CHAPTER 9

WIND FARMS AND THEIR INFLUENCE ON THE OCCURRENCE OF ICHTHYOPLANKTON AND SQUID LARVAE

S. Vandendriessche[*], A. M. Ribeiro da Costa & K. Hostens

Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Animal Sciences, Aquatic Environment and Quality, Ankerstraat 1, 8400 Oostende, Belgium.

[*]Corresponding author: Sofie.Vandendriessche@ilvo.vlaanderen.be

ABSTRACT

Changes in the seafloor structure induced by the introduction of wind farms are expected to influence fish populations depositing their eggs on the seafloor, since they are known to require a specific substrate for spawning. Hard substrate creation can also influence egg deposition opportunities for invertebrates, such as the commercially important cuttlefish and different species of squids. So, wind farm construction is expected to have positive effects on fish and invertebrate species that require hard substrates for spawning, and this is expected to manifest as higher densities of early life stages at the hard substrates (eggs) and in the water column (larvae) at the wind farms. This was investigated at the Thorntonbank wind farm by repeatedly sampling three impact

stations and three reference stations with a Bongo net from 2010 to 2013. The results do not show significant effects of the wind farm on fish eggs, fish larvae and squid larvae. Nevertheless, the data provide good baseline information about ichthyoplankton and squid

larvae at offshore stations that can be used in future monitoring. When planning future monitoring activities at this site, adaptations to the design should be incorporated and alternative methods for quantifying squid larvae should be considered.

9.1. INTRODUCTION

With the establishment of the 2020 targets by the EU, wind farms have been constructed throughout the North Sea, with more than 500 turbines being foreseen just in the Belgian part. Changes in the seafloor structure induced by the introduction of wind farm turbines are expected to influence fish populations depositing their eggs on the seafloor, since they are known to require a specific substrate for spawning. Hard substrate creation can also influence egg deposition opportunities for invertebrates, such as the commercially important cuttlefish and different species of squids. So, wind farm construction is expected to have positive effects on fish and invertebrate species that require hard substrates for spawning, and this is expected to manifest as higher densities of early life stages at the hard substrates (eggs) and in the water column (larvae) at the wind farms.

Herring is one of the species which is expected to benefit from hard substrate addition since it requires rocks, vegetation or gravel for spawning. In the Belgian part of the North Sea (BPNS) the known spawning grounds of *Clupea harengus* have disappeared, but the introduction of wind turbines in the area may trigger their recovery and even establish new ones (Di Marcantonio *et al.,* 2006). In addition to fish species, squids (Cephalopoda) are also expected to increase

in density with the introduction of wind farms since they require hard substrate for spawning and they usually deposit their eggs on rocky bottoms (e.g. loliginid species, Hastie *et al.,* 2009). Some of the species previously observed in the North Sea include *Sepia officinalis*, *Sepiola atlantica, Loligo vulgaris* and *Allotheutis subulata.* Several monitoring reports concerning the effects of wind farms on marine biota have included observations regarding (adult) squids. A short-term decrease in squid density (Degraer, 2014; Vandendriessche *et al.,* 2013b) and a long-term increase, although limited, in species richness have been reported (Lindeboom *et al.,* 2011 in Rumes *et al.,* 2013). Nonetheless, there is still a need to increase the information available regarding the effects of wind farm construction on this group. Their increasing importance for fisheries and as food for other organisms make them important targets for research.

The expected effects of hard substrate creation at wind farms on spawning activities of fish and squids have triggered a study to (a) gather baseline information on the composition and variability concerning ichthyoplankton and squid larvae at offshore stations and (b) determine whether wind farms influence the density of ichthyoplankton and squid larvae.

9.2. METHODS

A total of six stations were sampled on the Thorntonbank, three within the C-Power concession area (impact) and another three in the reference area (control). The positions of the stations were approximate and were slightly adapted based on weather conditions and safety guidelines given by the wind farm companies. At the impact stations, we tried to obtain a minimal distance to the nearest turbine, thereby respecting the safety buffer of 500m .
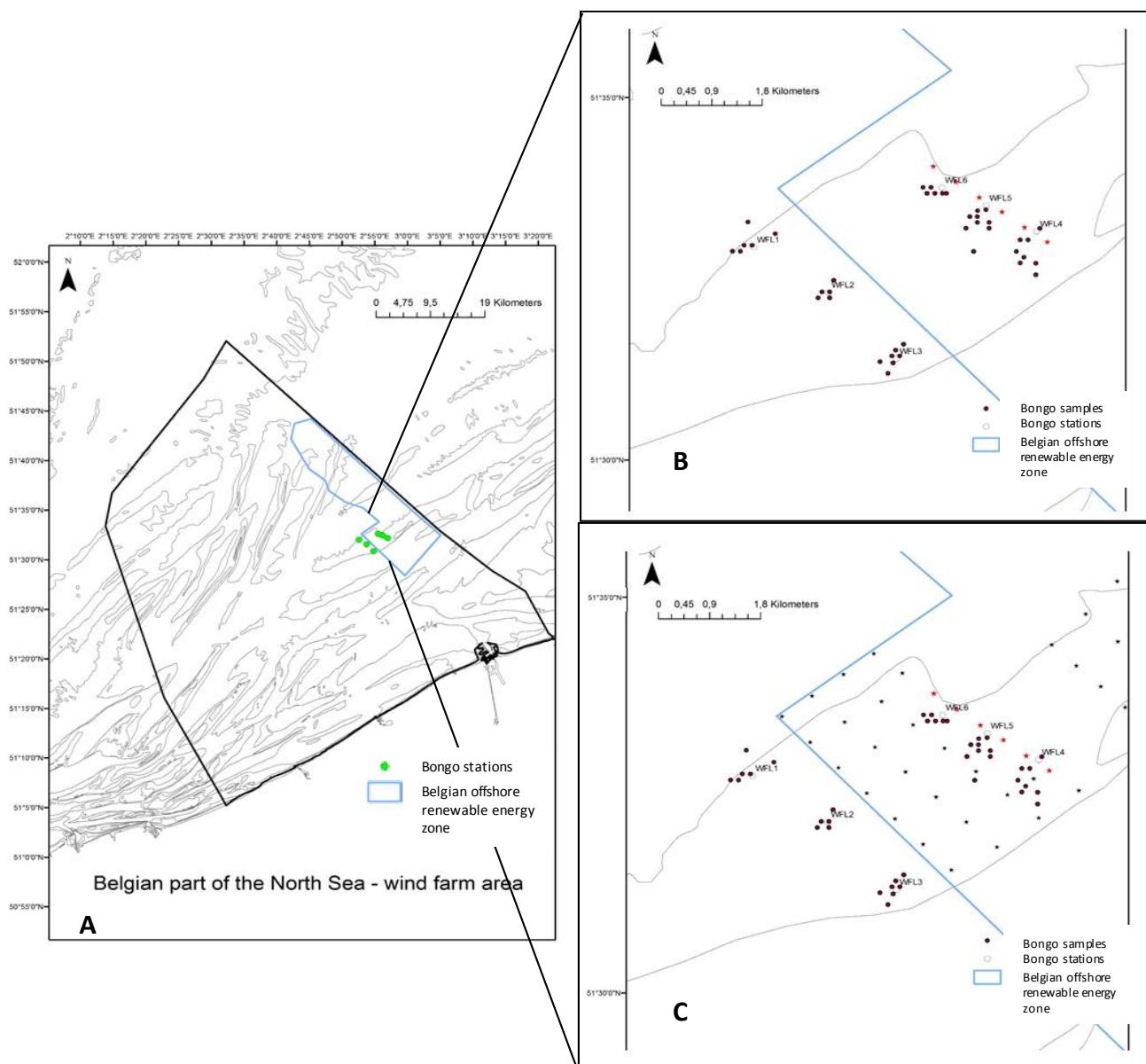

Figure 1. A) Overview of the Belgian part of the North Sea (BPNS) with the delimitation of the Belgian offshore renewable energy zone (blue) and the six sampling stations (green) at the Thorntonbank; B) detailed location of sampling stations, both in the reference (WFL1, WFL2, WFL3) and impact (WFL4, WFL, WFL 6) areas in the beginning of the study period; C) detailed location of the same sampling stations in the end of the study period, with the newly installed foundations(small black dots).

