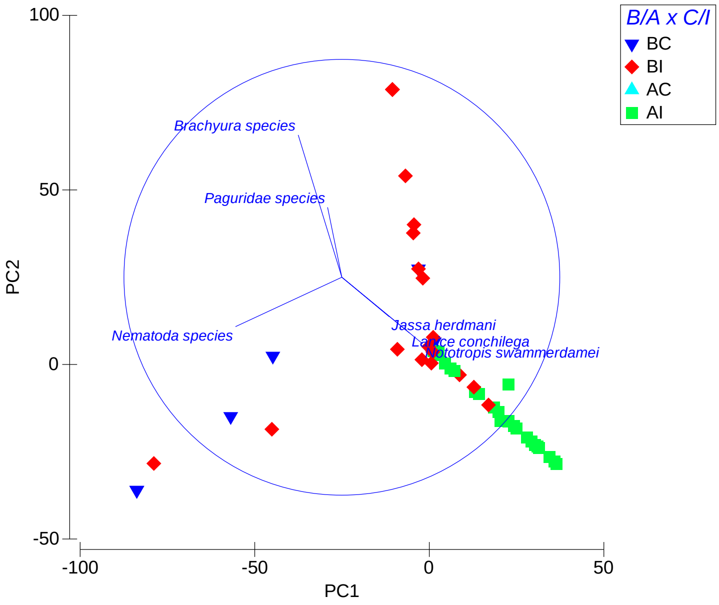

Figure 13. PCA (Principal Component Analysis) plot based on numerical prey abundances of dab (L. limanda) with indication of the most important prey taxa. Axes 1 and 2 explain 15% and 12.2 % of the total variation, respectively.

10.4. DISCUSSION

In this study the feeding behaviour of two demersal fish species (dab *Limanda limanda* and lesser weever *Echiichthys vipera*) was examined in and around the C-Power wind farm located on the Thorntonbank. To investigate whether the wind farm had an effect on the diet of the fishes, stomach content analyses were performed and changes in stomach fullness or diet composition were investigated in a Before After Control Impact (BACI) design.

LESSER WEEVER (*ECHIICHTHYS VIPERA*)

We encountered a relatively low percentage of **empty stomachs** for lesser weever (*E. vipera*). Quiniou (1978), Dauvin (1988), Creutzbert & Witte (1980), Vasconcelos *et al.* (2004) and Derweduwen *et al.* (2012) all found higher percentages of empty stomachs. Different authors noted that the time of sampling may play a role, since the nocturnal activity of lesser weever leads to fuller stomachs towards the morning (Lewis, 1976; Wheeler, 1978 in Vasconcelos *et al.*, 2004). Also the type of prey may influence the stomach fullness (Derweduwen *et al.*, 2012).

No significant differences in **fullness index** could be denoted between the wind farm area and the control area, neither before nor after the construction of the wind farm. This may partially be due to the use of a conversion formula to obtain the ash free dry weight (AFDW) of a number of fishes, leading to a rough estimation of the real AFDW.

The number of prey species and the **prey diversity** in the diet of lesser weever seemed not to be influenced by the wind farm nor by the individual factors ‘time’ and ‘area’. Notable however is that the long clawed porcelain crab *Pisidia longicornis* was found for the first time in the stomachs of lesser weever. This species is a common inhabitant of hard substratum communities (Ingle, 1980; Zintzen *et al.*, 2006; Zintzen *et al.*, 2008b) and abundantly present on the wind turbines almost directly after construction (Kerckhof *et al.*, 2010; De Mesel *et al.*, 2013; De Mesel *et al.*, 2015),

The **diet** of lesser weever in the control and impact area mainly consisted of Brachyura and Mysida, both numerically and concerning the frequency of occurrence. In a previous study, Derweduwen *et al.* (2012) found that lesser weever mostly foraged on Mysida, which was also found by Vasconcelos *et al.* (2004). Other studies also reported that the diet of lesser weever mainly consists of Crustacea, including Decapoda (Creutzberg and Witte, 1989; Quiniou, 1978; Sorbe, 1981; Dauvin, 1988; Collignon and Aloncle, 1960).

After the construction of the wind farm, the importance of Brachyura and Mysida decreased, while Amphipoda emerged in the diet and became the most important prey taxon in the impact area. The species, responsible for this was *Jassa herdmani*. This is the first observation of *J. herdmani* in the diet of any soft substrate demersal fish in and around the wind turbines. In a previous study, this amphipod species was not yet encountered in the fish diet (Derweduwen *et al.*, 2012). *Jassa herdmani* is a dominant species of the epifaunal community on the foundation of wind farms (Kerckhof *et al,* 2010; Kerckhof *et al.*, 2012; De Mesel *et al*, 2013; De Mesel *et al.,* 2015) . Based on the studies of Bergström *et al.* (2012, 2013) and Wilhelmsson *et al*. (2006), Vandendriessche *et al.* (2015) hypothesized that increases or changes in density, biomass, diversity, or community structure of the soft sediment communities between the turbines would remain limited or that it would take a long time before the reef effect expands into the sandy space between the turbine rows. Given the dominance of *J. herdmani* both on the hard substrate and in the diet of lesser weever (and dab, see further) and the relatively high abundance (up to 809 ind/m^2) of the species on the soft sediment near the turbines (Coates *et al.*, 2013), we can conclude that - at least to some extent - the reef effects already did expand into the soft sediments between and beyond the wind mills, *circa* 200 m from the turbines.

There was a substantial wind farm effect on the **prey species composition** of lesser weever which was mainly caused by two species: *J. herdmani* and *Gastrosaccus spinifer*. The Mysida, including *G. spinifer*, were dominant in the fish stomachs from both areas before the wind farm was constructed. Coates *et al.* (2016) also found high abundances (up to 42 ind/m² after construction) of *G. spinifer* in the soft sediment near the turbines, but only in the wind farm area and not in the control area.

The high number of *J. herdmani* encountered in the fish stomachs from the wind farm after construction was responsible for the community differences between the control and wind farm area after construction. The species is a highly available prey item in the wind farm with densities of more than 10 000 ind/m^2 on the turbines (De Mesel *et al.*, 2015) and up to 809 ind/m² in the soft sediment near the turbines (Coates *et al.*, 2013). Hyslop *et al.* (1980) and De Crespin *et al.* (2000) stated that the total abundance of prey items in a stomach depends on food availability and the prey digestion rate, but also that the hierarchical interactions among predators should be kept in mind. So, it is very likely that the dominance of *J. herdmani* in the diet of lesser weever originating from the wind farm, is due to the fact that this amphipod is a very abundant and thus easily accessible prey species in the soft sediment around the turbines. Furthermore, Lindeboom *et al.* (2011) and Vandendriessche *et al.* (2015) observed a decrease of lesser weever in the wind farm compared to the control area which may have led to a decrease in intraspecific competition and hence a relatively higher food availability. *Jassa herdmani* was also regularly found in the fish stomachs from the control area which might indicate that the wind farm effect is expanding into the surrounding area.

DAB (*LIMANDA LIMANDA*)

The percentage of **empty stomachs** of dab (*L. limanda*) was relatively low.

The **fullness index** showed some differences between control and impact area, before and after the construction of the wind farm. The fullness index was higher in the impact area - which is in accordance to our previous diet study (Derweduwen *et al.,* 2012) - and lower after the construction, both in impact and control area. However, none of these differences were significant. So, it can be stated that the observed differences in fullness index are not caused by the presence of the wind farm and the altered surrounding habitat.