Figure 2. Bongo nets

Sampling was carried out from 2010 until 2013, on board of the research vessels Zeeleeuw and Belgica. Samples were taken opportunistically: monthly when possible, but at least twice a year (in spring and autumn). At each station, a Bongo net (Figure 2) was deployed, fitted with 500 µm mesh nets, a fly weight and a flow meter (Smith *et al.,* 1968). At first, the nets were towed in an oblique continuous haul from the bottom to the surface, but this occasionally resulted in a very small filtered water volume. Consequently, we adapted the sampling method to 10 min undulating tows from March 2011 onwards. At each station, a CTD was used to obtain vertical profiles of temperature and salinity and to assess the level of water mixing. Turbidity was measured with a Secchi disk and chlorophyll *a* (Chl a) data were obtained from fixed fluorimeters on board of the research vessels Zeeleeuw and Belgica. Missing data were obtained from Modis/Meris satellite data [3] and from Van Ginderdeuren (2013).

All samples were rinsed on board and transferred into a 4% formaldehyde solution, since this allows for the best fixation of the melanophores (essential in identification), and body morphology (Munk and Nielsen, 2005). In the laboratory, samples were transferred to an ethanol solution for lab analyses. Using a Leica® stereomicroscope, all ichthyoplankton and squids (Cephalopoda) were identified to the lowest possible taxonomical level. Identification was done with the aid of Munk *et al.* (2005), Russell (1976) and Sweeney *et al* (1992). All larval individuals were identified and counted. When larvae were very abundant (more than 100 individuals of the same family), a subsample of 100 individuals was identified per larval stage and extrapolated. Fish eggs present in the samples were counted. The pseudoreplicates of each haul were kept separate during processing, but the results were averaged before analysis.

The obtained dataset was highly asymmetric, since not all seasons were analyzed in each year and the number of sampling events was not uniform, giving rise to an unbalanced design. Statistical analyses were performed using the package PRIMER 6 with PERMANOVA (Clarke 1993, Clarke and Gorley 2006). In first instance, the impact of wind farms was tested using a crossed design including control/impact and sampling event. Temporal patterns were then further explored using season and year as factors. In all analyses, a type III sum of squares (SS) was used to correct for an unbalanced design. In case of significant interaction effects, pairwise tests were done to detect the specific differences between groups. When the number of unique permutations in the output file turned out to be lower than 100, the Monte Carlo test option was selected *a priori*.

Community analysis was done on fish larvae data only; data of fish eggs and squid larvae were subjected to univariate analyses only. The larval density data were fourth-root transformed and a zero-adjusted Bray-Curtis similarity matrix (Bray and Curtis, 1957) was constructed.

In terms of biodiversity analysis, the DIVERSE function was used on raw density data. Species richness was obtained by calculating the number of species (S) and the expected number of species ES(50), while the Shannon-Wiener index (H', $\log_e$) was used to calculate species diversity. Although the latter is known to be sensitive to sample size, it was maintained to allow further comparisons with previous studies. Spatial and temporal differences for species diversity were also verified using PERMANOVA, based on Euclidean distance resemblances.

The environmental variables (water temperature, Chl a, salinity and secchi depth) were correlated with the patterns in species composition and abundance. The first step was to normalize the abiotic data and to check for collinearity using a draftsman plot. A Euclidean distance resemblance matrix was drafted and distance-based linear models (DistLM; step-wise model with BIC criteria) were used to see which predictor variables (or combination of) best explained the data patterns.

9.3. RESULTS

GENERAL RESULTS

The 66 samples analyzed in this study were spread over different years and seasons. The data were consequently unbalanced, with information from 8 different months (see table 1). Within each sampling event, samples were collected in both the control (reference) and the impacted areas (wind farm).

Table 1. Description of the included sampling events. During each event, three stations were sampled per location (reference and control).

Year	Month	Season	Cruise
2010	March	Winter	Zeeleeuw 10-090
2010	April	Spring	Zeeleeuw 10-210
2010	June	Spring	Zeeleeuw 10-310
2010	July	Summer	Zeeleeuw - 10-410
2010	September	Summer	Belgica 2010/25b
2010	December	Autumn	Zeeleeuw 10-750
2011	January	Winter	Zeeleeuw - 11/050
2012	January	Winter	Belgica 2012/01
2012	February	Winter	Belgica 2012/6a
2013	March	Winter	Belgica 2013/08A
2013	September	Summer	Belgica 2013/26A

Within the Bongo net samples, 38 species of fish and squids were encountered (29 identified to species level). Two groups of squids were found, more precisely *Sepiola atlantica* (Figure 6) and the species complex Loliginidae (Figure 7)*,* which is composed of the species *Loligo vulgaris* and *Allotheutis subulata*. These species are indistinguishable at larval stage. In terms of fish larvae, the most abundant species were Ammodytidae sp. (42%), *Clupea harengus* (36%) and Clupeidae sp. (8%). Table 2 gives an overview of the encountered species list, with average and maximum densities, and monthly occurrence. Some specimens could not be identified to species level mostly due to their small size and/or degree of degradation (e.g. disappearing melanophores).

Figure 3. Image of the squid *Sepiola atlantica*

Figure 4. Image of Loliginidae sp.

Table 2. List of all taxa found in this study with scientific and common names, their average density (ind.m^{-3}), maximum density (ind.m^{-3}) and seasonal occurrence.

| Fish taxa/species	CommonName	Average density	Maximum density	Seasonal occurence
Pisces eggs		22,04	192,59	Dec-April, Jun, Jul, Sep
Pisces sp.		0,38	12,03	Feb, April, Jun, Jul, Sep
Ammodytidae				
Ammodytidae sp.	sandeel	0,52	7,69	Feb-April, Jun
Ammodytes marinus	Raitt's sandeel	<0.1	2,33	Jan-March
Hyperoplus lanceolatus	greater sandeel	<0.1	0,20	March, April
Ammodytes tobianus	lesser sandeel	<0.1	1,52	Jan, March, Dec
Hyperoplus immaculatus	Corbin's sand eel	<0.1	0,20	March
Bothidae				
Arnoglossus laterna	scaldfish	<0.1	<0.1	March, Sep
Callionymidae				
Callionymus reticulatus	reticulated dragonet	<0.1	0,98	Jun, Jul
Callionymus lyra	common dragonet	<0.1	0,34	Jul
Callionymus sp.	dragonet	<0.1	0,32	Jan, Jul
Carangidae				
Trachurus trachurus	Atlantic horse mackerel	0,11	2,25	Jun, Jul, Sep, Dec
Clupeidae				
Clupea harengus	Herring	0,60	5,40	Dec-April, Jul
Clupeidae sp.	shads	0,59	17,33	Jan-April, Jun, Jul
Sprattus sprattus	Sprat	0,30	5,38	Feb, Jun, Jul, Dec
Sardina pilchardus	European pilchard	<0.1	0,80	Jul
Cottidae				
Myoxocephalus scorpius	short-horn sculpin	<0.1	0,20	March
Gadidae				
Gadus morhua	cod	<0.1	<0.1	March
Merlangius merlangus	whiting	<0.1	0,20	March
Gadidae sp.	codfishes	<0.1	1,28	Feb, March, Jun, Jul
Pollachius pollachius	pollack	<0.1	0,35	Jul
Trisopterus luscus	Bib	<0.1	0,10	Feb, Dec
Pollachius virens	saithe	<0.1	<0.1	Feb
Gobiidae				
Pomatoschistus microps	common goby	<0.1	<0.1	Sep
Pomatoschistus minutus	sand goby	<0.1	<0.1	Sep
Gobiidae sp.	gobies	<0.1	0,59	Jun, Jul, Sep, Dec
Gobius niger	black goby	<0.1	0,20	Jul, Sep
Pomatoschistus pictus	painted goby	<0.1	0,32	March, Jul, Sep
Liparidae				
Liparis liparis	sea snail	<0.1	<0.1	Jan, March
Pleuronectidae				
Pleuronectes platessa	plaice	<0.1	0,24	Jan-March
Pleuronectidae sp.	Righteyed Flounders	0,21	5,31	Jan, March, Jun
Limanda limanda	dab	<0.1	<0.1	Feb, March
Soleidae				
Solea solea	common sole	<0.1	0,79	Jan, April, Jun, Jul
Syngnathidae				
Syngnathus rostellatus	Nilsson's pipefish	<0.1	0,05	Sep
Trachinidae				
Echiichthys vipera	lesser weever	<0.1	0,32	Jul
Triglidae				
Chelidonichthys lucerna	tub gurnard	<0.1	0,29	Jul
Squid taxa/species	**CommonName**	**Average density**	**Maximum density**	**Seasonal occurence**
Loliginidae				
Loliginidae sp.	pencil squids	<0.1	<0.1	Sep
Sepiolidae				
Sepiola atlantica	Atlantic bobtail	<0.1	<0.1	Jan
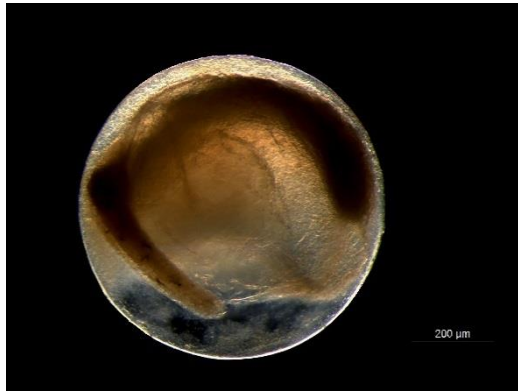
Figure 5. Image of a fish egg.