The number of prey species and the **prey diversity** in the diet of dab was significantly higher in the impact area compared to the control area. Since this higher diversity in the impact area was already present before the wind farm was constructed, we may expect no overall wind farm effect. However, the non-parallelism in number of prey species - with a higher number of prey species in the impact area after construction and a lower number in the control area after construction - indicates that the wind farm does have an effect on species richness.

The following studies may help explain this increased diversity of the diet of dab. As a well-known ecosystem-engineer, the tube-building polychaete *Lanice conchilega* has the potential to increase habitat complexity and heterogeneity (Rabaut *et al.*, 2007; Van Hoey *et al.*, 2008). Both authors also indicated a significant and positive correlation between the macrobenthic abundance, diversity and biomass with increasing densities of *L. conchilega.* Furthermore, Coates *et al.* (2013) found increased densities of *L. conchilega* in the C-Power wind farm and demonstrated its dominance close to the turbines. So, the combination of the ecosystem-engineer capacities of *L. conchilega* and its increased density in the wind farm, might explain the higher diversity of the diet of dab observed in the wind farm.

A few other encountered taxa/species of which the distribution was limited to the wind farm area, were Hydrozoa and the dwarf swimming crab *Liocarcinus pusillus*, the former is a typical hard-substratum taxon (Kerckhof *et al.*, 2010; De Mesel *et al.*, 2013; De Mesel *et al.*, 2015) and the latter likes coarser sediments (Froglia and Manning, 1982) and was already found in the wind farm area (Derweduwen *et al.*, 2012). Two other typical hard-substratum species, *Phtisica marina* and *P. longicornis* (Kerckhof *et al.*, 2010; De Mesel *et al.*, 2013; De Mesel *et al.*, 2015) were hardly or not encountered in the stomachs, although they have been found previously (Derweduwen *et al.*, 2012).

The **diet** of dab strongly varied between areas (control and impact) and before and after construction. Most dietary studies of dab have classified the species as a general feeder with a relatively wide prey spectrum (Hinz *et al.*, 2005).

The **prey species composition** in the diet was significantly affected by the wind farm. Before the construction of the wind farm, a similar taxon composition in control and impact area could be observed but with different proportions. The appearance of Nematoda is notable and the taxon did not yet occur in our previous diet study (Derweduwen *et al.,* 2012). It is not clear whether the Nematoda are ingested preys or free-living parasites in the stomachs of dab. Significant differences between control and impact area could mainly be attributed to Nematoda and Brachyura. Also Mysida - only present in the control area - and Amphipoda - only present in the impact area - contributed to these differences. After the construction of the wind farm, a completely different picture emerged for the taxon composition in the control area, which mainly can be explained by the higher abundance of Nematoda before construction and of Cirripedia after construction. The taxon composition in the impact area after and before construction differed, particularly in altered proportions, i.e. more Brachyura before and more Amphipoda after construction. Differences between control and impact were particularly caused by higher abundances of Cirripedia - which also did not occur in our previous study (Derweduwen *et al.,* 2012) - in the control area and higher abundances of the Amphipoda *Nototropis swammerdamei* in the impact area. The presence of Amphipoda in the wind farm area was already described in Derweduwen *et al.* (2012). However, its presence has evolved into a dominance in the impact area after the construction of the wind farm.

Coates *et al.* (2013) already revealed altered macrofaunal communities in close vicinity to a wind turbine foundation. These altered macrofaunal communities can partly elucidate the observed changes in prey species composition in the diet of dab. However, the most abundant prey species in the stomachs from the wind farm area – *N. swammerdamei* - only occurred on the hard substratum of the foundations (De Mesel *et al.*, 2013) and did not on the soft sediment (Coates *et al.*, 2013). The second and third most important species in the wind farm area – *L. conchilega* and *J. herdmani* – did occur on the soft sediment (Coates *et al.*, 2013), the latter to a lesser extent more than 15 m away from the turbines. Due to the opportunistic feeding strategy of dab, this species can be expected to be highly adaptable in respect to habitat and ecosystem change (Hinz *et al.*, 2005) and may profit from the wind farm area as a new habitat with its associated fauna. It is most likely that dab not only was foraging on the soft sediment but also actively foraged on the hard substratum. Since several hard substratum species (i.e. *L. pusillus* and *N. swammerdamei*) were already found in the stomachs from the impact area before the wind farm was constructed, the occurrence of those hard-substratum species cannot exclusively be explained by the wind farm.