Overall, the trends observed for fish eggs and fish larvae were similar, with peaks around March (2010, 2013) and June (2010). The maximum egg density was recorded in March 2013 with 73.73 ind.m^{-3} and fish larvae had their major peak in June, reaching 0.37 ind.m^{-3}. Squid larvae, on the other hand, were only present on two sampling occasions: January 2012 (density so small it is not visible in Figure 9) and in September 2013, where they reached their maximum density of 0.02 ind.m^{-3}. In these two occasions the species were different, in January the species observed was *Sepiola atlantica* while in September it was Loliginidae sp.

WIND FARM IMPACT

PERMANOVA tests in a *CI x event* design were done for total densities of fish eggs, fish larvae and squid larvae, for fish larvae composition and for densities of the most abundant fish species and groups (Clupeidae sp., Pleuronectidae sp., *Clupea harengus*, *Sprattus sprattus*, Ammodytidae sp., Gobiidae sp.) and for fish larvae diversity (number of species S, expected number of species ES, Shannon-Wiener diversity index). Significant interaction effects were seen for *C. harengus* densities, for fish larvae composition and for the expected number of species (see table 3). Pairwise tests, however, indicated that these were more the result of event-related factors rather than wind farms related impact.

Table 3. PERMANOVA results of overall tests including treatment (control versus impact) and sampling event. Significant results are indicated as bold.

	Source	df	SS	MS	Pseudo-F	P(perm)	unique perms
composition fish larvae	CI	1	586,94	586,94	0,74762	0,5672	9938
	ev	10	79387	7938,7	15,693	0,0001	9872
	CIxev	10	7850,8	785,08	1,5519	**0,0072**	9863
	Res	44	22258	505,87			
	Total	65	1,10E+05				
density *Clupea harengus*	CI	1	1,17E-02	1,17E-02	3,88E-02	0,8549	9835
	ev	10	12,528	1,2528	45,264	0,0001	9942
	CIxev	10	3,0176	0,30176	10,903	**0,0001**	9943
	Res	44	1,2178	2,77E-02			
	Total	65	16,775				
Density Ammodytidae sp.	CI	1	8,66E-02	8,66E-02	2,2004	0,1719	8006
	ev	10	15,345	1,5345	61,853	0,0001	9953
	CIxev	10	0,39344	3,93E-02	1,5859	0,1364	9920
	Res	44	1,0916	2,48E-02			
	Total	65	16,917				
Density Clupeidae sp.	CI	1	7,10E-03	7,10E-03	6,49E-02	0,8025	9812
	ev	10	11,026	1,1026	14,851	0,0001	9946
	CIxev	10	1,0946	0,10946	1,4744	0,1722	9937
	Res	44	3,2667	7,42E-02			
	Total	65	15,395				
density Gobiidae sp.	CI	1	3,78E-02	3,78E-02	0,87298	0,397	946
	ev	10	1,9468	0,19468	5,2808	0,002	999
	CIxev	10	0,43314	4,33E-02	1,1749	0,341	999
	Res	44	1,6221	3,69E-02			
	Total	65	4,0399				
density *Sprattus sprattus*	CI	1	2,92E-02	2,92E-02	0,33289	0,5473	2587
	ev	10	7,9397	0,79397	16,612	0,0001	9946
	CIxev	10	0,8768	8,77E-02	1,8345	0,0638	9927
	Res	44	2,1029	4,78E-02			
	Total	65	10,949				
density Pleuronectidae sp.	CI	1	3,55E-02	3,55E-02	3,7022	0,0816	2498
	ev	10	5,3453	0,53453	9,1838	0,0001	9932
	CIxev	10	9,60E-02	9,60E-03	0,16494	0,998	9917
	Res	44	2,561	5,82E-02			
	Total	65	8,0379				
total density fish larvae	ci	1	0,15066	0,15066	3,0968	0,0868	9845
	ev	10	17,779	1,7779	36,544	0,0001	9936
	cixev	10	0,89055	8,91E-02	1,8305	0,0834	9924
	Res	44	2,1406	4,87E-02			
	Total	65	20,961				
fish larvae S	CI	1	1,52E-02	1,52E-02	4,66E-03	0,9637	3719
	ev	10	299,27	29,927	15,553	0,0001	9935
	CIxev	10	32,485	3,2485	1,6882	0,121	9948
	Res	44	84,667	1,9242			
	Total	65	416,44				
fish larvae ES(50)	CI	1	1,2273	1,2273	1,4261	0,2635	961
	ev	10	104,12	10,412	42,95	0,0001	9921
	CIxev	10	8,6061	0,86061	3,55	**0,0014**	9957
	Res	44	10,667	0,24242			
	Total	65	124,62				
fish larvae Shannon-Wiener	CI	1	3,13E-02	3,13E-02	0,17072	0,685	9807
	ev	10	14,994	1,4994	13,316	0,0001	9942
	CIxev	10	1,8352	0,18352	1,6297	0,1287	9947
	Res	44	4,9547	0,11261			
	Total	65	21,816				
total density fish eggs	ci	1	0,33141	0,33141	3,9081	0,0522	9823
	ev	10	77,041	7,7041	90,848	0,0001	9934
	cixev	10	0,9596	9,60E-02	1,1316	0,3721	9937
	Res	44	3,7313	8,48E-02			
	Total	65	82,063				
total density squid larvae	ci	1	6,67E-05	6,67E-05	1,56E-02	0,9071	9774
	ev	10	0,56116	5,61E-02	13,13	0,0002	9616
	cixev	10	3,64E-02	3,64E-03	0,85108	0,6767	9923
	Res	44	0,18805	4,27E-03			
	Total	65	0,78564				

TEMPORAL VARIATION

Since "event" appeared to have more important structuring effect on fish eggs, fish larvae and squids than wind farm presence, we further explored the temporal variation within the samples (Figure 6). In terms of fish larval density, we observed a significant seasonal and inter-annual variability, and an interaction between the two (PERMANOVA SxY: pseudo F of 4.72, p<0.001). The species contributing most to the dissimilarity between seasons were Ammodytidae sp., Clupeidae sp., *Ammodytes marinus, Clupea harengus* and Pisces sp.. In terms of interannual variability, the main contributors were Ammodytidae sp., Clupeidae sp., *Ammodytes marinus, Clupea harengus* and Gobiidae sp.

For fish egg density, a significant inter-annual and inter-seasonal difference was also detected, but without interaction between the terms (PERMANOVA pseudo F of 3, p=0.0869). Seasonal variability was statistically significant between all seasons with the exception of winter versus spring, while inter-annual variability was significant among all years except between 2010 and 2013.

Squid larval densities were significantly different between seasons and years and there was a significant interaction between the two. However, the extremely low encounter rate of these larvae hampered further statistical exploration.
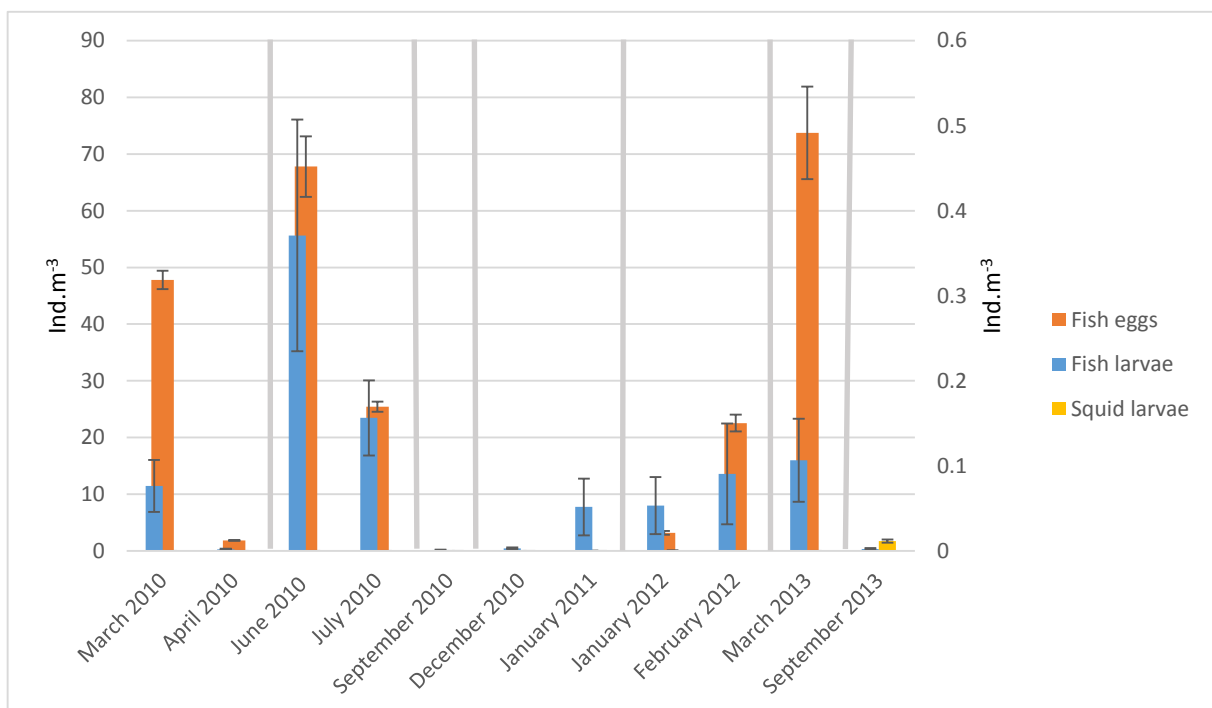
Figure 6. Density (ind.m^{-3}) of fish eggs (left axis) and fish and squid larvae (right axis) per sampling event. Grey vertical lines represent the periods (months) where data is missing.

Species richness *S* ranged between 0.3 to 7 species per sampling event, while the expected number of species in 50 individuals (ES_{50}) varied between 0 and 4. Shannon Wiener diversity (H') ranged between 0 and 1.6 (Figure 7). Species richness S showed both inter-seasonal and inter-annual significant differences. The seasonal differences were observed between winter-autumn and spring-autumn, while the annual ones were between 2011-2012, 2011-2013 and 2012-2013. ES_{50} on the other hand only exhibited a significant variability between seasons and not years. This asymmetry was between winter-autumn and summer-autumn and it was similar to the one observed in S. The Shannon-Wiener index showed interannual variability, with the differences lying between 2010-2011, 2011-2013 and 2012-2013.
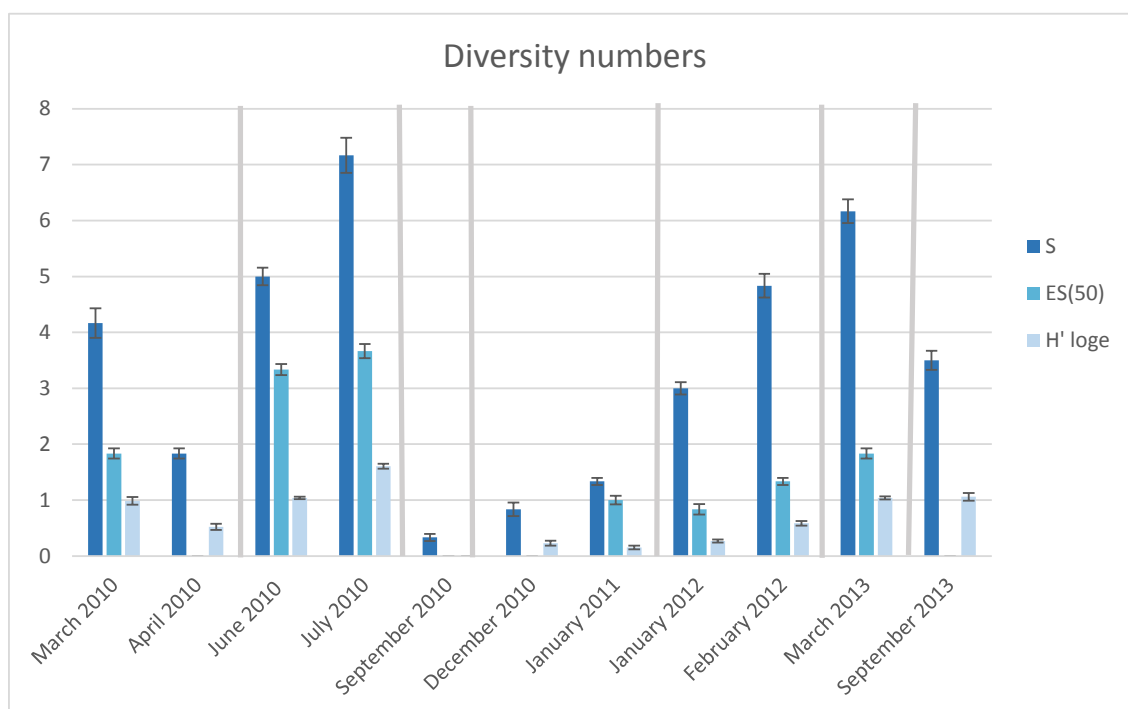
Figure 7. Column chart of diversity numbers for all sampling events considered in this study. Diversity represented as the number of species (S), the expected number of species in 50 individuals (ES(50)) and the Shannon-Wiener diversity index (H'). Grey vertical lines represent the periods (months) where data is missing.

Figure 8. Larvae of *Sprattus sprattus*, *Clupea harengus*, *Ammodytes marinus* and *Trachurus trachurus*.

The analysis of community structure of fish larvae also showed a clear temporal structure: in January the community composition was mainly *Clupea harengus* (87.82%) and Clupeidae sp*.* (10.29%). February was dominated by Ammodytidae sp. (87.35%) and *Clupea harengus* (9.62%). In March, *Ammodytes marinus* (23.85%) Ammodytidae sp. (40.19%) *Clupea harengus* (27.82%) had the highest relative abundance. In April the group with higher abundance was "other", which refers to amongst others *Arnoglossus laterna, Callionymus lyra, Pollachius pollachius,* and *Syngnathus rostellatus*. Besides this group, *Clupea harengus* represented 14.29% of the total community and Clupeidae sp*.* 21.43%. In June the group with highest abundance was also "other", but in terms of the species selected,