So, it seems that the feeding behaviour of dab is not only influenced by the presence of the wind farm. There are other factors playing in the impact area and in the control area, independently of the construction of the wind farm. However, the construction of the wind farm probably has enlarged the observed effects.

FUTURE RESEARCH

For future research, it is recommended to analyse a larger number of stomachs to increase the statistical power. For example, the wind farm effect for dab might have been proven statistically significant if the number of ‘After Control’ samples was higher. Secondly, we could not yet analyse the diet of whiting, another commercially important fish species, due to the limited number of individuals in the control area. The main reasons for these limitations were the limited number of beam trawl samples that could be taken within the foreseen ship time and the logistic problems encountered during sampling in and around the wind concession zones.

10.5. CONCLUSION

Lesser weever showed no significant differences in fullness index, between control and impact areas, nor before and after construction. Also the diversity of the diet was not affected by the presence of the wind farm. However, the diet composition did change: lesser weever consumed significantly more of the species *Jassa herdmani* both in the control and to a greater extent in the impact area and less of the mysid *Gastrosaccus spinifer* and Brachyura. This amphipod species *J. herdmani* is typically associated with hard substrates and was highly available in the wind farm (De Mesel *et al.*, 2015). This was the first record of *J. herdmani* to be found in the diet of a demersal fish species in this area.

The fullness index of dab also displayed no significant differences. Although the impact values were slightly higher, both before and after construction, the fullness index decreased after construction, both in impact and control areas. The diversity and the composition of the diet of dab were influenced by the presence of the wind farm. The number of prey species was higher in the impact than in the control area, after construction. Species that were responsible for these differences were *Nototropis swammerdamei, J. herdmani* and *Lanice conchilega.* The latter species is a well-known ecosystem-engineer with the potential to enhance habitat complexity and heterogeneity. Its presence might have led to a significant higher prey diversity in the wind farm and hence in the stomachs of dab. The prey species composition of dab was variable since dab is known as an opportunistic feeder (Hinz *et al.*, 2005). Still, some differences were observed that could be related to the presence of the wind farms. However, since some of the hard-substratum prey species found in the impact area did already occur before the wind farm was constructed, other factors must play a role.

To summarise, we can state that the observed differences in prey diversity and diet composition of the fish in the wind farm and the direct vicinity of the wind concession zone are clearly induced by the presence of the hard substrates of the wind farm and its associated fauna. Consequently, it can be stated that the reef effect, related to the introduction of hard substrates (and its attraction for associated hard sub fauna), is expanding into the surrounding soft sediments.

REFERENCES

Anderson, M.J., Gorley, R.N. & Clarke, K.R. (2007). PERMANOVA+for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth: 214 pp.

Bergstrom, L., Sundqvist, F., Bergstrom, U. (2012). Effekter av en havsbaserad vindkraftpark pa fordelningen av bottennara fisk. Naturvardsverket, 39p, ISBN 978-91-620-6485-3.

Bergström, L., Sundqvist, F., & Bergström, U. (2013). Effects of an offshore wind farm on temporal and spatial patterns in the demersal fish community. *Marine Ecology Progress Series*, *485*, 199-210.

Berkenhagen, J., Döring, R., Fock, H.O., Kloppmann, M.H.F., Pedersen, S.A. & Schulze, T. (2010). Decision bias in marine spatial planning of offshore wind farms: Problems of singular versus cumulative assessments of economic impacts on fisheries. Marine policy, Band 34 (3), 733-736.