Clupeidae sp. had a relative abundance of 38.45%, Pleuronectidae sp*.* 14.79% and *Sprattus sprattus* 12.01%. In July it was mainly *Sprattus sprattus* (39.33%) and *Trachurus trachurus* (20.08%), with a smaller percentage of *Clupea harengus* (9.62%). September was dominated by Gobiidae sp. (48.87%) and December by *Clupea harengus* (20%), *Sprattus sprattus* (20%) and Gobiidae sp. (20%). Since the individuals identified as Clupeidae sp. were the ones where a proper identification to species level was not possible, the interpretation of figure 9 suggests that they were most likely *C. harengus* in the first half of the year and *S. sprattus* in the second half. However, it's important to keep in mind the discontinuity of this study when interpreting the results.
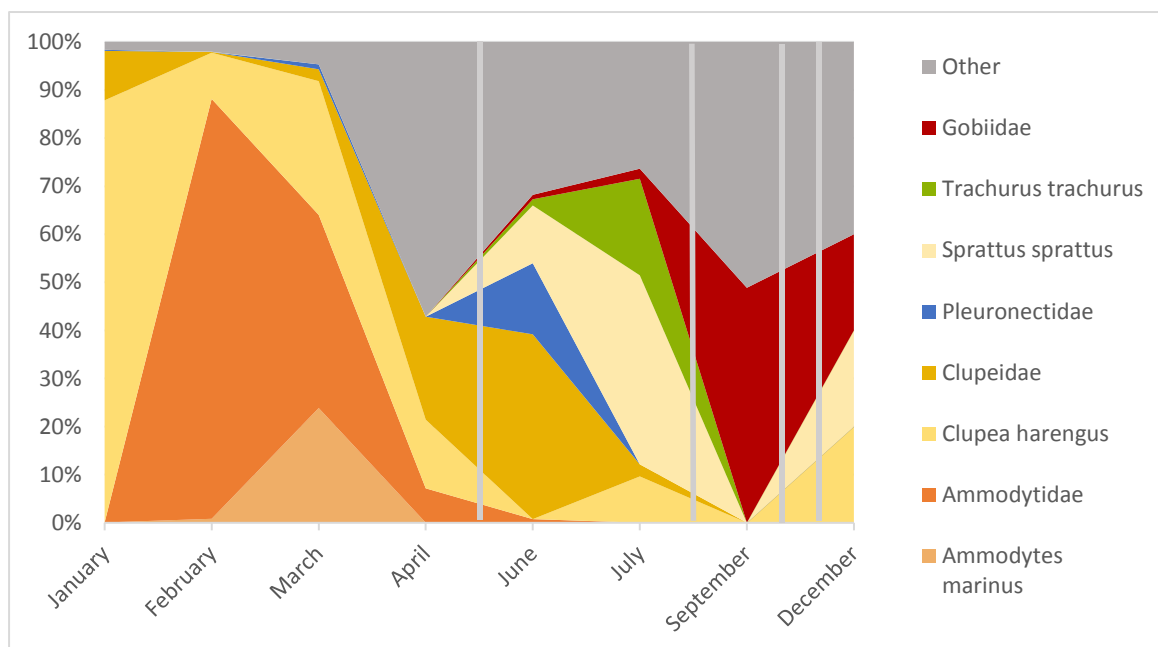
Figure 9. Relative seasonal abundance (%) of the most abundant fish larvae taxa. Grey vertical lines represent the periods (months) where data is missing.

ENVIRONMENTAL VARIABLES AND THEIR INFLUENCE ON FISH LARVAE

The profiles of all environmental variables show that the water column was generally well-mixed throughout the year. Water temperature showed peaks around July and September. Values fluctuated between 4 ºC in February 2012 (winter) and 17 ºC in July 2010 (summer). Salinity was practically constant throughout the year and ranged between 31 PSU and 34 PSU, with lowest values in March 2013 and highest values in January 2012 (Figure 10). Chl *a* and secchi depth (a measure of turbidity) showed largely opposite profiles. Chl *a* varied between 0.14 µg/L (June 2010) and 6.01 µg/L (April 2010)

and Secchi depth had as lowest value 2 m (March 2013) and highest 8 m (June 2010).

To investigate the relationship between environmental variables and observed densities, a DistLM model was used. The output showed that the model that best fitted the data was the one including water temperature, salinity and secchi depth. Between the three variables, water temperature most explained the variation in the data (19%, p=0.0001), while Secchi depth was responsible for 12% (p=0.0001) and salinity for less than 0.1% (p=0.0001).
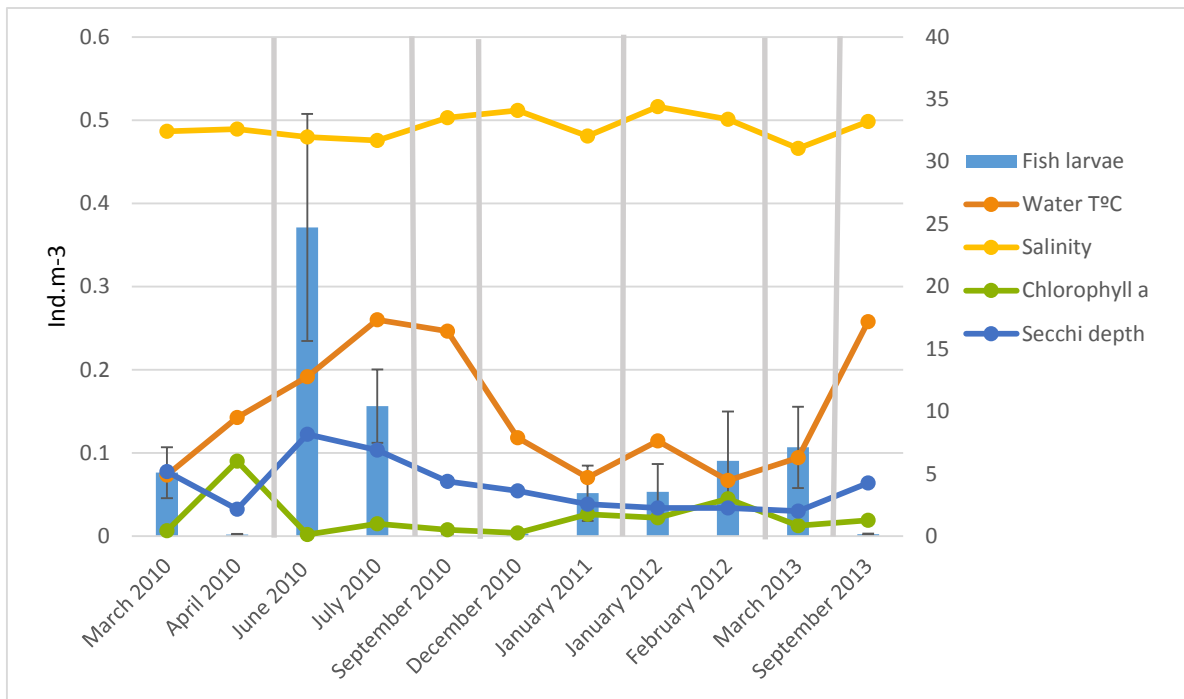
Figure 10. Fish larval densities (ind.m-3) per sampling event, averaged over all stations (with SE) in the left axis. On the right axis: Water temperature (ºC), salinity (PSU), chlorophyll a (µg/L) and secchi depth (m). Grey vertical lines represent the periods (months) where data is missing.