Coates, D. A., Deschutter, Y., Vincx, M., & Vanaverbeke, J. (2013) Macrobenthic enrichment around a gravity based foundation. *Degraer, S., R. Brabant, B. Rumes (eds)*.

Coates, D. A., Deschutter, Y., Vincx, M., & Vanaverbeke, J. (2014). Enrichment and shifts in macrobenthic assemblages in an offshore wind farm area in the Belgian part of the North Sea. *Marine environmental research*, *95*, 1-12.

Coates, D. A., Kapasakali, D. A., Vincx, M., & Vanaverbeke, J. (2016). Short-term effects of fishery exclusion in offshore wind farms on macrofaunal communities in the Belgian part of the North Sea. *Fisheries Research*, *179*, 131-138.

Collignon, J.; Aloncle, H., 1960: Le re´gime alimentaire de quelques poissons benthiques des coˆ tes marocaines. Bull. Inst. Pêches Marit. Maroc. 5, 17–29.

Creutzberg, F.; Witte, J. I. J., 1989: An attempt to estimate the predatory pressure exerted by the lesser weever, Trachinus vipera Cuvier, in the southern North Sea. J. Fish Biol. 34, 429–449.

Dauvin, J. C., 1988: Rôle du macrobenthos dans l'alimentation des Poissons de´mersaux vivant sur les fonds de sediments fins de la Manche occidentale. Cah. Biol. Mar. 29, 445–467.

De Crespin de Billy, V., Doledec, S., Chessel, D., 2000. Biplot presentation of diet composition data: an alternative for fish stomach contents analysis. J. Fish Biol. 56, 961–973.

Degraer, S. (2014, March). Environmental impacts of offshore wind farms in the Belgian part of the North Sea: Learning from the past to optimize future monitoring programmes. In *BOOK OF ABSTRACTS*.

De Mesel, I., Kerckhof, F., Rumes, B., Norro, A., Houziaux, J. S., & Degraer, S. (2013). Fouling community on the foundations of wind turbines and the surrounding scour protection. *Degraer, S., R. Brabant, B. Rumes (eds)*.

De Mesel, I., Kerckhof, F., Norro, A., Rumes, B. & Degraer, S. (2015). Succession and seasonal dynamics of the epifauna community on offshore wind farm foundations and their role as stepping stones for non-indigenous species. *Hydrobiologia* (2015): 1-14

Derweduwen, J., Vandendriessche, S., Willems, T. & Hostens, K., (2012). The diet of demersal and semi-pelagic fish in the Thorntonbank wind farm: tracing changes using stomach analyses data. pp. 73-84. In Degraer, S., Brabant, R. & Rumes, B., (Eds.) (2012). Offshore wind farms in the Belgian part of the North Sea: Heading for an understanding of environmental impacts. Royal Belgian Institute of Natural Sciences, Management Unit of the North Sea Mathematical Models, Marine ecosystem management unit. 155 pp. + annexes..

Edgar, G. J., and Shaw, C. 1995. The production and trophic ecology of shallow-water fish assemblages in southern Australia, II: diets of fishes and trophic relationships between fishes and benthos at Western Port, Victoria. Journal of Experimental Marine Biology and Ecology, 194: 83–106.

Froglia, C. A. R. L. O., & Manning, R. B. (1982). Notes on *Liocarcinus pusillus* (Leach) and related species. *Quad. Lab. Tecnol. Pesca*, *3*(2-5), 257-266.

Gray, J.S., 2006. Minimizing environmental impacts of a major construction: the Oresund Link. Integrated Environmental Assesment and Management. 2(2) 196-199.

Hinz, H., Kröncke, I., & Ehrich, S. (2005). The feeding strategy of dab *Limanda limanda* in the southern North Sea: linking stomach contents to prey availability in the environment. *Journal of Fish Biology*, *67*(sB), 125-145.