A distance-based redundancy analysis (dbRDA) plot was combined with a Multi-Dimensional Scaling (MDS) plot of fish larvae composition in order to visualize, in context, the results from the DistLM analysis. This ordination plot illustrates the relationship between the predictor (environmental) variables that best explain the fish larvae density data variation. The vectors within the circle represent the effect of the explanatory variables included in the model, with the length of the vector corresponding to how much a variable explains the data. The longer the vector the best a certain variable explains it (Anderson *et al.,* 2008). Accordingly, the variables which best explained the fish larvae density data were water temperature, Secchi depth and salinity (Figure 11).
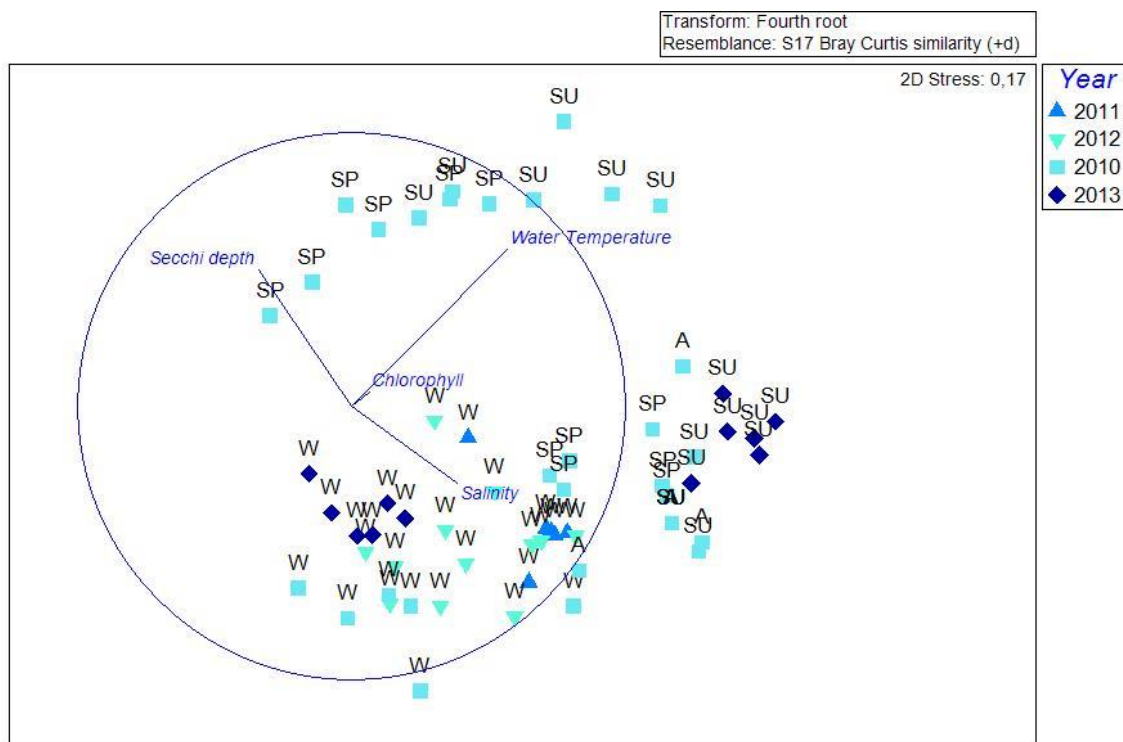
Figure 11. Two-dimensional non-metric MDS plot (stress value=0.17) of all samples, with indication of different shades of blue for year (2010-2013) and different symbols for spring (April-June), summer (July-September), autumn (October-December) and winter (January-March). Adjusted to the MDS plot is the distLM output graph (dbRDA plot), with the environmental variables that best explain the outcome. The bigger the line the more likely that variable is to explain the pattern observed in the MDS plot.

9.4. DISCUSSION

GENERAL

This study focused on the offshore zooplankton community of the Thorntonbank, and a total of 38 fish and 2 squid species were found within the sampling area. Similar results were obtained in previous studies conducted in the BPNS and the larger North Sea (e.g. Dewicke et al., 2002; Taylor et al., 2007; Van Ginderdeuren et al., 2013), with the exception of the presence of larvae of *Gobius niger*, which were not recorder yet in Belgian waters. Adults, however, are found abundantly near wind turbine foundations (Andersson and Öhmann, 2010).

WIND FARM IMPACT

Up to now, research has been mainly focusing on the impacts of wind farms on the adult stages of fish (e.g. Derweduwen *et al.,* 2010; Lindeboom *et al.,* 2011; Reubens *et al.,* 2010, 2011; Vandendriessche *et al.,* 2011-2013a). The present study, on the other hand,

specifically focused on the impact of wind farms on the early life stages of fish and squids in order to detect the specific effects of the introduction of turbines (hard substrate) on such an important part of their life cycle. Potential effects include an increase in eggs and larvae of species which require hard substrates to spawn (e.g. herring, gobies), and a decrease in the species which need sandy bottoms.

Despite the expected effects of the wind farm on fish and squid larvae, PERMANOVA tests did not detect significant differences between the two sampling locations: control (reference area) and impact (concession area), in terms of density, species composition and diversity. The absence of statistical evidence of impact, however, should be seen in the light of the following facts:

- The continuous construction of turbines after the beginning of this study (see Figure 1), leading to an expansion of the concession area which almost reached the reference area by the end of the study period. This caused the conditions between the two sampling locations to become increasingly similar with time, reducing the probability of finding significant differences among them. The reference stations could have been affected by the disturbance caused during the construction phase, which influenced the usual dynamics of the area. However, effects take longer to establish with increasing distance from the impact so some distinction between the sampling stations (control and impact)

would probably still exist but the disturbance decreased the probability of that difference being significant. Therefore, in future studies it is imperative to move the reference area further away from the C-Power concession in order increase the possibility of detecting the impacts of the introduction of such artificial structures while still maintaining similar environmental conditions (to avoid confounding factors).

- The large distance between turbine rows which delays the detection of changes within the sandy substrate, and the safety measures (i.e. distance from turbines) which prevented the detection of effects in the areas where they most occur (i.e. closer to the turbines, Coates *et al.,* 2012).

- The wind farm is relatively recent and it has been shown that stable biotic communities take some time to be fully established following the introduction of an artificial structure (new habitat) (i.e. 3 to 5 years) delays the detection of impacts (Degraer *et al.,* 2012; Jensen, 2002; Gray, 2006; Petersen and Malm, 2006). Therefore, effects of spawning substrate are still small but may increase over the following years and with the increase of the number of turbines.

In terms of squid larvae, an increase in density within the wind farms in comparison with the reference area was expected, due to their preference for hard substrate as

spawning ground. The results, however, showed that the densities were rather low and that only few individuals were caught with the Bongo net. Hence, other sampling methods should be considered in order to capture more individuals and allow a proper comparison between different locations. One of these methods could include visual census (performed by divers) of spawning adults and egg clusters. Diver observations are already being carried out within the framework of the environmental monitoring of the wind farms in the BPNS (Kerckhof *et al.,* 2012), and an additional focus on squids and their egg clusters could be considered. Egg clusters have been seen on video footage (Francis Kerckhof, pers. comm.) and on loggers recovered from the wind farm area (Jan Reubens, pers. comm.) but these were not investigated in detail. Moreover, literature has been mainly focusing on adults and on the species with high commercial value (e.g. *Loligo forbesi*) and there is a general lack of information regarding the community composition, prey, spawning periods, geographic range and temporal distribution of these animals and their young. There is a need to expand the focus of research and encompass a wide range of species. A fundamental baseline study on the distribution of larval and juvenile squids in the southern North Sea is desirable. An impact study of wind farms and other anthropogenic activities can only be successful when detailed information on the ecology of these species is available.

Moreover, other than detecting the anthropogenic impact of wind farms on the density and diversity of ichthyoplankton and squids, it is important to confirm if the wind farms are, in fact, spawning grounds. Usually this can be detected by mapping the distribution of species and observing which areas have higher aggregation of individuals, the presence of both eggs and larvae, and the presence of mature individuals with running eggs or sperm (Ellis *et al.,* 2012). Therefore, ichthyoplankton studies, such as this one, provide valuable information for tracing the changes derived from the introduction of a new type of substrate (creation of a new spawning ground), but should be complemented with more information (e.g. spawning stock biomass).

OTHER SOURCES OF VARIATION

Fish larvae

Significant wind farm effects were not detected, but significant differences among seasons and years were very clear, reflecting a temporal variability in density, composition and diversity of fish larvae. The temporal variability was best reflected in the succession of species throughout the year with *Clupeidae* present year-round, alternating between *C.harengus* and *S. sprattus. Ammodytidae* were mostly confined to February and March, while *Pleuronectidae* appeared mainly in June, followed by *T. trachurus* and *Gobiidae* in the last months of the year. Other than this seasonal succession, also interannual differences were detected. These types of variability are related to the time of spawning and to the time at which the main food sources of fish larvae are most available, so that the hatching matches the period of higher food availability. Since fish larvae are small in size, their swimming capacities are not yet well developed (i.e. slow swimming

speed), which makes finding prey more difficult. Therefore, the higher the abundance in prey, the higher the encounter rates will be and feeding will succeed instead of fail (Bailey *et al.,* 2004). This synchronization is vital for the recruitment success of high trophic level species in the temperate southern North Sea (Hjort 1914, Cushing 1990, Eilertsen and Wyatt 2000, Kirby *et al.* 2007).