Hyslop, E.J. (1980). Stomach contents analysis – a review of methods and their application. J. Fish Biol. 17, 411-429

Ingle, R.W., 1980. British crabs. London, British Museum (Natural History), 222p

Jensen, A., 2002. Artificial reefs in Europe: Perspective and future. ICES Journal of Marine Science 59, 3-13

Kaiser, M. J., & Ramsay, K. (1997). Opportunistic feeding by dabs within areas of trawl disturbance: possible implications for increased survival. *Marine Ecology Progress Series*, (1-3).

Kerckhof, F., Norro, A., Jacques, T., & Degraer, S. (2009). Early colonisation of a concrete offshore windmill foundation by marine biofouling on the Thornton Bank (southern North Sea). *Offshore wind farms in the Belgian part of the North Sea: State of the art after two years of environmental monitoring, 39Ŕ51*.

Kerckhof, F., Rumes, B., Norro, A., Jacques, T.G. & Degraer, S. (2010) Seasonal variation and vertical zonation of the marine biofouling on a concrete offshore windmill foundation on the Thornton Bank (southern North Sea). In Degraer, S., Brabant, R. & Rumes B. (Eds.) (2010). Offshore wind farms in the Belgian Part of the North Sea: Early environmental impact assessment and spatio-temporal variability. Royal Belgian Institute of Natural Sciences, Management Unit of the North Sea Mathematical Models. Marine ecosystem management unit. 184 pp. + annexes.

Kerckhof, F., Rumes, B., Norro, A., Houziaux, J.-S., Degraer, S. (2012). A comparison of the first stages of biofouling in two offshore wind farms in the Belgian part of the North Sea. In: Degraer, S., Brabant, R., Rumes, B., (Eds.). 2012. Offshore wind farms in the Belgian part of the North Sea: Heading for an understanding of environmental impacts. Royal Belgian Institute of Natural Sciences, Management Unit of the North Sea Mathematical Models, Marine Ecosystem Unit. pp. 17-39.

Lincoln, R. J. (1979). British marine amphipoda: Gammaridea (No. 818). British Museum (Natural History).

Lindeboom, H.J., H.J. Kouwenhoven, M.J.N. Bergman.,S. Bouma, S. Brasseur, R. Daan, R.C. Fijn, D. de Haan, S. Dirksen, R. van Hal, R. Hille Ris Lambers, R. ter Hofstede, K.L. Krijgsveld, M. Leopold, M. Scheidat, 2011. Short-term ecological effects of an offshore wind farm in the Dutch coastal zone; a compilation. Environmental Research Letters 6, 035101.

Marine Species Identification Portal (http://species-identification.org/)

May, J. (2005). Post-construction results from the North Hoyle offshore wind farm. Paper for the Copenhagen offshore wind international conference, Project Management Support Services Ltd, 10pp.

Petersen, J. K., Malm, T. (2006). Offshore Windmill Farms: Threats to or Possibilities for the Marine Environment. Ambio Journal of the Human Environment 35(2), 75-80.

Quiniou, L., 1978: Les poisons démersaux de la baie de Douarnenez. Alimentation et écologie. Thèse 3e cycle, Océanogr. Biol., Univ. Brest, Brest, France, 222 p.

Rabaut, M., Guilini, K., Van Hoey, G., Magda, V., Degraer, S. (2007). A bio-engineered soft-bottom environment: the impact of Lanice conchilega on the benthic species-specific densities and community structure. Estuar. Coast. Shelf Sci. 75, 525e536.

Reubens, J.T.; Degraer, S.; Vincx, M. (2011). Aggregation and feeding behaviour of pouting (*Trisopterus luscus*) at wind turbines in the Belgian part of the North Sea. *Fisheries Research 108(1)*: 223-227.