Fish larvae are very susceptible to not only biological, but also physical changes in the environment (Taylor *et al.,* 2002, Voigt *et al.,* 2003, Hays *et al.,* 2005). These changes affect their physiological metabolism and reproduction, so individuals tend to search for appropriate conditions (e.g. temperature, salinity) which may differ to some degree between species (Rose, 2005; van der Kooij *et al.,* 2008; Munk *et al.,* 2009; Hillgruber *et al.,* 1997, Erftemeijer *et al.,* 2009). These environmental conditions also have the capacity to affect fish larvae indirectly, by affecting the timing of both phyto- and zooplankton blooms (food sources). For instance, temperature and salinity have a major effect on the stratification or mixing of the water column, which consequently influence the nutrient supply to the layers occupied by phytoplankton. Moreover, warmer temperatures are known to affect the timing of both blooms (Genner *et al.,* 2010; Smayda *et al.,* 2004; Beare *et al.,* 2002).

Surface water temperature during the study period ranged between 4ºC in February 2012 (winter) and 17ºC in July 2010 (summer). Similar results were obtained by Van Ginderdeuren *et al.* (2013) with a minimum of 2ºC also in February and a maximum of 21ºC in August, and O'Brien *et al*. (2011) with the minimum temperature being recorded in February and the maximum in August. Salinity ranged from 31 to 34 PSU,

remaining constant throughout the year, which matched the observations made by Van Ginderdeuren *et al.* (2013) where the range was from 30-35 PSU. These results are identical to the "typical values for salinity and temperature of water masses in the North Sea" (OSPAR report, adapted from NSTF, 1993). Secchi depth, as a measure of turbidity, showed an opposite pattern in relation to Chl *a*. This is logical since an increase in phytoplankton density increases the amount of particles in the water, thereby decreasing visibility (increasing turbidity). An increase in turbidity corresponds to a lower secchi depth. However, it is not accurate to assume an exclusive correlation between Chl *a* and secchi depth, since visibility/turbidity is also affected by other factors (e.g. sediment resuspension).

In this study, the model which best fitted the density of fish larvae was the one with all variables except Chl *a*. This was not expected, since fish larvae are dependent on phytoplankton, and Chl *a* is an indirect measure of phytoplankton abundance. Many studies, performed on merohyperbenthos (i.e. individuals which spend only part of their early-life history on the water layer closer to the seabed, including fish larvae), have come to the conclusion that the two variables which explained the data best were Chl *a* and temperature (Dewicke *et al.,* 2002; Russell 1976; Lindley 1998). In the present study, large chl *a* differences were not expected, since control and impact stations were quite close to each other. Nevertheless, chl *a* values were obtained from in situ fluorimeter measurements. In the case of missing or aberrant measurements, values were obtained from satellite data. The spatial and temporal resolution of these data were not always optimal, which could have obscured small-scale spatial variation. Hence, chl *a* was

not retained in the statistical model. However, the remaining variables within the model were expected to have an influence on the ichthyoplankton data. For instance, Harris and Cyrus (1996) reported temperature and turbidity (secchi depth) as the most relevant explanatory variables in terms of fish larvae density.

Fish eggs

A significant inter-seasonal and inter-annual variability was also detected in terms of fish egg density. They were observed throughout the study period and showed a similar pattern as fish larvae, with peaks occurring in March (2010, 2013) and June (2010). The year-round occurrence of fish eggs was expected since no distinction was made between eggs from different species, and spawning periods are spread throughout the year with occasions where the spawning period of more than one species overlap. This may explain the higher densities in those months. These observations supported the ones made by Dewicke *et al*. (2002), who found the highest numbers of fish eggs around the same time of the year in the Flemish Banks, and by Beyst *et al*. (2001) where fish eggs were observed around March and April. Like fish larvae, the peaks in egg density were most likely related with the synchronization between the time of spawning and of higher food availability for the newly-hatched larvae. The analysis of fish eggs is important since it provides valuable insight in terms of spawning of important fish species, sometimes more precise than the data obtained from adult individuals (Fox *et al.,* 2005). This is of extreme importance for fisheries biology and management in order to, for instance, outline important habitats and determine the cause of shifts in population abundance (Munk *et al.,* 2009). Therefore, in future studies, the identification to species level should be conducted not only for larvae, but for eggs as well.

9.5. CONCLUSION

Based on the data of 2010-2013, no clear evidence could be provided for positive impacts of wind farms on early life stages of fish and squid. The study, however, provides a good baseline for future monitoring and allows to fine-tune research methodology. Recommendations for future studies include an optimal geographical spacing of the sampling stations, and an extension of data collection through visual census. Additionally, data on early life stages should be complemented with data on spawning stock biomass.

ACKNOWLEDGEMENTS

REFERENCES

Andersson, M. H., & Öhman, M. C. (2010). Fish and sessile assemblages associated with wind-turbine constructions in the Baltic Sea. *Marine and Freshwater Research* 61, 642–650.

Bailey, K. M., Nakata, H., & Van der Veer, H. W. (2004). The planktonic stages of flatfishes: physical and biological interactions in transport processes. Flatfishes: Biology and exploitation, 94-119.

Beare, D.J., Batten, S., & Edwards, M. (2002). Prevalence of boreal Atlantic, temperate Atlantic and neritic zooplankton in the North Sea between 1958 and 1998 in relation to temperature, salinity, stratification intensity and Atlantic inflow. Journal of Sea Research 48: 29–49

Beyst, B., Buysse, D., Dewicke, A., & Mees, J. (2001). Surf zone hyperbenthos of Belgian sandy beaches: seasonal patterns. Estuarine, Coastal and Shelf Science. 53: 877-895.

Bray, J.R., & Curtis, J.T. (1957). An ordination of the upland forest communities of Southern Wisconsin. Ecological Monographs 27: 325-349.

Clarke, K., & Gorley, R. (2006). PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth, 190 pp.

Clarke K.R. (1993). Non-parametric multivariate analysis of changes in community structure. Australian Journal of Ecology. 18: 117-143.

Coates, D., Vanaverbeke, J. & Vincx, M. (2012). Enrichment of the soft sediment macrobenthos around a gravity based foundation on the Thorntonbank pp. 41-54. 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.

Cushing, D.H. (1990). Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. Advances in Marine Biology 26: 249-293.

Degraer, S., Brabant, R., Coates, D., Courtens, W., Derweduwen, J., Haelters, J., Hostens, K., Houziaux, J.-S., Kerckhof, F., Norro, A., Onkelinx, T., Rumes, B., Stienen, E.W.M., Vanaverbeke, J., Vandendriessche, S., Van de walle, M., Vanermen, N., Van Roy, W., Verschelde, P., Verstraete, H., Vigin, L., Vincx, M., & Willems, T. (2012). Executive Summary. pp. 1-8 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.

Degraer, S. (2014). Environmental impacts of offshore wind farms in the Belgian part of the North Sea: Learning from the past to optimize future monitoring programmes in: Mees, J. et al. (Ed.), 2014. Book of abstracts – VLIZ Young Scientists' Day. Brugge, Belgium, 7 March 2014. VLIZ Special Publication, 67: pp. 164-168.

Derweduwen, J., Vandendriessche, S., & Hostens, K. (2010). Monitoring the effects of the Thorntonbank and Bligh Bank wind farms on the epifauna and demersal fish fauna of soft bottom sediments: Thorntonbank: status during construction (T2) Bligh Bank: status during construction (T1). Pp 106-132. 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.

Dewicke, A., Cattrijsse, A., Mees, J., & Vincx M. (2002). Structuring patterns of the hyperbenthos communities of the Belgian and Zeeland subtidal sandbanks (North Sea) in Dewicke, A., 2002. Hyperbenthic communities of the North Sea: De hyperbenthische gemeenschappen van de Noordzee. PhD Thesis. Universiteit Gent.

Di Marcantonio, M., Brabant, R., Haelters, J., Kerckhof, F., Schallier, R., Van den Eynde, D., Vigin, L., & Jacques, T.G. (2007). Milieueffectenbeoordeling van het BELWIND offshore windmolenpark op de Bligh Bank. BMM, Koninklijk Belgisch Instituut voor natuurwetenschappen.

Eilertsen, H., & Wyatt, T. (2000). Phytoplankton models and life history strategies. South African Journal of Marine Science 22: 323-338.

Ellis, J.R., Milligan, S.P., Readdy, L., Taylor, N., & Brown, M.J. (2012). Spawning and nursery grounds of selected fish species in UK waters. Sci. Ser. Tech. Rep., Cefas Lowestoft, 147: 56 pp.

Erftemeijer, P.L.A., van Beek, J.K.L., Bolle, L.J., Dickey-Collas, M., & Los, H.F.J. (2009). Variability in transport of fish eggs and larvae. I. Modelling the effects of coastal reclamation. Marine Ecology Progress Series 390: 167–181.

Fox, C., Taylor, M., Dickey-Collas, M., van Damme, C.J.G., Bolle, L., Daan, N., Rohlf, N., Kraus, G., Munk, P., Fossum, P., & Bailey, N. (2005). Initial results from the 2004 ichthyoplankton survey of the North Sea. ICES CM 2005/AA:04.

Genner, M.J., Halliday, N.C., Simpson, S.D., Southward, A.J., Hawkins, S.J., & Sims, D.W. (2010). Temperature-driven phonological changes within a marine larval fish assemblage. Journal of Plankton Research 32: 699–708.

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

Hastie, L.C., Pierce, G.J., Wang, J., Bruno, I., Moreno, A., Piatkowski, U. & Robin, J-P. (2009). 'Cephalopods in the north-eastern Atlantic: Species, biogeography, ecology, exploitation and conservation'. Oceanography and Marine Biology, vol 47, pp. 111-190.

Hays, G.C., Richardson, A.J., & Robinson, C. (2005). Climate change and plankton. Trends in Ecology and Evolution 20: 337–344.

Hillgruber, N., Kloppmann, M., Wah, l.E., & von Westernhagen, H. (1997). Feeding of larval blue whiting and Atlantic mackerel: a comparison of foraging strategies. Journal of Fish Biology 51: 230–249.

Hjort, J., (1914). Fluctuations in the great fisheries of northern Europe. Rapp. P. – v des Réun. Cons. Int. Explor. Mer., 20: 1-128.

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

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. et al. (Ed.) (2012). Offshore wind farms in the Belgian part of the North Sea: Heading for an understanding of environmental impacts. pp. 17-39.

Kirby, R.R., Beaugrand, G., Lindley, J.A., Richardson, A.J., Edwards, M., & Reid, P.C. (2007). Climate effects and benthic-pelagic coupling in the North Sea. Marine Ecology Progress Series 330: 31–38.

Lindeboom, H.J. , Kouwenhoven, H. .J, Bergman, M.J.N., Bouma, S., Brasseur, S., Daan, R., Fijn, R.C. , de Haan, D. , Dirksen, S., van Hal, R., Lambers, R.H.R., ter Hofstede, R., Krijgsveld, K.L., Leopold, M., & Scheidat, M. (2011). Short-term ecological effects of an offshore wind farm in the Dutch coastal zone; a compilation. Environmental Research Letters. 6: 13 pp.

Lindley, J.A. (1998). Diversity, biomass and production of decapod crustacean larvae in a changing environment. Invertebrate Reproduction and Development 3 3 : 209 -219.

Munk, P., Fox, C.J., Bolle, L.J., van Damme, C.J.G., Fossum, P., & Kraus, G. (2009). Spawning of North Sea fishes linked to hydrographic features. Fisheries and Oceanography 18: 458–469.

Munk, P., & Nielsen, J.G. (2005). Eggs and larvae of North Sea fishes. Biofolia.

O'Brien, T. D., Wiebe, P. H., & Hay, S. (Eds), (2011). ICES Zooplankton Status Report 2008/2009. ICES Cooperative Research Report No. 307. 152 pp.

Petersen, J.K., & Malm, T. (2006). Offshore windmill farms: threats to or possibilities for the marine environment. Ambio 35(2).

Reubens, J., Degraer, S., & Vincx, M. (2010). The importance of marine wind farms, as artificial hard substrata, for the ecology of the ichthyofauna. In Degraer S., Brabant R., Rumes B. (Eds.), Offshore wind farms in the Belgian part of the North Sea : early environmental impact assessment and spatio-temporal variability. Brussels, Belgium: Royal Belgian Institute of Natural Sciences. Management Unit of the North Sea Mathematical Models. pp. 69–82.

Reubens, J., Degraer, S. & Vincx, M. (2011). Spatial and temporal movements of cod (Gadus morhua) in a wind farm in the Belgian part of the North Sea using acoustic telemetry, a VPS study, in: Degraer, S. et al. (Ed.) (2011). Offshore wind farms in the Belgian part of the North Sea: Selected findings from the baseline and targeted monitoring. pp. 39-46.

Rose, G.A., (2005). On distributional responses of North Atlantic fish to climate change. ICES Journal of Marine Science 62: 1360–1374

Rumes, B., Coates, D., De Mesel, I., Derweduwen, J., Kerckhof, F., Reubens, J., & Vandendriessche, S. (2013). Does it really matter? Changes in species richness and biomass at different spatial scales in: Degraer, S. et al. (Ed.), 2013. Environmental impacts of offshore wind farms in the Belgian part of the North Sea: Learning from the past to optimise future monitoring programmes. pp. 183-189.

Russell, F.S. (1976). The Eggs and Planktonic Stages of British Marine Fishes. Academic Press, London,524pp.

Smayda, T.J., Borkman, D.G., Beaugrand, G., & Belgrano, A. (2004). Responses of marine phytoplankton populations to fluctuations in marine climate. In: Stenseth NC, Ottersen G, Hurrell J, Belgrano A (eds) Marine ecosystems and climate variation: the North Atlantic. Oxford University Press, Oxford, p 49–58.

Sweeney, M.J., Roper, C.F.E., Mangold, K.M., Clarke, M.R., & Boletzky, S.V. (1992). "Larval" and juveline cephalopods: a manual for their identification. Smithsonian Institution Press.

Taylor, A.H., Allen, I.J., & Clark, P.A. (2002). Extraction of a weak climatic signal by an ecosystem. Nature 416: 629–632.

Taylor, N., Fox, C.J., Bolle, L., Dickey-Collas, M., Fossum, P., Kraus, G., Munk, P., Rolf, N., Van Damme, C., & Vorbach, M. (2007). Results of the spring 2004 North Sea ichthyoplankton surveys. ICES Cooperative Research Report No. 285, 59 pp.

van der Kooij, J., Scott, B.E., & Mackinson, S., (2008). The effects of environmental factors on daytime sandeel distribution and abundance on the Dogger Bank. Journal of Sea Research 60:201–209.

Van Ginderdeuren, K., Hostens, K., Van Hoey, G., & Vincx, M. (2013). The mesozooplankton species association in the Belgian part of the North Sea. Journal of Sea Research. In 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. Ghent University (Ugent), 226pp.

Vandendriessche, S., Reubens, J., Derweduwen, J., Degraer, S., Vincx, M., 2013a. 'Offshore wind farms as productive sites for fishes?' in S Degraer, R Brabant & B Rumes (eds), Environmental impacts of offshore wind farms in the Belgian part of the North Sea: Learning from the past to optimise future monitoring programmes. Royal Belgian Institute of Natural Sciences, pp. 152-161.

Vandendriessche, S., Derweduwen, J., & Hostens, K. (2011). Monitoring the effects of offshore windmill parks on the epifauna and demersal fish fauna of soft-bottom sediments: baseline monitoring. pp. 65-81 in Degraer, S., Brabant, R. & Rumes, B., (Eds.), 2011. Offshore wind

farms in the Belgian part of the North Sea: Selected findings from the baseline and targeted monitoring. Royal Belgian Institute of Natural Sciences, Management Unit of the North Sea Mathematical Models. Marine ecosystem management unit. 157 pp. + annex.

Vandendriessche, S., Derweduwen, J., & Hostens, K. (2013b). Between the turbines: soft substrate epibenthos and fish in: Degraer, S. et al. (Ed.), 2013. Environmental impacts of offshore wind farms in the Belgian part of the North Sea: Learning from the past to optimise future monitoring programmes. pp. 99-113.

Voigt, W., Perner, J., Davis, A.J., Eggers, T. and others (2003). Trophic levels are differentially sensitive to climate. Ecology 84: 2444–2453.