Reubens, J. T., Vandendriessche, S., Zenner, A. N., Degraer, S., & Vincx, M. (2013). Offshore wind farms as productive sites or ecological traps for gadoid fishes?–Impact on growth, condition index and diet composition. *Marine environmental research*, *90*, 66-74.

Rijnsdorp, A. D., & Vingerhoed, B. (2001). Feeding of plaice *Pleuronectes platessa L*. and sole *Solea solea (L.)* in relation to the effects of bottom trawling. *Journal of Sea Research*, *45*(3), 219-229.

Schückel, S., Sell, A., Kröncke, I., & Reiss, H. (2011). Diet composition and resource partitioning in two small flatfish species in the German Bight. *Journal of Sea Research*, *66*(3), 195-204.

Smith, E.P., Orvos, D.R., Cairns, J. (1993). Impact assessment using the before-after-control-impact (BACI) model: concerns and comments. Canadian Journal of Fisheries and Aquatic Sciences 50, 627-637.

Sorbe, J. C., 1981: Rôle du benthos dans le régime alimentaire des poissons demersaux du secteur Sud-Gascogne. Kieler Meeresforsch. Sonderh. 5, 479–489.

Vandendriessche, S., Reubens, J., Derweduwen, J., Degraer, S., & Vincx, M. (2013). Offshore wind farms as productive sites for fishes? Degraer, S., Brabant, R., & Rumes, B.(2013). Environmental Impacts of Offshore Wind Farms in the Belgian Part of the North Sea: Learning From the Past to Optimise Future Monitoring Programs. Royal Belgian Institute of Natural Sciences, Brussels, 153-161.

Vandendriessche, S., Derweduwen, J., & Hostens, K. (2015). Equivocal effects of offshore wind farms in Belgium on soft substrate epibenthos and fish assemblages. *Hydrobiologia*, *756*(1), 19-35.

Vandepitte L., Decock, W. & Mees J. (Eds.) (2010). Belgian Register of Marine Species, compiled and validated by the VLIZ Belgian Marine Species Consortium. VLIZ Special Publication, 46. Vlaams Instituut voor de Zee (VLIZ): Oostende, Belgium. 78 pp. ISBN 978-90-812900-8-1. Online available at http://www.marinespecies.org/berms/

Van Ginderdeuren, K. (2013). Zooplankton and its role in North Sea food webs: community structure and selective feeding by pelagic fish in Belgian marine waters (Doctoral dissertation, Ghent University).

van Hal, R., Couperus, A. S., Fassler, S. M. M., Gastauer, S., Griffioen, B., Hintzen, N. T., ... & Winter, H. V. (2012). *Monitoring-and Evaluation Program Near Shore Wind farm (MEP-NSW): Fish community* (No. C059/12, p. 161). IMARES.

Van Hoey, G., Guilini, K., Rabaut, M., Vincx, M., & Degraer, S. (2008). Ecological implications of the presence of the tube-building polychaete Lanice conchilega on soft-bottom benthic ecosystems. *Marine Biology*, *154*(6), 1009-1019.

Vasconcelos, R., Prista, N., Cabral, H. and Costa, M. J. (2004), Feeding ecology of the lesser weever, *Echiichthys vipera* (Cuvier, 1829), on the western coast of Portugal. Journal of Applied Ichthyology, 20 (2004): 211–216.

Wilhelmsson, D., Malm, T. & Öhman, M.C. (2006). The influence of offshore windpower on demersal fish. ICES Journal of Marine Science 63(5): 775–784.

Zintzen, V., Massin, C., Norro, A., & Mallefet, J. (2006). Epifaunal inventory of two shipwrecks from the Belgian Continental Shelf. *Hydrobiologia*, *555*(1), 207-219.

Zintzen, V., Norro, A., Massin, C., Mallefet, J., 2008b. Spatial variability of epifaunal communities from artificial habitat: Shipwrecks in the Southern Bight of the North Sea. Estuarine, Coastal and Shelf Science 76, 327-344.